New value-adding opportunities for natural therapeutic products in the Australian Ginger Industry

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Craig Davis and Jeff Herse.

Research Provider: Queensland Department of Primary Industries.

FINAL REPORT

Queensland Government
Department of Primary Industries

Horticulture Australia Project Number: VG 00068.

Authors: Craig Davis and Jeff Herse

This is the final report for the above project that identifies the potential bioactivity of up to 30 compounds, including the *in vitro* and *in vivo* anti-inflammatory properties of ginger oil (a literature review, compound review, reports from *in vitro* and *in vivo* studies, and purification protocols as appendices).

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Date of report: 28 February 2003

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MEDIA SUMMARY

Ginger (*Zingiber officinale*) is one of the best-known and most important spices with a long history of the use of the ginger rhizome in Chinese and Ayurvedic medicine. It is an ingredient in more than half of all traditional Chinese medicines, and it has a wide range of pharmacological effects. In western alternative medicine, ginger is used to prevent nausea and motion sickness and to treat inflammatory (rheumatic) conditions.

In this study, ginger oil produced by supercritical fluid extraction with pressurised CO_2 was further fractionated using preparative HPLC. The resultant fractions were assessed for their anti-inflammatory activity using cell culture and animal model systems. The results showed that the whole ginger oil and some of the isolated fractions were able to reduce the inflammatory reactions in the model systems used. This information gives support to the use of ginger and its extracts for the treatment of medical conditions such as arthritis and rheumatism.

Further work in the area should involve a more thorough fractionation and characterisation of the major (and minor) components of the ginger oil. While this study has identified groups of compounds with (and without) bioactivity, the isolation, characterisation and assessment of individual components may provide a greater understanding of the mode of action of this widely used traditional medicine.

TECHNICAL SUMMARY

Ginger has been recognised for thousands of years as a medicine in the Chinese and Indian pharmacopoeias. More recently, modern science has attempted to dissect the components of the ginger that are responsible for the many and varied effects observed in medicines that contain ginger. This study has focused on the anti-inflammatory potential of ginger and the research effort has been directed to the activity of the 200 or more chemicals that make up the essential oil extract of the ginger rhizome.

Careful extraction of the oil from the dried ginger rhizome ensures that the heat-sensitive chemicals in the ginger oil are not adversely affected. This extraction is best undertaken using supercritical fluid extraction because the product is subjected to minimal temperature and the extract is not exposed to chemical solvents. The composition of the ginger oil produced using supercritical fluid extraction can be manipulated by varying the temperature and pressure of the dried ginger extraction.

In this study, two supercritical fluid extracted ginger oils were evaluated – the first was an oil while the other was a viscous ginger resin. Both of these ginger extracts were shown to have anti-inflammatory activity when assessed using a cell culture model system. This model assessed the release of prostaglandins (which are involved in the inflammation process) from cells with and without ginger oil added to the growth media. Both oils were shown to produce significant inhibition of prostaglandin release, with inhibitory potentials greater than currently used anti-inflammatory medications (such as aspirin and ibuprofen). One of these oils was then processed using preparative HPLC into ten discrete fractions, including four known ginger components (6-, 8- and 10-gingerol, and 6-shogaol). These fractions were presented to the *in vitro* model system and the results confirm that all of the ginger fractions retained some anti-inflammatory activity. The greatest activity (twice the level observed for aspirin) was observed in the 10-gingerol fractions.

Having confirmed the *in vitro* potential of the ginger oil and its various fractions, the project evaluated the anti-inflammatory activity of the oils using whole animals. For these studies, the whole ginger oils were presented to animals with a developing inflammation in the paw. As with the *in vitro* study, both oils were shown to have anti-inflammatory activity. One of the oils was then fractionated using preparative HPLC to produce gram quantities of the various fractions. While the fractionation of the ginger oil for the *in vitro* studies required a single run with a small (200mg) load, the fractionations for the *in vivo* studies required multiple 1 gram loads onto the HPLC. The ultimate volume of each of the 5 fractions that were produced was many litres. The material required for the *in vitro* study was able to be collected from a single chromatographic run and each fraction was analysed as it was produced for composition and purity. For the *in vivo* study, the final fractions were in large volumes and each of these fractions contained a range of compounds. The concentration of these fractions to pure oils (with no solvent or water) presented a major challenge. The final method that was adopted used selective adsorption of the fractions onto the preparative column and their concentrated elution in pure solvent. The solvent was then removed under vacuum and the oil was separated from the remaining water by solid phase extraction. Losses of over 70% of the oil (by weight) were observed during the various vacuum concentration steps. Since the material that was lost was the more volatile components of the ginger oil, the ginger pungents (6-, 8- and 10-gingerol, and 6-shogaol) were effectively concentrated during this step.

Similar to the observations in the cell culture system, the animal model showed that most of the ginger oil fractions were able to inhibit the progression of the inflammatory disease. The surprising exception to this was the 6-gingerol that appeared to promote the oedema associated with the inflammation (this result is contrary to previous published investigations). Some of the fractions that eluted early from the HPLC (e.g. those that contained the 8- and 10-gingerol and the 6-shogaol) were the most active, while the fractions that eluted late from the HPLC (e.g. the sesquiterpenes) presented with a comparatively small amount of anti-inflammatory activity. There is also some evidence to suggest that the disease process rebounds when the treatment with ginger or ginger fractions is ceased.

INTRODUCTION

The *Zingiberaceae* is a large family of perennial herbaceous plants. Ginger (*Zingiber officinale* Roscoe) has thick-lobed, pale yellowish, tuberous rhizomes from which an above-ground stem rises about 1 metre. Ginger is among the most frequently and heavily consumed spices throughout the world. The pungent, spicy rhizome has been used in the Indian sub-continent and the orient for at least 25 centuries for culinary and medicinal purposes (Denniff and Whiting, 1976). Ginger is also a common additive in a large number of compounded foods and beverages due to its flavour and pungency (Mascolo *et al.*, 1989). Zingiberaceous plants have been used in traditional or herbal medicine throughout the tropical world and members of the family have attracted continuous phytochemical interest for their biological and pharmaceutical activities (Pancharoen *et al*., 2000). Of the different constituents of ginger, some compounds seem to be responsible for the distinctive taste (Murata *et al*., 1972), while others have specific pharmacological effects (*e.g.* anti-emetic, anti-inflammatory).

Ginger (*Zingiber officinale*) is one of the best-known and most important spices. It is sold commercially as a fresh rhizome, as a glazed confectionary, as preserved ginger, as a pungent oleoresin extract and as a steam-distilled essential oil. Dried ginger consists of starch (40-60%), protein (10%), lipid (5-10%), fibre (2-5%), inorganic material (6%), residual moisture (10%) and essential oils (1-4%) (Van Beek *et al.*, 1987). The ginger variety, the geographic region, the maturity at harvest, the agroclimatic conditions, the method of analysis, and the market requirements can all influence the composition of different ginger varieties (Mustafa *et al*., 1993).

Zingiberaceous plants are capable of producing a wide range of chemical structures that include monoterpenoids, sesquiterpenoids, diarylheptanoids, arylalkanoids, phenylpropanoids, phenylbutanoids, cyclohexane oxides and flavonoids (Pancharoen *et al*., 2000). Ginger is valued both for its aromatic odour and its spicy, pungent taste. Ginger oil consists of a mixture of more than 200 terpenes and some non-terpenoid compounds (Lawrence, 1984a). The extraction and composition of the essential oil and oleoresins from ginger has been extensively studied.

Chemicals which have been isolated from ginger (*Zingiber officinale* Roscoe) include gingerols and shogaols (Wu *et al.*, 1998; Kiuchi *et al.*, 1992; Kikuzaki *et al.*, 1992), diarylheptanoids (Endo *et al.*, 1990; Kikuzaki *et al.*, 1991a;b), phenylbutenoids, flavonoids (Nakatani *et al.*, 1991), glycosides and gingesulphonic acids (Yoshikawa *et al.*, 1994), cassumunaquinones and sesquiterpenes (Akhila and Tewari, 1994; Terhune *et al.*, 1975). Minor compounds which have been identified in ginger include neral, geranial, geraniol, geranyl acetate, linalool, citronyl acetate, α-terpineol, borneol, isoborneol, bornyl acetate, zingiberone, sesquiphenandrene, curcumene, 2,6-dimethyl hepten-1-ol, α-gurjunene, linalool oxide, isovaleraldehyde, 2-pentanone, cadinol, α- and γ-calacorene, eremophyllene, *t*-muurolol, α-himachallene, α-cubebene, pinanol, α-santalene, geranyl propionate, geranoic acid, α-farnesene, *n*-methyl pyrrole and geranic acid (Nigam *et al.*, 1964; Connell, 1970; Connell and Sutherland, 1966; Kami *et al.*, 1972; Smith and Robinson, 1981; MacLeod and Pieris, 1984; Nishimura, 2001; Agarwal *et al*., 2001; Onyenekwe and Hashimoto (1999).

The pungent principles of ginger have been thoroughly investigated. Ginger oleoresin is generally obtained by solvent extraction of dried unpeeled ginger, since peeled ginger loses much of its essential oil content. The major non-volatile phenolics (the pungent constituents)

found in *Z. officinale* are the gingerols and their dehydrated products, the shogaols (Balladin *et al.*, 1998). Other non-volatile components of ginger include 3-, 4-, 5- and 12-gingerol, 6-paradol, 4-, 6-, 8- and 10-gingerdiol, 6-methyl gingerdiol, 4- and 6-gingerdiacetate, 6-methyl gingerdiacetate and hexahydrocurcumin (Connell and Sutherland, 1969; Connell and McLachlan, 1972; Harvey, 1981; Kikuzaki *et al.*, 1992; Masada *et al.*, 1973; Nomura and Iwamoto, 1928; Mustafa *et al*., 1993).

Ginger bioactivity

The history and use of ginger is well documented. Ginger has been used since the 4th Century BC (Tyler, 1981), is an ingredient in more than half of all traditional Chinese medicines (Awang, 1992), and has been in medical use in Europe at least since the 12th Century. Ginger is listed in modern pharmacopoeias and repertories and has a wide range of confirmed pharmacological properties (Weidner and Sigwart, 2000). Its chemistry, pharmacology and pharmacokinetics have been investigated (Emig, 1931; Backon, 1986; Ding *et al.*, 1991). There is a long history of the use of the ginger rhizome in Chinese and Ayurvedic medicine. In western alternative medicine, ginger is used to prevent nausea and motion sickness and to treat inflammatory (rheumatic) conditions. *In vitro* evidence suggests that ginger may also have anti-cancer effects (Surh *et al*., 1999).

A literature review was prepared on the "Medicinal Properties of Ginger" as a part of this project and is included as Appendix 1. A further search of the literature was performed late in the project to find relevant references about compounds found in ginger which have had reported bioactive principlen (see Appendix 7).

In traditional medicine, the rhizome of ginger is believed to possess medicinal properties. More recently, the fresh or powdered rhizome, the aqueous or organic ginger extracts, and volatile ginger oils have been scientifically investigated for their bioactivity. Although ginger and its components have a wide range of pharmacological effects, the precise mechanisms of the various actions described is generally unknown. The bioactivity of ginger has recently been grouped into 4 main areas by Mustafa *et al*. (1993) – the metabolism of arachidonic acid, the prevention of nausea and vomiting, effects on blood pressure and heart rate, and cytoprotective and cytotoxic effects.

l. Metabolism of arachidonic acid. The pungent components of ginger (gingerdione, shogaol, gingerol and dehydroparadol) have been shown to inhibit cyclooxygenase and lipoxygenase activity in the arachidonic acid metabolic pathway and thereby probably reduce inflammation and relieve pain in rheumatic disorders and migraine headache (Flynn *et al*., 1986; Srivastava, 1986; Kiuchi *et al.*, 1982). Consumption of ginger also reduces plasma thromboxane B2 (TxB2) levels in humans. The inhibition of arachidonic acid metabolism can explain reduced platelet aggregation via inhibition of platelet thromboxane production (Srivastava, 1984; 1986), amelioration in rheumatic symptoms by reduced arachidonic acid metabolites (Srivastava and Mustafa, 1989; 1992), alleviation of migraine by reduced formation of oxygen radicals, prostaglandins and thromboxane (Mustafa and Srivastava, 1990) and anti-inflammatory effect via interaction with histamine and bradykinin leading to reduced vascular permeability and oedema.

2. Prevention of nausea and vomiting. Ginger is reported to reduce nausea, vertigo and vomiting for which the mechanism of action is, however, not yet understood. The anti-emetic activity of ginger may be due to an increase in gastric motility and absorption of acids (Bone *et al*., 1990), reduced vertigo by dampening of vestibular impulses to the autonomic

CNS (Mowery and Clayson, 1982), reduced vomiting and cold sweats by both gastrointestinal and CNS effects (Grontved *et al*., 1988), and antagonism of 5-HT3 by galanolactone (Huang *et al*., 1991).

3. Blood pressure and heart rate. (6)-Shogaol is known to reduce blood pressure by both a centraI and a peripheral action (Suekawa *et al*., 1986) and (8)-gingerol may be cardiotonic via enhancement of the Ca2+-pumping activity in the cardiac sarcoplasmic reticulum (Kobayashi *et al*., 1987).

4. Cytoprotective and cytotoxic effects. Gastrointestinal system effects include increased bile secretion and an anti-emetic action. Given orally, (6)-shogaol has been shown to accelerate gastrointestinal movement in mice, but when given intravenously, it inhibits such movement. Galanolactone antagonises 5-HT3 receptors which may explain the anti-emetic and gastrointestinal movement enhancing effects. Zingiberone and (6)-gingerol are reported to protect against gastric mucosal lesions. The ethanolic extract of ginger, zingiberene and (6)-gingerol inhibit the development of gastric lesions (Yamahara *et al*., 1988) and the whole extract is more effective than the isolated components, gingerol and shogaol are mutagenic while zingerone is anti-mutagenic in Salmonella strains (Abraham *et al*., 1976).

The anti-inflammatory properties of ginger.

The particular focus of this investigation has been the fractionation of the ginger oil and the assessment of the various fractions for their anti-inflammatory activity, both *in vitro* and *in vivo*.

The active constituents in ginger have also been shown to inhibit the enzymes of arachidonic acid metabolism (Flynn *et al.*, 1986; Kiuchi *et al.*, 1982). The major rate-limiting factor in the generation of prostaglandins and other metabolites of arachidonic acid is the availability of free arachidonic acid. These eicosanoids are generated in response to various physiological, pharmacological or pathological stimuli. Arachidonic acid is released from membrane phospholipids by the action of phospholipases. Arachidonic acid is then converted to prostaglandin endoperoxides by the action of the enzyme, cyclooxygenase. Prostaglandin endoperoxides are subsequently converted into Thromboxane A_2 by the action of another enzyme, thromboxane synthetase, or into prostaglandins (see Figure 4). *In vitro* model systems often use platelet aggregation to assess the effect of various compounds on the prostaglandin pathway. Ginger can increase the production of platelet lipoxygenase products (Rattan, 1988).

Mechanical, chemical or immunological challenge stimulates cyclooxygenase activity. Living tissue responds to irritation and injury by way of inflammation. Oxygenation of arachidonic acid is increased in inflamed tissues, and prostaglandins and leukotriene levels are elevated (Zurier, 1985). Increased levels of prostaglandins are always observed in inflamed tissues. Prostaglandin E_2 is the predominant product, although Prostaglandin $F_{2\alpha}$, Prostaglandin D_2, Thromboxane B_2 and 6-keto-Prostaglandin $F_{1\alpha}$ have also been detected in inflamed tissues.

Present-day therapy for osteoarthritis is directed at symptoms, since there is no established disease-modifying therapy. Treatment programs involve a combination of non-pharmacological and pharmacological measures, utilising a combination of analgesic, anti-inflammatory and intra-articular programs (Altman and Marcussen, 2001). More than 200 drugs (ranging from non-steroidal anti-inflammatory drugs, corticosteroids, gold salts,

disease modifying anti-rheumatic drugs, methotrexate, cyclosporine) have been tested for their anti-inflammatory potential. All of the synthetic drugs are known to produce mild to serious side-effects. For example, the prolonged use of acetaminophen (one of the most widely advocated pain killer in osteoarthritis) can result in serious gastrointestinal damage. Corticosteroids and non-steroidal anti-inflammatory drugs (which inhibit the cyclooxygenase) are used to treat such disorders. Both types of drugs produce adverse side-effects on prolonged use (Zurier, 1985).

Currently, ginger is one of the most popular herbal medications for rheumatic diseases. While the beneficial effects of ginger have been widely reported (Srivastava and Mustafa, 1989a), only a few controlled studies have been performed. Ginger has been reported to have a suppressive effect in arthritic rats (Weidner, 1997) and chemical substances found in ginger have been shown to have an anti-inflammatory potential (Kiuchi *et al.*, 1992). Ginger is described in Ayurvedic and Tibb systems of medicine to be useful in inflammation and rheumatism (Srivastava and Mustafa, 1992). Ginger has been postulated to interfere with the cyclooxygenase and lipoxygenase enzymes of the prostaglandin and leukotriene biosynthetic pathways (Srivastava and Mustafa, 1989b).

Mascolo *et al.* (1989) found potential anti-inflammatory, antipyretic and hypoglycaemic properties in extracts from *Zingiber officinale*. The severity of pedal oedema was reduced (to be comparable with acetylsalicilic acid) in an animal model system. In acute inflammation, prostaglandins (and other arachidonate metabolites) interact with substrates (such as histamine and bradykinin) to augment vascular permeability (Williams *et al.*, 1983) and produce oedema (Moncada *et al.*, 1973). The theory which best explains the anti-inflammatory activity of aspirin-like drugs is based on the discovery that the drugs inhibit prostaglandin biosynthesis through their interaction with prostaglandin synthetase (Vane, 1971).

Alternative medicine is used extensively by patients with, for example, chronic osteoarthritic pain. The effect of an extract of ginger (one of the most popular herbal medications) has been tested in a controlled setting (Bliddal *et al.*, 2000). Ginger extracts were compared with the most relevant clinical alternative (Ibuprofen) in a randomised, placebo-controlled, cross-over study of osteoarthritis.

Oral consumption of dried, powdered ginger for between 3 months and 2.5 years by patients with rheumatoid arthritis, osteoarthritis or muscular discomfort has been shown to result in relief of pain and swelling (Srivastava and Mustafa, 1989a; Srivastava and Mustafa, 1992). The investigators of these studies suggested that the ameliorative effects of ginger could be related to the inhibition of prostaglandin and leukotriene biosynthesis. In other studies, ginger oil (obtained by steam distillation of dried ginger) has been shown to be an inhibitor of both cyclooxygenase and lipoxygenase activities (Kiuchi *et al.*, 1982; Flynn *et al.*, 1986). The oral administration of ginger oil to rats for 26 days has been shown to significantly reduce paw and joint swelling (Sharma *et al.*, 1994), suggesting that ginger oil possesses anti-inflammatory properties. Pungent components of ginger inhibit cyclooxygenase and lipoxygenase activity in the arachidonic acid metabolic pathway and thereby probably reduce inflammation and relieve pain in rheumatic disorders and migraine headache (Mustafa and Srivastava, 1990). Consumption of ginger has also been shown to reduce plasma thromboxane B_2 levels in humans.

Ginger contains a number of compounds (*e.g.* 6-gingerol) which have been shown to be able to inhibit prostaglandin synthetase *in vitro* (Kiuchi *et al.*, 1982). Four other components (6-dehydrogingerdione, 10-dehydrogingerdione, 6-gingerdione and 10-gingerdione) were found to be more potent than indomethacin as prostaglandin inhibitors. A ginger extract has also been shown to inhibit the production of thromboxane and prostaglandin in a dose-dependent manner (Srivastava, 1984a, Srivastava, 1984b). Hydroxy-methoxy-phenyl compounds are dual inhibitors of cyclooxygenase and 5-lipoxygenase (Bliddal *et al.*, 2000). In a carrageenan-induced paw swelling assay (Mascolo *et al.*, 1989) and several enzyme assays (Kiuchi *et al.*, 1992), extracts of *Zingiber officinale* were shown to potently inhibit the inflammatory processes (comparable to non-steroidal anti-inflammatory drugs).

Seventeen pungent oleoresin principles of ginger (*Zingiber officinale*, Roscoe) and synthetic analogues were evaluated for inhibition of cyclooxygenase-2 enzyme activity in the intact cell. These compounds exhibited a concentration- and structure-dependent inhibition of the enzyme. *In vitro* cyclooxygenase enzyme activity was strongly inhibited by 8-paradol and 8-shogaol, and by two synthetic ginger analogues. Analysis of these phenolic compounds revealed three important structural features that affect cyclooxygenase inhibition: (i) the lipophilicity of the alkyl side-chain, (ii) the substitution of hydroxy and carbonyl groups on the side-chain, and (iii) the substitution of hydroxy and methoxy groups on the aromatic moiety (Tjendraputra *et al.*, 2001).

6-gingerol exhibits diverse pharmacological activities, including inhibition of cyclooxygenase and lipoxygenase activities (Kiuchi *et al.*, 1982; Kiuchi *et al.*, 1992; Flynn *et al.*, 1986). 6-gingerol and four gingerdione derivatives (6- and 10-dehydrogingerdione and 6- and 10-gingerdione) were isolated from ginger rhizome and shown to be potent inhibitors of prostaglandin biosynthesis. Some were more potent inhibitors than indomethacin which is known to be one of the strongest inhibitors (Kiuchi *et al.*, 1982).

Ginger is well-known as a crude drug with several pharmacological functions. An aqueous extract of ginger has been shown to inhibit the biosynthesis of thromboxane and prostaglandin (Srivastava *et al.*, 1984a, Srivastava *et al.*, 1984b; Srivastava, 1986). The inhibitory principles have been reported to be gingerol analogues (Kiuchi *et al.*, 1982). Inhibitors of platelet aggregation have been reported to require *o*-methoxyphenol-components (*e.g.* as found in the gingerol analogues) for their activity (Kawakishi *et al.*, 1994).

Besides producing cyclooxygenase products (prostanoids), it has been found that human rheumatoid and osteoarthritic synovium can generate 5-lipoxygenase products. Leukotrienes C_4, D_4 and E_4 were identified in the synovial membranes from rheumatoid and osteoarthritic patients. The level of Leukotriene B_4 in the fluids of rheumatoid arthritis patients is slightly in excess of that in the fluids from osteoarthritis patients. Interestingly, the levels of Leukotriene B_4 have been found to be increased six-fold in the synovial fluids from patients with gout compared to the Leukotriene B_4 levels in corresponding fluids obtained from patients with rheumatoid arthritis and osteoarthritis (Rae *et al.*, 1982).

Monohydroxy lipoxygenase products show weak chemokinetic and chemotactic properties both *in vitro* and *in vivo* in human and rabbit polymorphonuclear leucocytes (Palmer *et al.*, 1980). The dihydroxy product, Leukotriene B_4, on the contrary, shows powerful effects on the polymorphonuclear leucocytes. It produced degranulation of polymorphonuclear leucocytes of several species *in vitro* and accumulation of these cells *in vivo* (Bray, 1983;

Higgs *et al.*, 1981). Klickstein *et al.* (1980) showed that synovial fluid of patients with rheumatoid arthritis and spondyloarthritis contained higher levels of Leukotriene B_4 and 5-HETE (from which leukotrienes are derived).

During the last 45 years many chemical investigations have been carried out on the constituents of the essential oil (Van Beek *et al.*, 1987; Chen and Ho, 1988). All together more than 200 different volatile compounds have been identified in essential oil wherein the pharmacological activity is confined (Lawrence, 1984a). The essential oil contains mixture of various terpenes as well as some other non-terpenoid compounds. It is likely that crude ginger powder intake brings about amelioration of symptoms by, for example, interfering with the production and release of products of lipid membranes (eicosanoids, reactive oxygen), peptides and proteins (lysosomal enzymes, growth factors, Iymphokines, bradykinin), and amino acids (histamine, serotonin). Ginger has been proposed to inhibit both the cyclooxygenase (Srivastava, 1986; Kiuchi *et al.*, 1982) and lipoxygenase products (Flynn *et al.*, 1986). Ginger may be a dual inhibitor of eicosanoid synthesis and has been reported to contain anti-histaminic and anti-oxidant factors (Duke and Ayensu, 1985).

Non-steroidal anti-inflammatory drugs have three major actions, all of which are related to inhibition of cyclooxygenase resulting in decreased formation of prostanoids. Firstly, an anti-inflammatory action, achieved by reduced production of vasodilator prostaglandins (Prostaglandin E_2, Prostaglandin I_2), means less vasodilation and indirectly less oedema. Secondly, an analgesic effect is achieved by reduced prostaglandin production (less sensitisation of nociceptic nerve endings to the inflammatory mediators bradykinin and 5-hydroxytryptamine). Thirdly, an anti-pyretic effect is probably due to a decrease in the mediator Prostaglandin E_2 generated in response to inflammatory pyrogens, such as Interleukin-1. Since ginger inhibits prostanoid synthesis and also products of 5-lipoxygenase, its ameliorative effects in arthritis and muscular discomforts could be related to reduced formation of prostanoids and leukotrienes. Hence, a decrease in the carrageenan-induced oedema formation in the rat's paw after 3 hours of ginger extract administration has been demonstrated and the potency of the extract in the acute inflammation test appears to be comparable to that exhibited by acetyl salicylic acid reported in the same study (Mascolo *et al.*, 1989).

This investigation has attempted to fractionate ginger oil extracted under controlled conditions (with supercritical CO_2) and HPLC, and to assess the various fractions for anti-inflammatory activity using *in vivo* and *in vitro* model systems.

MATERIALS AND METHODS.

The ginger oil and resin.

This study utilised two different supercritical fluid extracted (SFE) ginger oils that were supplied by Buderim Ginger Limited. The first ginger oil "SV1" had a resinous consistency (like honey) and a rich gingery odour (typical of ginger nut biscuits). It had a golden colour with an orange to red hue and a very pungent flavour. The second ginger oil "SV2" had a thin oily consistency (like paraffin oil) and an aromatic, predominantly lemony but subtle gingery odour. It had a yellow to golden colour and a very pungent flavour.

The assessment of the solubility of ginger oil in methanol/water mixtures.

a) Method 1

Solutions of the ginger oils (SV1 and SV2) were prepared at 0.025g/mL before being diluted 10-fold with methanol and filtered for HPLC analysis. One gram of each of the ginger oils was accurately weighed into separate 100mL measuring cylinders, and then dissolved with 40mL of methanol. These solutions were then diluted to 100mL with water and vigorously mixed for 2 minutes. The sample was then transferred to tubes and centrifuged at 10000rpm at 25°C for 20 minutes. The aqueous and organic layers were sampled for HPLC analysis.

b) Method 2

100mg of ginger oil (SV1) into four separate 10ml volumetric flasks for each solvent to be assessed. Solvents were then added to each of the flasks (10, 8, 6 and 4 mL, respectively) and the ginger oil was allowed to fully dissolve. Water was then added to make each of the flasks up to 10mL total volume. The contents of each flask was then centrifuged at 10000rpm for 20min at room temperature and the physical appearance of each phase was recorded. Solvents used for this experiment were Acetic Acid, Glacial (AR grade, BDH P# 100015N), Acetone (HPLC grade, EM Science Merck AX0115-1), Acetonitrile (HPLC grade, EM Science Merck AX0145-46), Butan-1-ol (AR grade, Ajax 107), Butan-2-ol (AR grade, BDH P# 10316), Dimethylformamide (AR grade, BDH P# 10322), Dimethylsulphoxide (LR grade, BDH), Ethanol (AR grade, BDH), Isopropyl alcohol (HPLC grade, EM Science/Merck P# PX1838-1), Methanol (HPLC grade, EM Science/Merck P# MX0475-46), Methylacetate (LR grade, BDH) and Propan-1-ol (AR grade, BDH P# 10345).

Materials used in ginger chromatography.

All solvents were of HPLC grade and were purchased from EM Science, an affiliate of Merck KGaA (Darmstadt, Germany). HPLC grade water was obtained from a Milli-Q water purification system (Millipore, USA). Trifluoroacetic acid (TFA), (Auspep, Australia), peptide synthesis grade (Catalogue Number 79187). A ginger kit was purchased from ChromaDex USA (Catalogue Number 07615-5K) and it contained 1x5mg of each 6-Gingerol, 8-Gingerol, 10-Gingerol and 6-Shogaol. Ultra high purity helium and liquid nitrogen was from BOC gases (Rocklea, Australia). Syringe filters were 0.45µm, from Millipore, Millex-HV type (Catalogue Number SLHV013 NK).

HPLC analysis of SFE ginger oil.

The initial analytical HPLC method (Pham *et al.*) and the subsequent preparative LC method development were based on a previous method developed at the Centre for Food Technology (CFT) for a previous HRDC project "Evaluation of aroma and pungent principles in raw ginger" (Project Number VG310). Reversed phase HPLC analysis was subsequently used as the basis for all of the analytical HPLC and preparative LC of SFE ginger oil in this project.

A faster analytical method was subsequently developed to mimic the analytical method used by Southern Cross University (SCU).

The analytical characterisation of SFE ginger oil (CFT-modified method)

Samples of SFE ginger oil were dissolved in methanol at concentrations of 1 to 10 mg/ml. These were 0.45µm syringe filtered and 20µl aliquots were analysed by analytical HPLC (Shimadzu, Kyoto, CLASS-VP Version 6.10 software) - Shimadzu SCL-10Avp system controller, LC-10AD pump, FCV-10AL low-pressure gradient mixer, DGU-12A degasser, SIL-10A auto-injector, CTO-10AC column oven with 2.6mL solvent mixer and SPD-M10Avp photodiode array detector. The HPLC was fitted with a Vydac C18 guard column (Catalogue Number 218GD52), and a 5µm Vydac C18, 4.6 x 250mm column (Catalogue Number 218TP54). The column oven was maintained at 45^{o}C. The eluted peaks were detected at 282nm and, for the purpose of this project, various wavelengths were also assessed using this method. The mobile phase was water (A) and methanol (B) commencing with 45% B at 1ml/min. Immediately after injection gradient elution was applied: 45-55% B, 0-3min; 55% B, 3-20min; 55-65% B, 20-35min; 65-70B% B, 35-45min; 70% B, 45-55min; 70-100% B, 55-65min; 100% B, 65-75min; 100-45% B, 75-80min and then at 85min the flow rate stepped back to 1ml/min.

SCU analytical HPLC method

The SCU analytical HPLC method is described in detail in Appendix 2. In summary, an Agilent 1100 LC, fitted with a Phenomenex "aqua" 5µm C18 150 x 4.6 mm column was used. A gradient from 50% to 95% phase B, over 20 minutes was used (Solvent A: 0.05%TFA in H2O, Solvent B: 0.05%TFA in Acetonitrile). The flow rate was set at 1 mL per minute and the detector was set at 210 and 280nm.

Analytical HPLC method - CFT (SCU mimic)

This method used the same instrumentation as detailed previously in the Pham *et al*. analytical HPLC method. A 3µm Phenomenex C18 column (100Å, 30 x 4.6mm, Catalogue Number 00A-4251-E0) and a Phenomenex guard column (Catalogue Number AJO-4287) were used. The mobile phases were 0.05% TFA in water (A) and 0.05% TFA in acetonitrile (B). The flow rate was 1.5ml/min for the entire analysis. Typically, a 10µl injection volume was used. Equilibration was at 45% phase B followed by gradient elution: 45-95% B, 0-10min; 95-100% B, 10-10.1min; 100% B, 10.1-12min; 100-45B% B, 12-12.1min then 45% B, 12.1-16min to complete the analysis. Detection was performed at 210nm (plus diode array detection from 194 to 294nm).

CFT preparative LC methods

The strategy for preparative LC fractionation, purification and concentration becomes one of balancing the following factors; desired mass of final products, desired purity of compounds, PLC run times, number of PLC runs to be done, volumes of final poolings and further processing or treatment of pools. While each factor has numerous pros and cons to consider, we attempted to adopt methods that best suited our goals and limitations. These methods used a Shimadzu Prepstar 8000 prep LC instrument with CLASS-VP Version 6.10 software controlled by the same computer as the analytical system, two LC-8A pumps, a low-pressure switching valve with preparative solvent mixer and a UV/VIS detector, fitted with either an analytical or preparative detector flow cell. A Waters U6K manual switching valve with a 2mL sample loop was used to inject ginger samples in methanol. An in-line filter kit (Alltech Catalogue Number 27000) with a 4mm, 0.5µm filter element (Alltech Catalogue Number

28644), a preparative C18 guard column (Vydac Catalogue Number 218FSK1210) and a prep C18, 10µm, 22 x 250mm column (Vydac Catalogue Number 218TP1022) were fitted after the injector. The mobile phases were water (A) and methanol (B). One-minute fractions were collected from the beginning of each preparative run. Figure 1 is a diagrammatic representation of the preparative HPLC system used for this project. Mobile phases and load solutions were degassed by continuous helium sparging.

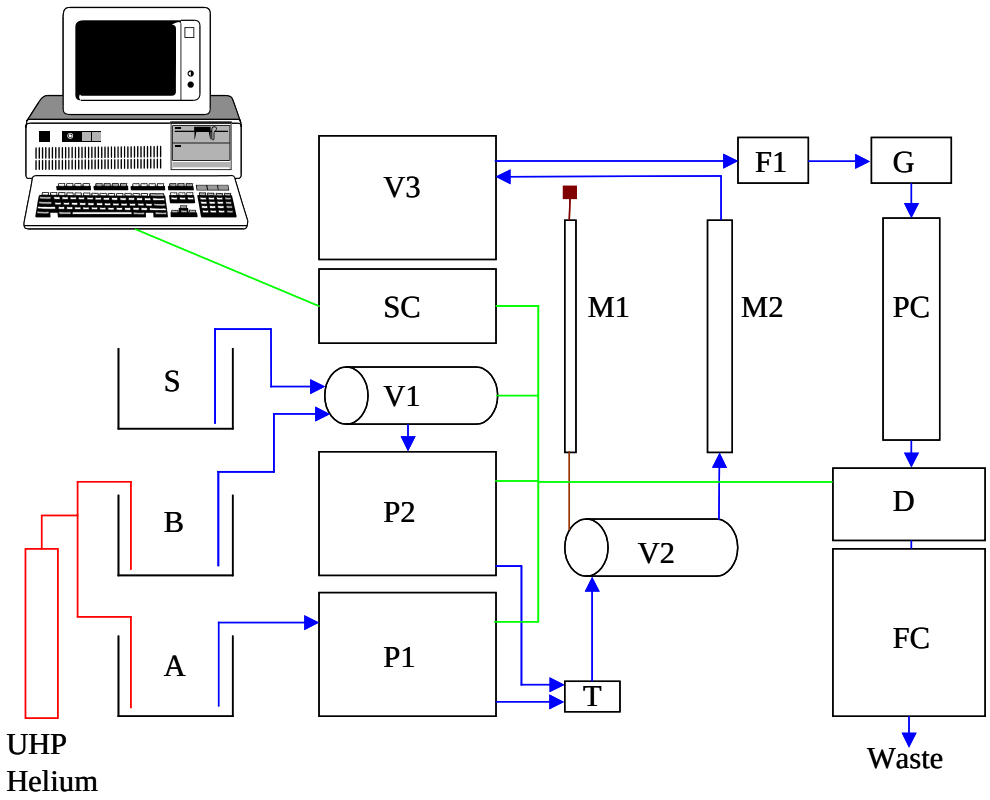

Figure 1. Scheme of the preparative HPLC system used for this project. Components are: A, phase A reservoir; B, phase B reservoir, S, sample reservoir; P1, phase A pump; P2, phase B pump; V1, 6 position switching valve; SC, system controller; V3, Waters U6K manual injector; T, flow junction; V2, manual switching valve; M1, analytical in-line mixer; M2, preparative in-line mixer; F1, in-line filter; G, guard column; PC, preparative column; D, detector and FC, fraction collector.

Initial *in vitro* anti-inflammatory activity of whole ginger oils (SV1 and SV2).

The methods used in the initial *in vitro* studies of the anti-inflammatory activity of the whole ginger oils (SV1 and SV2) are presented in detail in Appendix 2. The SV2 oil was selected for subsequent fractionation studies because it had a less resinous nature.

Preparative HPLC fractionation of SV2 for *in vitro* bioactivity testing.

Preparative HPLC at the Southern Cross University (Appendix 3) was used to produce fractions from SFE-extracted ginger oil (SV2). Sufficient material was generated to assay 10 fractions for anti-inflammatory activity using the methods outlined in the previous section.

Preparative HPLC fractionation of SV2 for *in vivo* bioactivity testing.

Initial fractionation of the ginger oil (SV2) using preparative HPLC produced four pools (#1-4). Pools 2-4 were subsequently concentrated and used for further bioactivity investigations. Pool 1 was fractionated to remove the 6-gingerol component (see Figure 2).

Initial *in vivo* anti-inflammatory activity of whole ginger oils (SV1 and SV2).

The methods used in the initial *in vivo* studies of the anti-inflammatory activity of the whole ginger oils (SV1 and SV2) are presented in detail in Appendix 4.

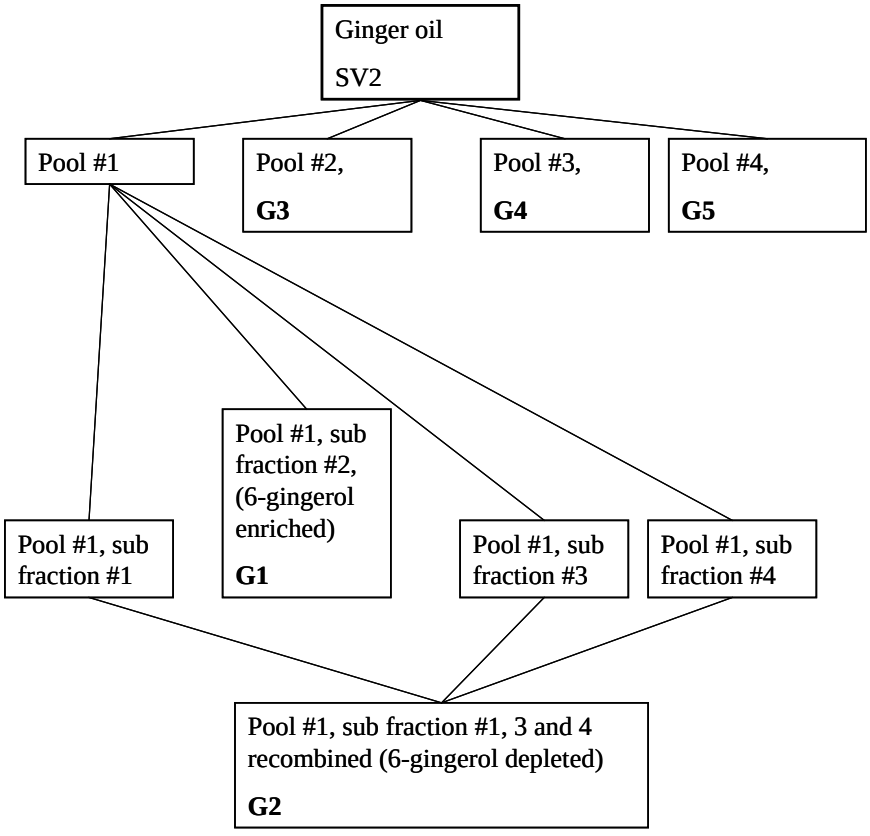

Figure 2. Schematic for the preparative fractionation of ginger oil (SV2).

Preparative HPLC fractionation of SV2

a) Initial fractionation of ginger oil (SV2) by Preparative HPLC (second set).

The method used for these fractionations was slightly altered from the first set of fractionations to simplify it and to improve resolution and fractionation run times. The column was equilibrated at 45% phase B at 15ml/min, and then increased to 22ml/min. After the injection, using the Waters U6K manual injector, of 2mL of ginger oil (1:1 v/v in methanol), a gradient elution (45-55% B, 0-1min; 55-65% B, 1-50min; 65-100% B, 50-60min; 100% B, 60-70min; 100-45% B, 70-70.01min, 45% B, 70.01-80min) was used. The flow rate was returned to 15ml/min after 80 minutes to complete the fractionation. A total of 16g of ginger oil (SV2) was fractionated using this method.

b) Re-fractionation of Pool #1 by preparative LC.

For re-fractionation of Pool #1 the Waters U6K manual injector was removed from the flow path, so that the outlet of the solvent mixer connected directly to the in-line filter. To bind the compounds in Pool #1 to the preparative LC column again, we had to dilute the methanol with water (calculated using the Pearson square method). The flow rate was 22ml/min for the entire procedure. The column was equilibrated with 5% phase B. Pool #1 was divided into one small load of 200ml for initial trials and five loads of 540ml for subsequent bulk fractionations. After loading the column, gradient elution was undertaken (30-37% phase B, 35-45min; 37-40% phase B, 45-160min; 40-100% phase B, 160-175min; 100% phase B, 175-180min). 1min fractions were collected from the start of the run.

c) Concentration of dilute fractions prepared by preparative LC.

The preparative LC was configured as described above. For all of these preparative LC concentrations, the primary objectives were to minimise the loss of unbound compounds (to less than 5%) during loading and to maximise recovery in a minimum volume of methanol. During the desorption step the real time absorbance profile was monitored. When the concentrated compounds began to desorb, the fraction collector was stepped up one tube to minimise the volume requiring final concentration.

(i) Concentration of the 6-gingerol fraction (G1).

The G1 solution was diluted with water so that the concentration of methanol in it was about 8%. The flow rate for this concentration method was 25ml/min. The column was conditioned with 10% methanol prior to commencement of loading. After all of the load solution had been pumped onto the column, the column was washed with 10% methanol. Then the concentration of methanol was stepped directly to 100% to desorb all of the bound compounds in a minimum volume. This G1 fraction derived from the ginger oil (SV2) was ultimately concentrated at a low temperature by rotary evaporation and solid phase concentration to produce the final solvent and water free oil fraction.

(ii) Concentration of the 6-gingerol-depleted fraction (pre- and post-6-gingerol) (G2)

Sub-fractions 1, 3 and 4 from Pool #1 were kept separate and individually made to 20% methanol concentration by dilution with the appropriate volumes of water. These sub-fractions were combined into one pool which was called G2. The flow rate for this concentration method was 25ml/min. The column was conditioned to 10% methanol prior to commencement of loading. After loading, the column was then washed with 10% methanol. Then the conentration of methanol was stepped directly to 100% to desorb all of the bound compounds in a minimum volume. The G2 fraction derived from the ginger oil (SV2) was ultimately concentrated at a low temperature by rotary evaporation and solid phase concentration to produce the final solvent and water free oil fraction.

(iii) Concentration of the 6-shogaol and 8-gingerol fraction (G3)
The G3 solution was diluted with water so that the concentration of methanol was about 25%. The flow rate for this concentration method was 25ml/min. The column was conditioned with 10% methanol prior to commencement of loading. After all of the load solution was pumped onto the column it was washed with 20% methanol. Then the concentration of methanol was stepped directly up to 100% to desorb all of the bound compounds in a minimum volume. The G3 fraction derived from the ginger oil (SV2) was ultimately concentrated at a low temperature by rotary evaporation and solid phase concentration to produce the final solvent and water free oil fraction.

(iv) Concentration of the 10-gingerol fraction (G4).
The G4 solution was diluted with water so that the concentration of methanol was about 25%. The flow rate for this concentration method was 25ml/min. The column was conditioned with 10% methanol prior to commencement of loading. After all of the load solution had been pumped onto the column, the column was washed with 10% methanol. Then the concentration of methanol was stepped directly to 100% to desorb all of the bound compounds in a minimum volume. The G4 fraction derived from the ginger oil (SV2) was ultimately concentrated at a low temperature by rotary evaporation and solid phase concnetration to produce the final solvent and water free oil fraction.

(v) Concentration of the sesquiterpene fraction (G5).
As the G5 fraction was eluted from the preparative column in a high percentage of methanol, it was concentrated by low temperature rotary evaporation and solid phase concentration to produce the final solvent and water free oil fraction.

Vacuum and solid-phase concentration of ginger fractions (G1-G5).
The most concentrated fractions from the preparative LC concentration step (generally Fractions #4 to 7) were combined and subjected to evaporation at 40^{o}C using a Rotorvapor (Buchii model number RE111) under house vacuum (maximum of –1 atmosphere). These super concentrated solutions were subjected to centrifugation and the free oil from this super-concentrate was collected into a pre-weighed beaker. The aqueous material that remained after the centrifuging step was pumped at a flow rate of 3ml/min through a SPE cartridge (Agilent, Zorbax C18, catalogue number 5184-3600) that had been pre-conditioned by flushing with several cartridge volumes of acetone followed by several volumes of water. After loading, the SPE cartridge was pumped dry to remove any free aqueous load solution. The walls of the centrifuge bottles were washed with several small volumes of acetone, to collect the oil that had adhered to the bottles. This acetone was then used to desorb the SPE cartridges into the pre-weighed beaker. The acetone was then evaporated off using a stream of nitrogen at room temperature.

Trials to assess the effect of freeze-drying on the composition of ginger oil.
a) Effect of freeze-drying on the composition of preparative HPLC fractions
Ginger oil (SV2) fractions prepared by preparative LC (GP1-GP5) were frozen in liquid nitrogen and freeze-dried over a period of 20 hours. The remaining oil was reconstituted in 10mL of methanol, theoretically giving a 10-fold concentration of the original oil.

b) Effect of freeze-drying on the composition of whole ginger oil (SV2)
1g samples of ginger oil (SV2) were weighed into four beakers. 10 mL of methanol was added to two of the beakers and mixed to dissolve the SV2, then 40 mL of water was added to these beakers and mixed. All of the beakers were placed in a –70°C freezer overnight and then subjected to freeze-drying for 96 hours.

Evaluation of the bioactivity of ginger extracts

a) In vitro anti-inflammatory activity
Ginger oils (SV1 and SV2) and the fractions produced in this study by preparative HPLC were assessed for anti-inflammatory activity *in vitro* (see Appendices 2 and 3).

b) In vivo anti-inflammatory activity
Ginger oils (SV1 and SV2) and the fractions produced in this study by preparative HPLC were assessed for anti-inflammatory activity *in vitro* (see Appendices 4 and 5).

RESULTS AND DISCUSSION

HPLC analysis of SFE ginger oils (SV1 and SV2)

The CFT-modified version of the analytical HPLC method developed by Pham et al. was used to analyse SV1 (Figure 3) and SV2 (Figure 4).

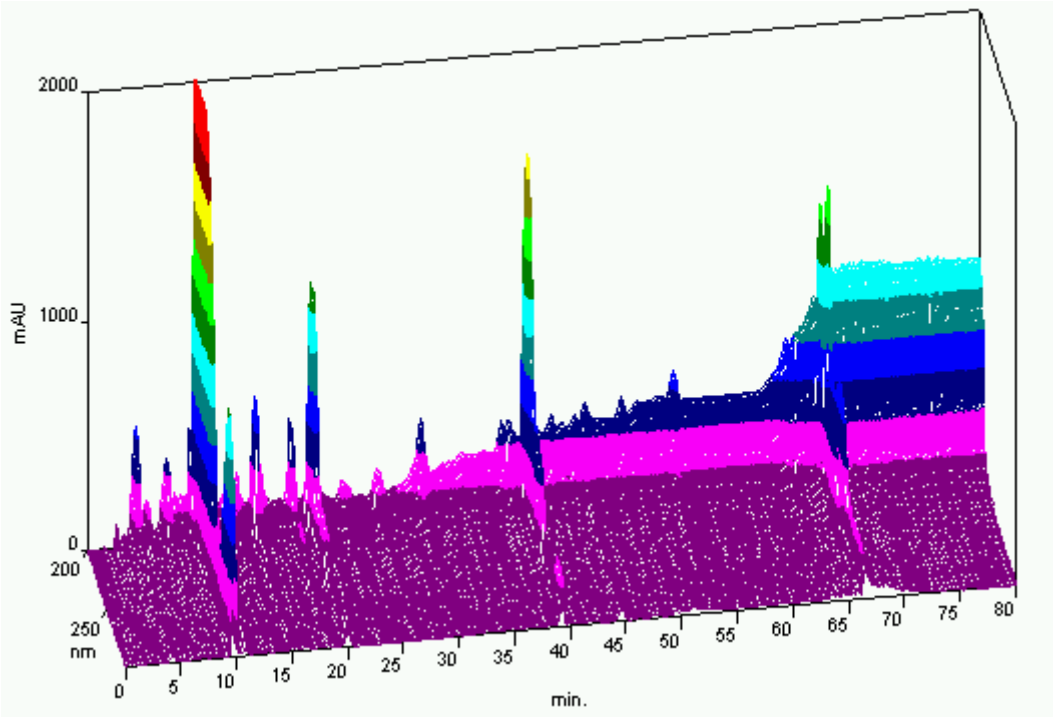

Figure 3a. Three-dimensional photodiode array profile using a 20ul injection of 10mg/ml SV1 in methanol.

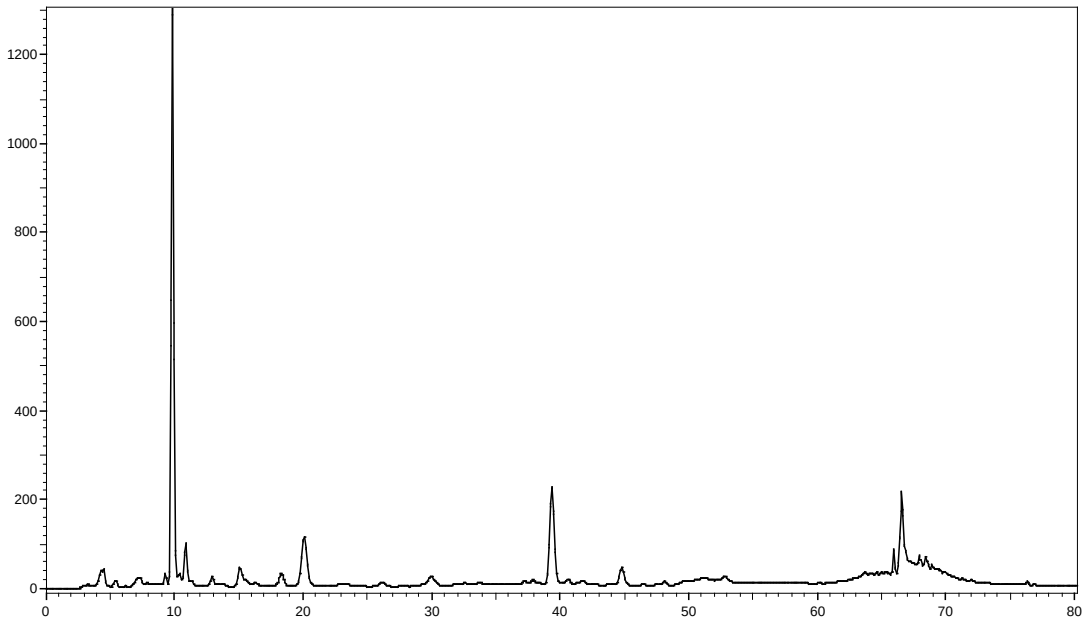

Figure 3b. 282nm profile using a 20ul injection of 10mg/ml SV1 in methanol.

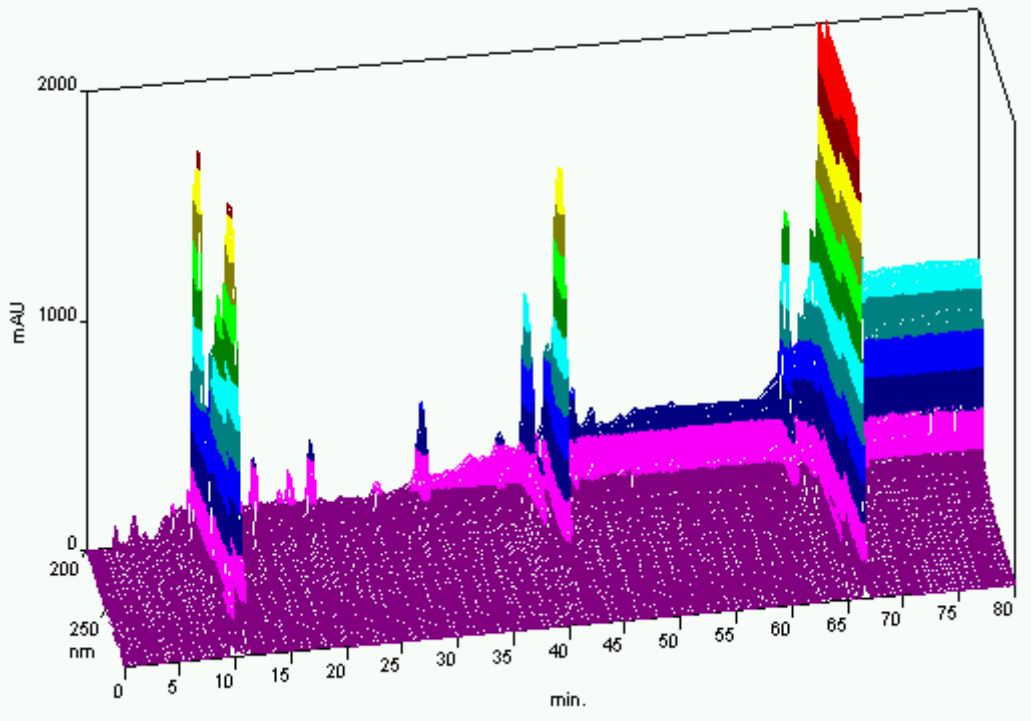

Figure 4a. Three-dimensional photodiode array profile using a 20ul injection of 10mg/ml SV2 in methanol.

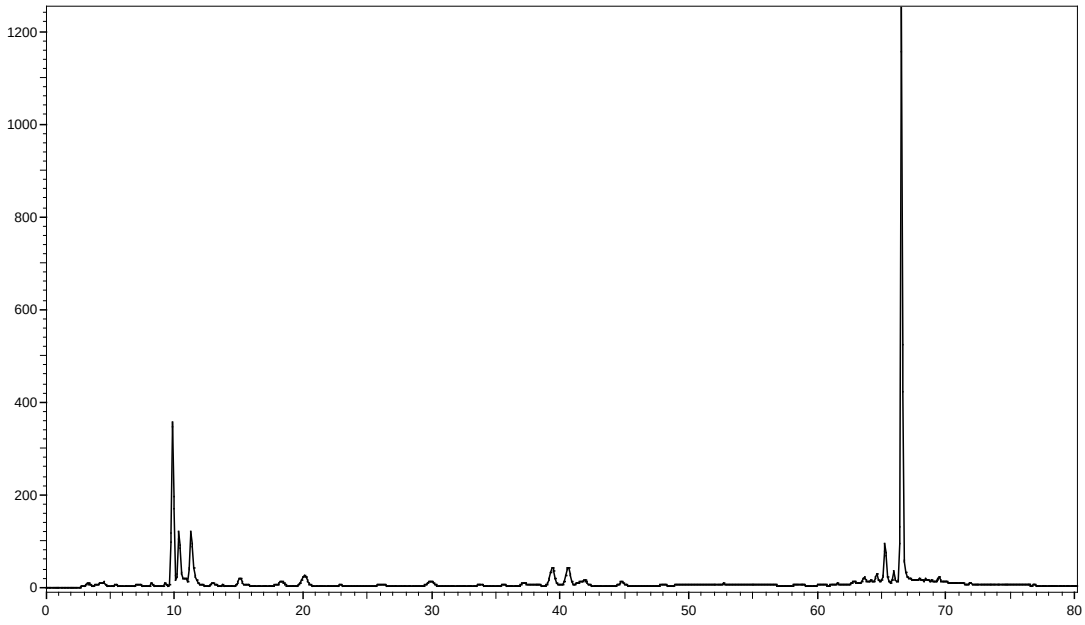

Figure 4b. 282nm profile using a 20ul injection of 10mg/ml SV2 in methanol.

These chromatographic runs showed that all of the compounds were common to both SV1 and SV2, but there were differences in the concentrations of the various compounds. Tentative identification of ginger components in these analytical HPLC profiles are tabulated in Table 1.

Tentative peak ID	Retention time (min)
6-Gingerol	11.8
6-Shogoal	22.5
8-Gingerol	24.2
8-Shogoal	43.4
10-Gingerol	44.6
10-Shogoal	50.9

Table 1. Tentative identification of ginger components in the analytical HPLC profile.

During the preparation of SV2 for chromatography this ginger oil was observed to be completely soluble in methanol. However, precipitates and phase separations occurred when water (even relatively small amounts of water) was added. This led us to investigate the solubility of SV1 and SV2 in methanol and water mixtures.

Solubility of SV1 and SV2 in methanol and water mixtures

Initial tests showed when methanol was added to ginger oils, a white-to-yellow cloudiness appeared which then disappeared rapidly after mixing. When 10g of ginger oil was dissolved in 20ml of methanol the solution had an opaque and slightly cloudy appearance. A white-to-yellow precipitate or cloudiness formed instantly when water was added to make the solution up to 50ml (i.e. when ginger oil represented 0.2g/ml of the solution). This solution quickly separated into two layers. After centrifuging, the supernatant was a pale yellow aqueous phase, and the precipitate was a dark red-to-brown organic layer. This led to more detailed tests.

The composition of the whole oil and the two phases (aqueous and methanol) for SV1 and SV2, from solubility assessment method 1, are presented in Figures 5 and 6 and Tables 2 and 3, respectively.

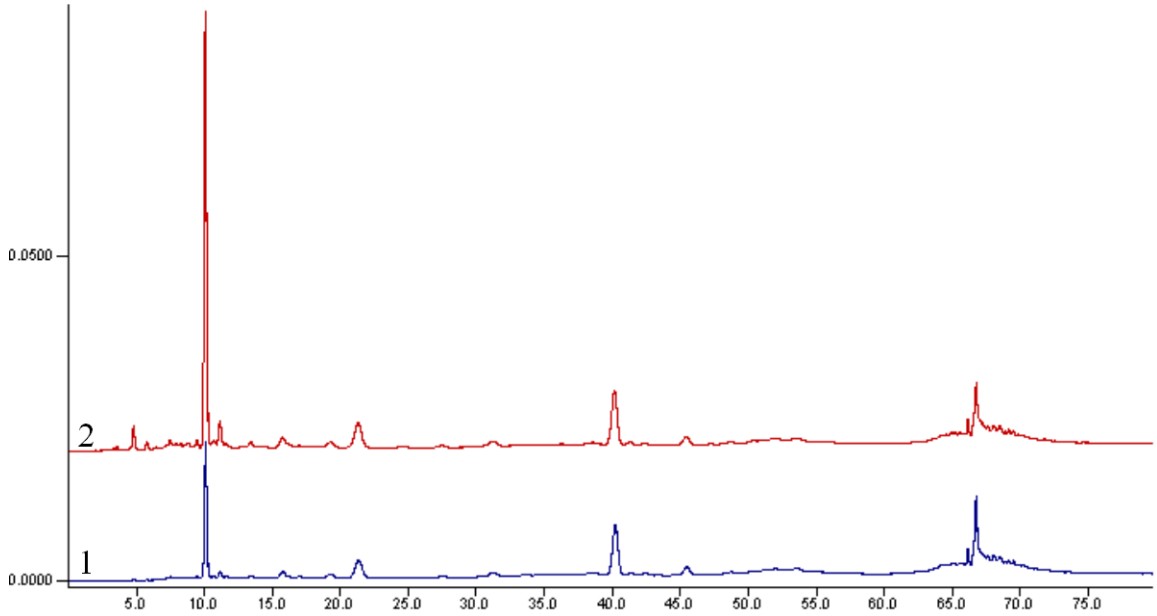

Figure 5a. Effect of water on solubility of SV1 in methanol: (1) SV1 organic layer and (2) whole SV1 in methanol.

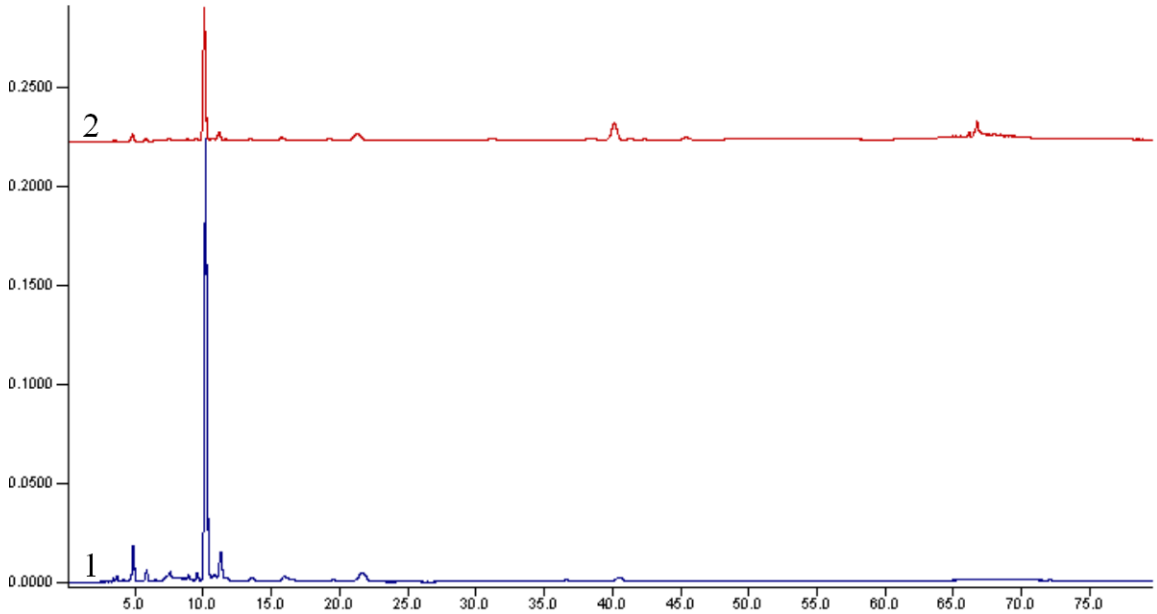

Figure 5b. Effect of water on solubility of SV1 in methanol: (1) SV1 aqueous layer and (2) whole SV1 in methanol.

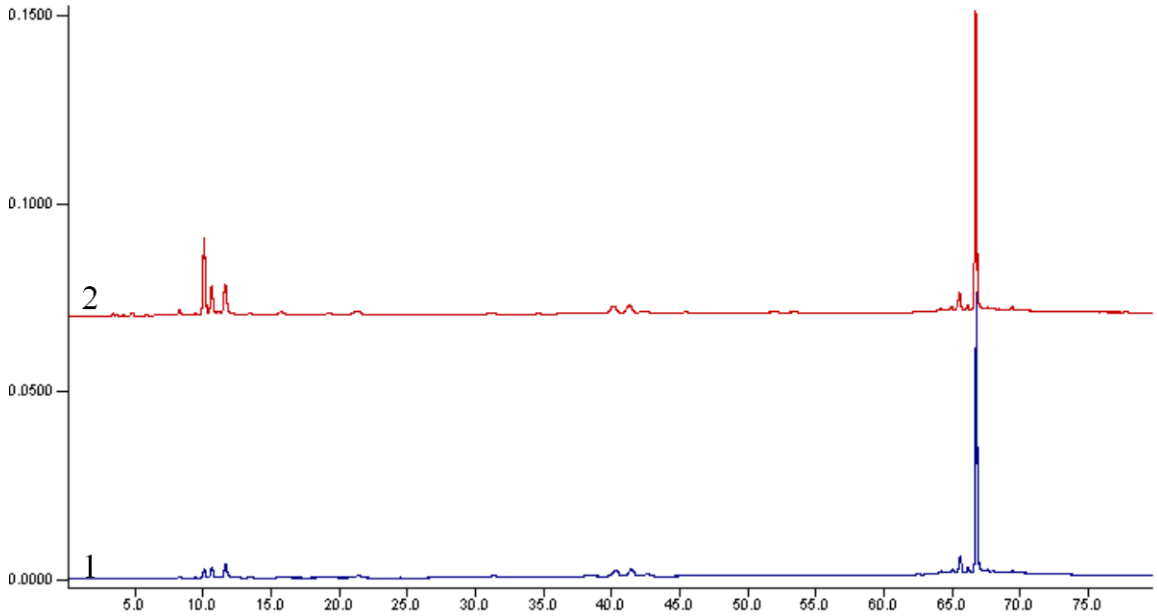

Figure 6a. Effect of water on solubility of SV2 in methanol: (1) SV2 organic layer and (2) whole SV2 in methanol.

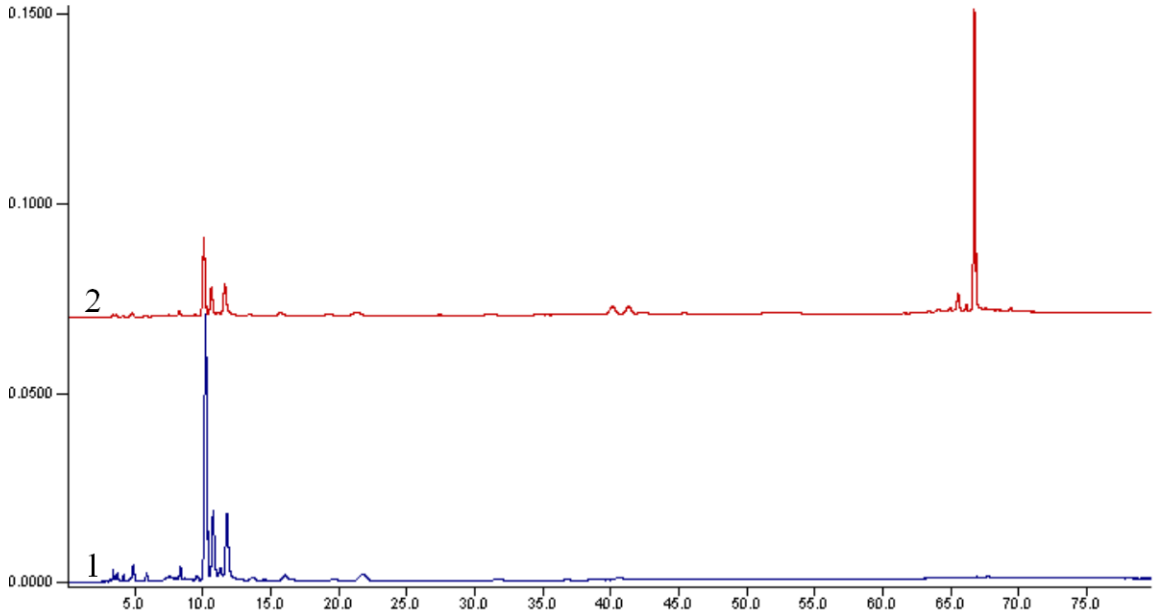

Figure 6b. Effect of water on solubility of SV2 in methanol: (1) SV2 aqueous layer and (2) whole SV2 in methanol.

Peak #	Retention time (min)	Peak area of SV1 Whole oil	Peak area of organic layer	Percentage remaining in organic layer.
6	10.04	851805	266057	31.2
9	11.60	3813	3243	85.1
11	15.70	54227	31571	58.2
14	21.30	139220	101704	73.1
15	31.16	68492	36760	53.7
20	38.55	64157	45300	70.6
21	40.10	266369	260765	97.9
22	41.28	28526	9188	32.2
23	42.40	28013	8931	31.9
24	45.40	75863	47989	63.3
25	48.70	42344	29986	70.8
26	50.39	49922	41177	82.5
27	51.90	121952	102588	84.1
34	64.10	85063	98578	115.9
39	65.53	48171	48394	100.5
40	66.10	64145	61784	96.3
41	66.72	325917	343859	105.5
44	68.02	70386	69696	99.0
45	68.47	75590	72202	95.5
47	69.06	55440	52505	94.7
48	69.40	44248	41868	94.6
49	69.80	83148	108677	130.7
52	71.78	47639	26338	55.3
53	73.60	16467	14643	88.9

Table 2. Effect of water on solubility of SV1 in methanol.

Peak #	Retention time (min)	Peak area of SV2 Whole oil	Peak area of SV2 organic layer	Percentage remaining in organic layer
6	10.04	250670	30285	12.1
7	10.60	101450	36370	35.9
9	11.60	137844	57685	41.8
13	19.27	12699	6237	49.1
14	21.30	37482	16898	45.1
15	31.16	25067	15643	62.4
21	40.10	71068	49789	70.1
22	41.28	74368	54156	72.8
33	63.40	25703	18003	70.0
34	64.10	35296	25287	71.6
35	64.60	14963	13245	88.5
36	65.00	36803	26153	71.1
39	65.53	93452	78853	84.4
40	66.10	36214	27041	74.7
41	66.72	991923	796506	80.3
43	67.50	2810	3072	109.3
44	68.02	2246	2161	96.2
45	68.47	2680	2358	88.0
48	69.40	9262	7380	79.7
52	71.78	1923		0.0

Table 3. Effect of water on solubility of SV2 in methanol.

All of these samples were analysed using the CFT modified Pham *et al*. HPLC method using detection at 282nm. Peaks or compounds that elute early in these profiles are more readily soluble in the aqueous layer of these mixtures. This data shows that fractionation of compounds using an aqueous/organic partitioning method may be possible. These results encouraged us to investigate the potential use of other solvents to fractionate compounds from ginger oil using organic/aqueous solvent partitioning. We chose solvents that may be suitable in the food processing industry and that are more commonly used in general chemical methods.

Solubility of ginger oil (SV1) in various solvent/water mixtures.

We chose to use SV1 for this work since the solubility in the methanol/water mix experiment showed that all compounds were present in both SV1 and SV2, but SV1 had higher levels of the less water-soluble compounds. The ginger oil was initially dissolved in the solvent before water was added to achieve the final concentration of solvent listed in Table 4. The samples were centrifuged, and the results suggest that some selective fractionation of the oils may be possible. However, the complexity of the data was beyond the scope of this project and it was decided to fractionate the oils using preparative HPLC.

Solvent	100%	80%	60%	40%
1. Acetic Acid	1, T, O.	1, SCL, O, SS.	1, CL, O/Y, SS.	1, CL, O/Y, OP, SS.
2. Acetone	1, T, G.	2, T, Y. PPT, TH, BR.	2, Y, SCL. PPT, TH, BR.	2, Y, CL. PPT, TH, BR.
3. Acetonitrile	1, T, G.	2, T, Y. PPT, TH, BR.	2, T, Y. PPT, TH, BR.	2, CL, Y. PPT, TH, BR.
4. Butan-1-ol	1, T, G.	2 (less than 1ml LAC). UOY, T, G.	2, LAC. UOY & T, G.	2, LAC. UOY & T, G.
5. Butan-2-ol	1, T, G.	1, T, G.	2, LAC. UOY & T, G.	2, LAC. UOY & T, G.
6. Dimethylformamide	1, T, G.	1, G, SO.	1, G, SO.	2, G. PPT, TH.
7. Dimethylsulphoxide	1, T, O, SO.	1, T, O, SO.	1, Y, CL, SO.	1, Y, CL, SO.
8. Ethanol	1, T, G.	1, T, G, PPT (very small).	1, Y, CL. PPT, TH, BR.	1, Y, VCL, OP. PPT, TH, BR.
9. Isopropanol	1, T, G.	1, T, G.	1, T, Y. PPT, TH, BR.	1, CL, Y. PPT, TH, BR.
10. Methanol	1, T, Y.	1, SCL, Y. PPT, TH, BR.	1, CL, Y. PPT, TH, BR.	1, VCL, Y. PPT, TH, BR. SN went clear when filtered.
11. Methylacetate	1, T, Y.	2, UOY, LAC.	2, UOY, IF, LAC.	2, UOY, IF, LAC.
12. Propan-1-ol	1, T, Y.	1, T, Y.	2, Y. PPT, TH, BR.	1, Y, SCL.

Table 4. The effect of different solvent and water mixtures on SV1 after centrifuging.

Description codes.

Phase separation

1 = 1 layer.
2 = 2 layers.
CH = Chunks.
IF = InterFace.
LAC = Lower (layer) Aqueous Clear (or only very pale in colour).
PPT = Precipitate.
UOY = Upper (layer) Organic Yellow (various shades from golden to nearly brown).
SO = Surface Oil.
SS = Surface Stuff (floating oily stuff or oily scum or chunky bits).
TH = Thick (viscous, slow to move when tilting the tube).

Transparency

OP = Opaque.
T = Transparent.
SC = Slightly Cloudy.
C = Cloudy.
VC = Very Cloudy.

Colours

B = Brown.
G = Golden.
O = Olive.
Y = Yellow.

Preparative HPLC method development

We decided to attempt to scale-up the CFT-modified Pham *et al*. analytical HPLC method by doing several runs, starting with a relatively small load and working up to 1g total SV2 load per run. For all of the preparative fractionation runs shown in this report, we used the preparative flow cell in the detector. The first preparative HPLC run (see Figure 7) used a 40µl (20mg of SV2) load of 100µl of SV2 in 100ul of methanol load solution.

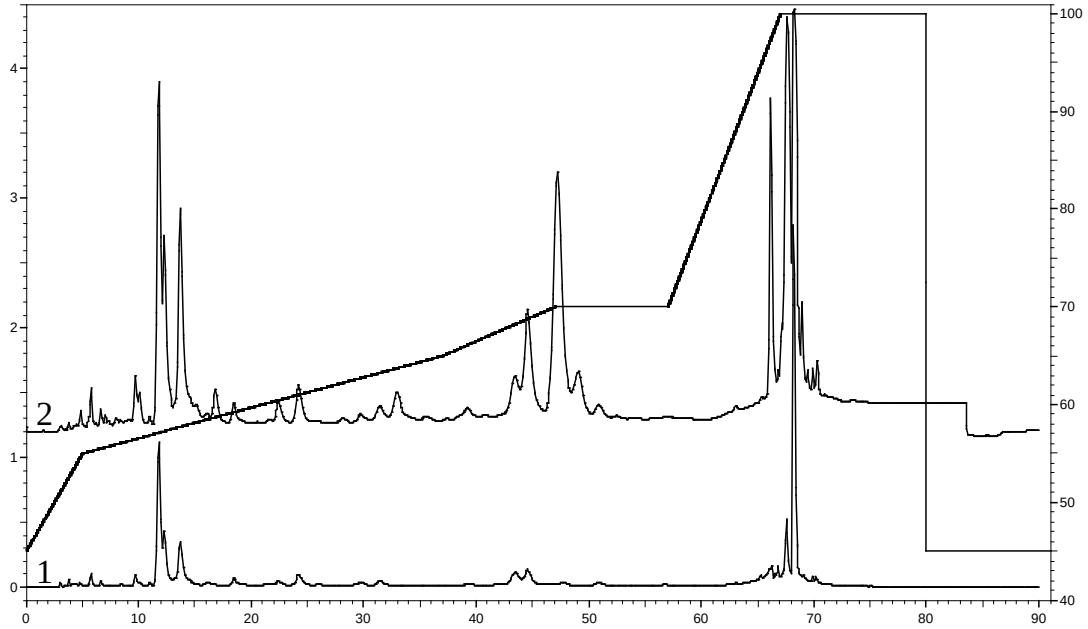

Figure 7. First preparative LC run, 20mg load of SV2; (1) 282nm and (2) 214nm profiles.

The resolution and general profile shown in the chromatogram above is very similar to that obtained by the CFT-modified Pham *et al*. analytical method. Tentative peak identifications based on retention times and peak profiles of this method are presented in Table 5.

Retention time (min)	Tentative peak ID
11.8	6-Gingerol
22.5	6-Shogoal
24.2	8-Gingerol
43.4	8-Shogoal
44.6	10-Gingerol
50.9	10-Shogoal

Table 5. Tentative peak identifications for first preparative LC run.

As this method showed potential for the fractionation of ginger oil, further runs with increasing loads of ginger oil were performed. Figure 8 presents the trial preparative LC run using an injection volume of 500µl (250mg of SV2), while Figure 9 presents the trial preparative LC run using an injection volume of 2000µl (1000mg of SV2).

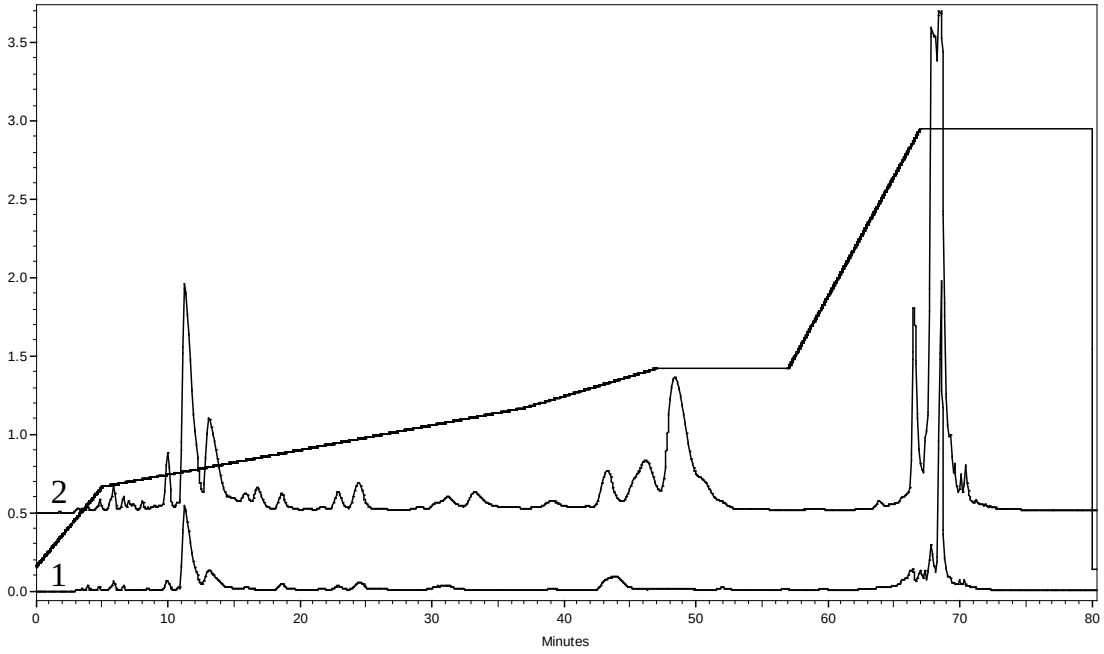

Figure 8. Preparative LC run, 250mg load of SV2 (1) 282nm and (2) 214nm profiles.

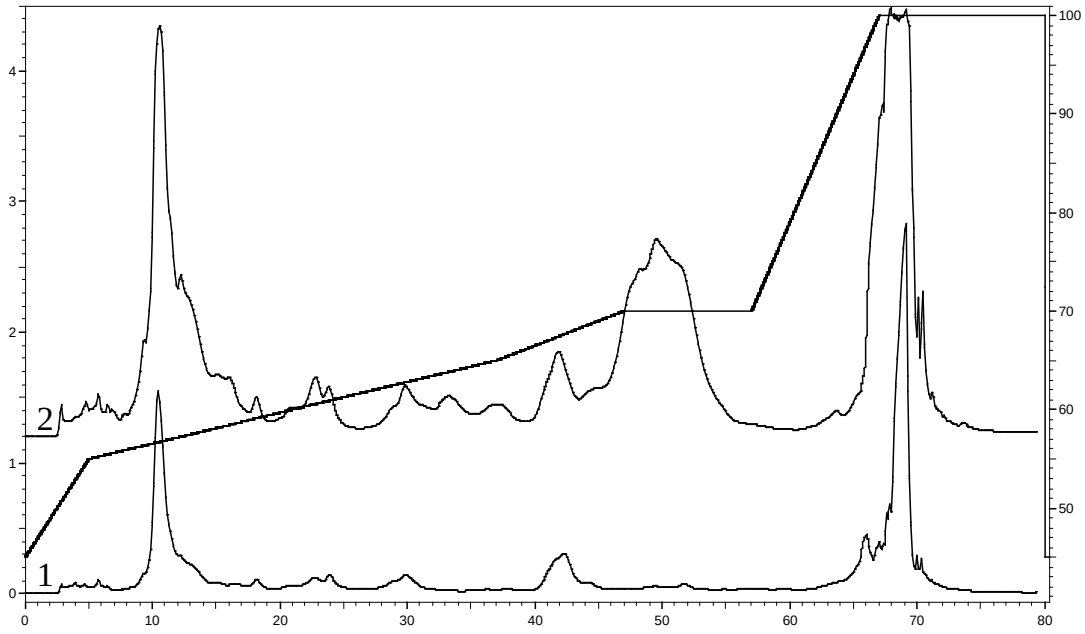

Figure 9. Preparative LC run, 1000mg load of SV2 (1) 282nm and (2) 214nm profiles.

The objective of this preparative LC fractionation procedure was to duplicate the fractionation done by Southern Cross University (Appendix 2) and produce fractions for in vitro and in vivo testing. A total of 16 replicates of the run shown in Figure 9 were fractionated into five pools, this was the first set of 16 fractionation runs (see Figure 10). Another 16 replicates were done later, this was the second set of fractionation runs. The final oils (G1 to G5) used for *in vivo* assessment were derived from this second set of fractionation runs.

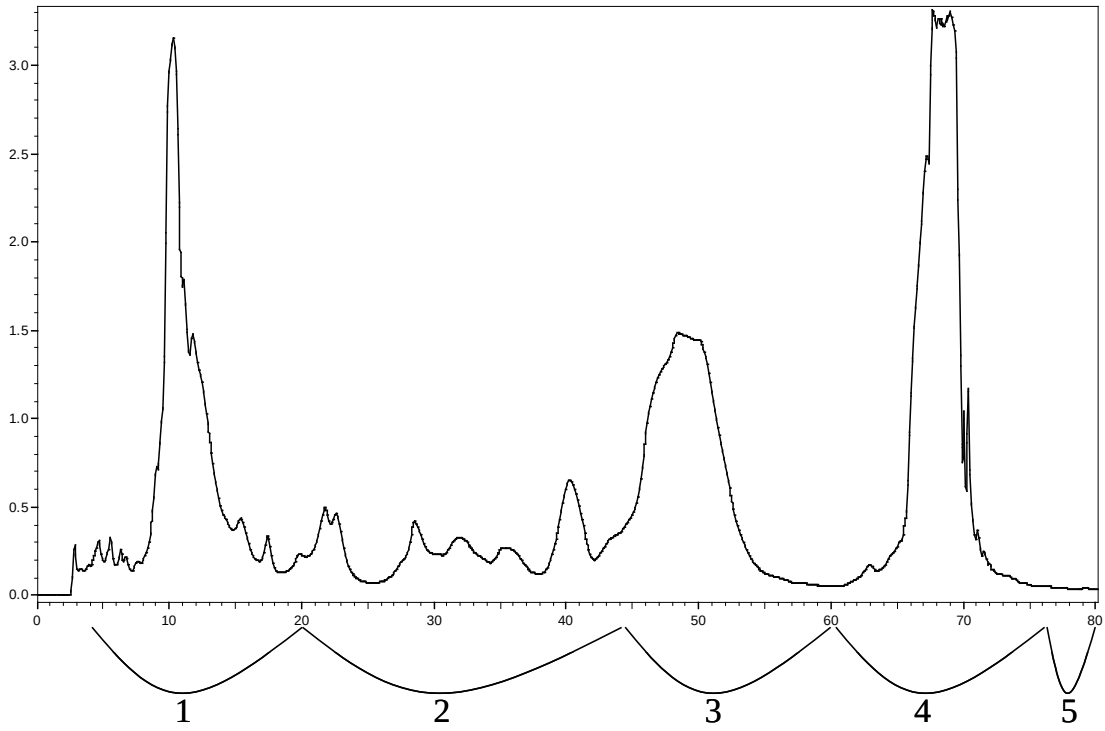

Figure 10. Typical pooling for first set of sixteen preparative LC fractionation runs.

Poolings for these preparative LC fractionation runs were typically: Pool #1, F# 4 – 19; Pool #2, F# 20 – 43; Pool #3, F# 44 – 60; Pool #4, F# 61 – 75 and Pool #5, F#76 - 90. Chromatographic profiles of these fractions are presented in Figure 11.

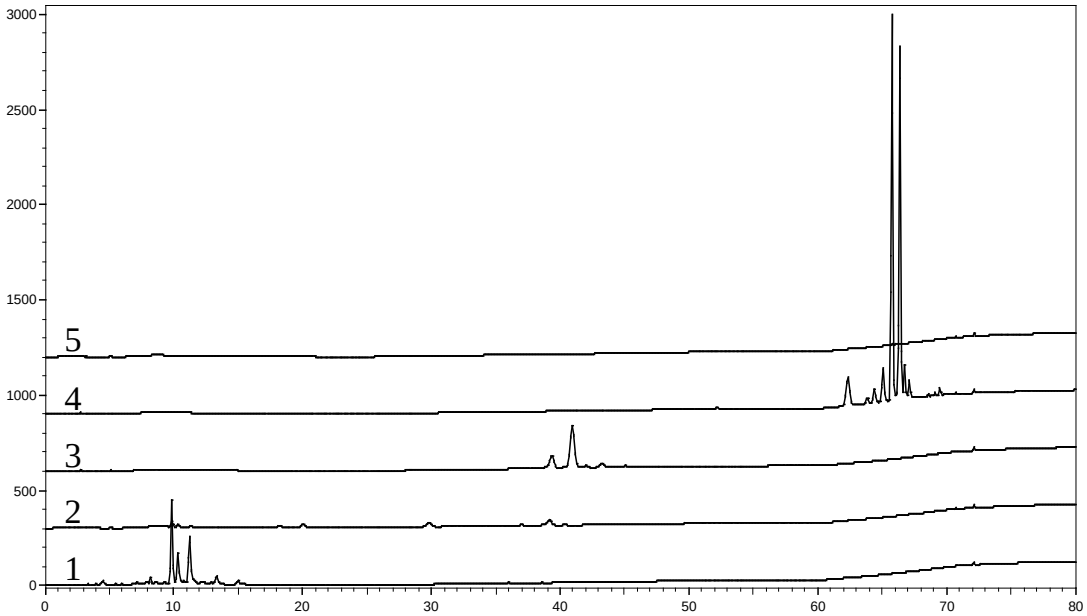

Figure 11. Analytical HPLC profiles of pools from the first set of 16 replicated preparative LC fractionation runs; (1) Pool 1, (2) Pool 2, (3) Pool 3, (4) Pool 4 and (5) Pool 5.

These pools (first set) were used to assess the effect of freeze-drying on the composition and recovery of their components. Initial trials suggested that a large proportion of the ginger oil components were lost during freeze-drying.

Effect of freeze-drying on the composition of ginger oil.

a) First freeze-drying trial

About 15 minutes after commencing freeze-drying, the ginger fractions (Pools 1 to 5) defrosted. Consequently, the drying process wasn't true lyophylisation, but they were evaporated to dryness after 20 hours in the freeze-drier. Generally, aqueous solutions do not defrost during freeze-drying. The high concentrations of methanol in the pools may have lowered the freezing point of the solutions. The reconstituted Pool 1 to 5 solutions should be 10 times more concentrated than the starting solutions. Figures 12 to 16 show the analytical HPLC profiles of the original ginger oil and the freeze-dried fractions.

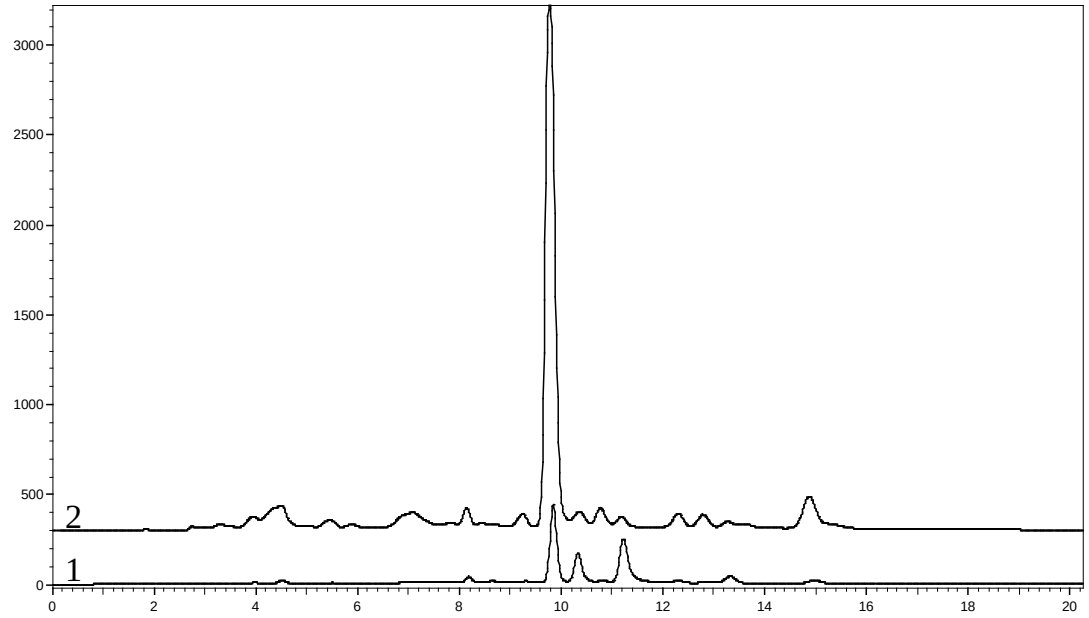

Figure 12. HPLC analyses of (1) Pool 1 and (2) Pool 1 after reconstituting the residue from the freeze-drying trial (at theoretically 10 times the original concentration).

This figure shows that most peaks in the Pool 1 fraction have been retained and concentrated by the freeze-drying process with the exception of the peaks at 10.3 and 11.2 minutes.

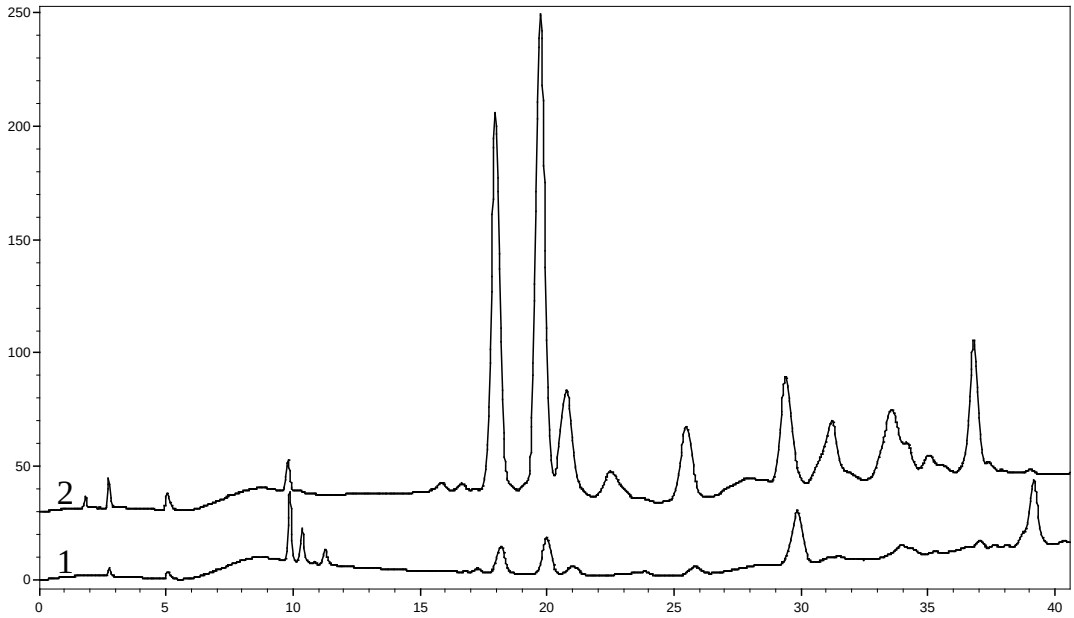

Figure 13. HPLC analyses of (1) Pool 2 and (2) Pool 2 after reconstituting the residue from the freeze-drying trial (at theoretically 10 times the original concentration).

Most peaks in the Pool 2 fraction have been retained and concentrated by the freeze-drying process with the exception of the peaks at 9.9, 10.3, 11.2 and 39.1 minutes.

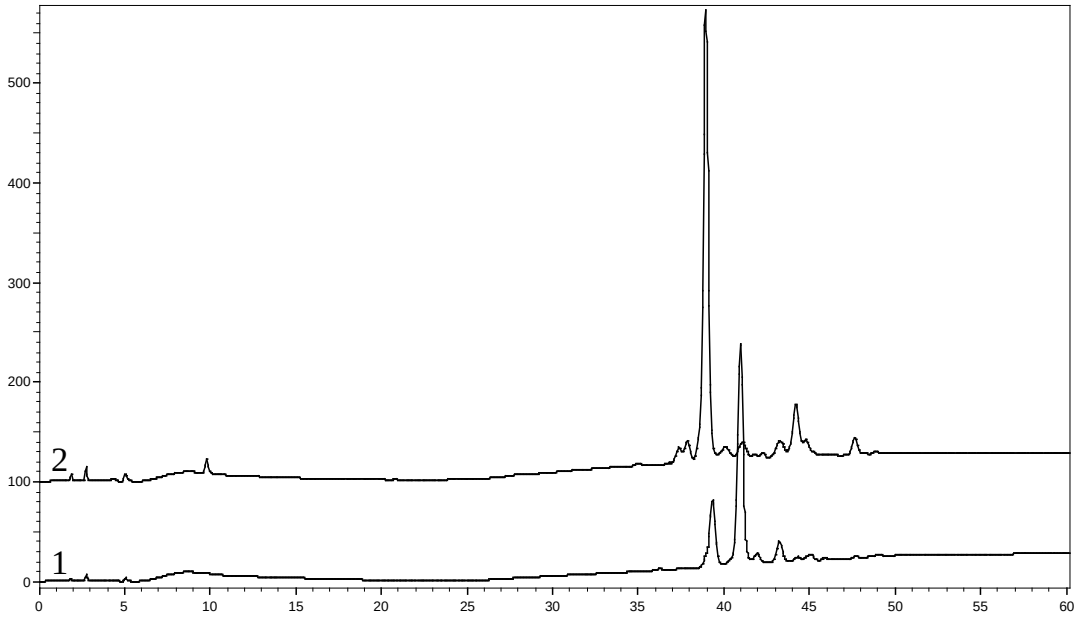

Figure 14. HPLC analyses of (1) Pool 3 and (2) Pool 3 after reconstituting the residue from the freeze-drying trial (at theoretically 10 times the original concentration).

This figure shows that most peaks in the Pool 3 fraction have been retained and concentrated by the freeze-drying process with the exception of the peak at 40.9 minutes.

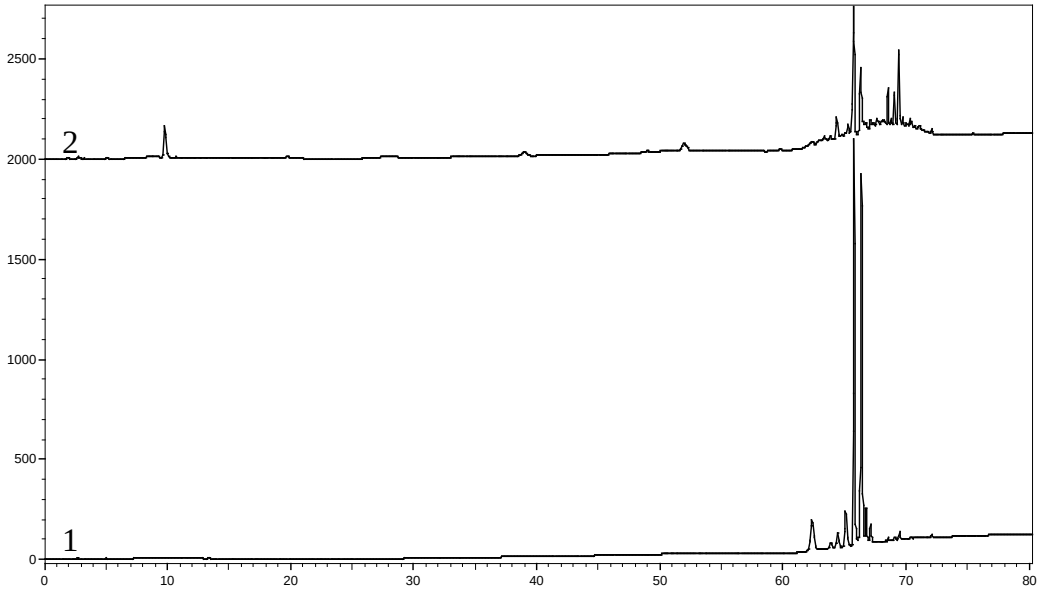

Figure 15. HPLC analyses of (1) Pool 4 and (2) Pool 4 after reconstituting the residue from the freeze-drying trial (at theoretically 10 times the original concentration).

This Figure shows that the group of peaks in the Pool 4 fraction that elute after 68 minutes have been retained and concentrated by the freeze-drying process while those that elute between 61 and 68 minutes have been removed from the oil residue.

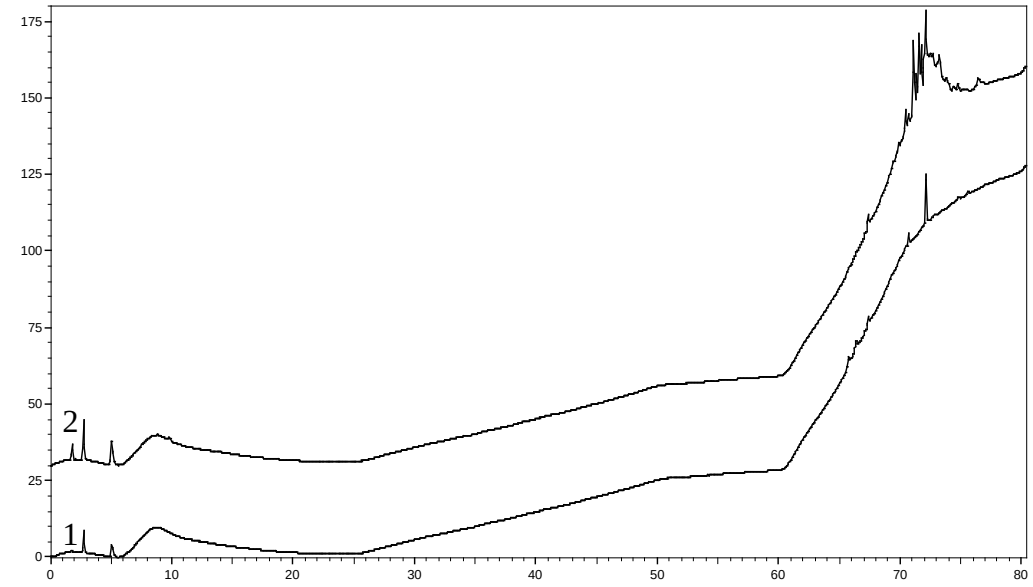

Figure 16. HPLC analyses of (1) Pool 5 and (2) Pool 5 after reconstituting the residue from the freeze-drying trial (at theoretically 10 times the original concentration).

This figure shows that most peaks in the Pool 5 fraction have been retained and concentrated by the freeze-drying process with the exception of the peaks at 65.7, 66.4 and 72.1min. These results indicate that many peaks are highly volatile and were lost from the oil residue during freeze-drying.

CFT (SCU-mimic) HPLC method

This method was created so that we could compare our HPLC profiles more easily with those in the SCU report (Appendix 2) and to speed up our HPLC analysis times. The numbers on the chromatograms in Figure 17 have been assigned to each of the larger peaks of both chromatograms based mainly on the similarity of the two profiles and are only provisional for the exercise of this comparison. The time scale of the CFT trace has been stretched to demonstrate the similarity between the resolution of the two methods. The CFT method is considerably quicker (16min total run time) than the SCU method (a run time of at least 25min).

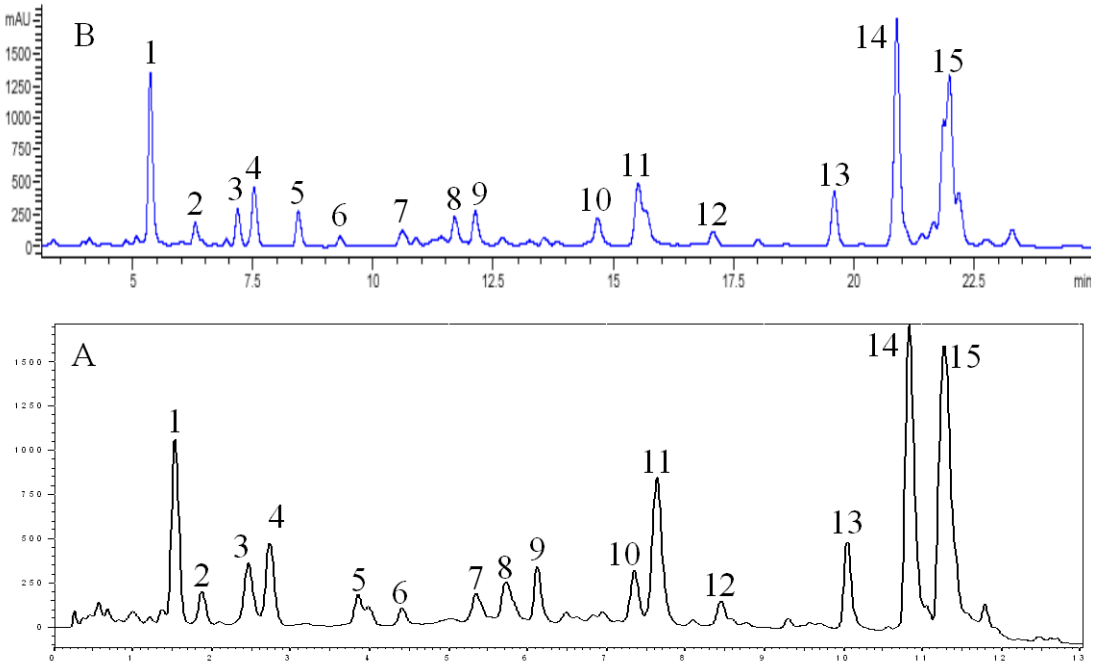

Figure 17. Comparison of analytical HPLC methods using SV2: (A) CFT (SCU-mimic) HPLC method and (B) SCU HPLC method.

Ginger standards were purchased from Chromadex and analysed using the SCU-mimic analytical HPLC method. The retention times for the four standards are presented in Table 6, and the representative chromatogram showing the four standards is presented in Figure 18.

Compound	Retention time (min)	Purity (%)
6-Gingerol	1.56	94.6
8-Gingerol	3.88	99.7
10-Gingerol	6.14	100.0
6-Shogaol	4.38	81.4

Table 6. Results of HPLC analysis of Chromadex ginger kit standards using the CFT HPLC (SCU-mimic) method.

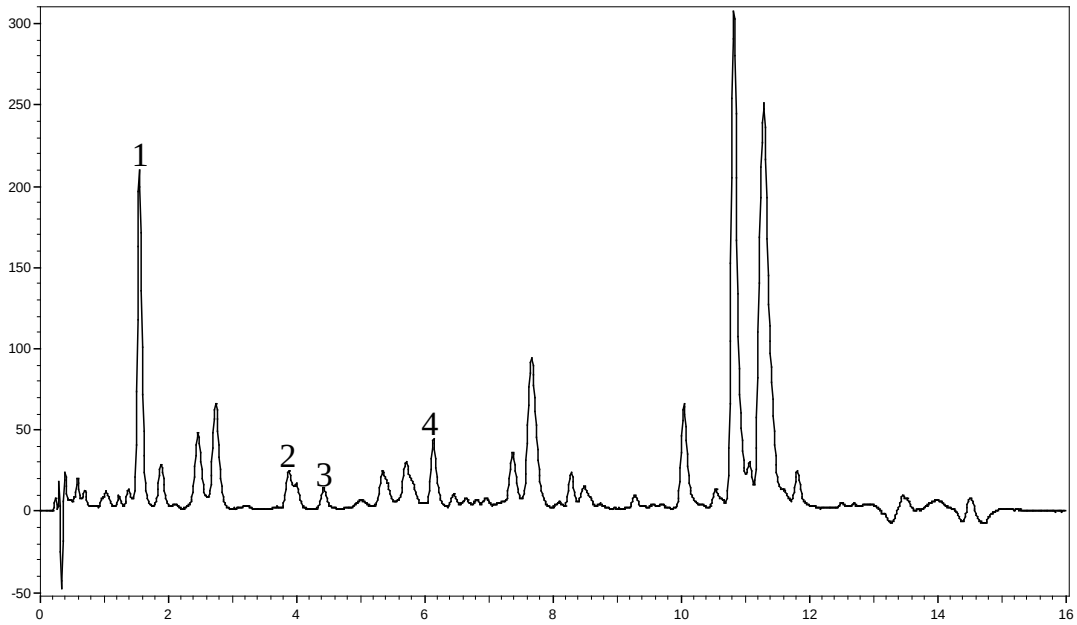

Figure 18. Baseline subtracted HPLC analysis of SV2 at 1mg/ml in methanol, Peak 1 is 6-gingerol, Peak 2 is 8-gingerol, Peak 3 is 6-shogaol and Peak 4 is 10-gingerol, analysed using the CFT (SCU-mimic) HPLC method (detection at 210nm) showing complete run time from 0 to 16min which includes the 100% phase B wash and re-equilibration steps.

b) Second freeze-drying trial.

When water was added to the methanol-dissolved ginger oil (SV2), the solutions went milky and had some oil which remained on top of the solution. The results in Table 7 suggest that over 70% of the SV2 ginger oil is lost during the freeze-drying process. Figure 19 presents a chromatographic profile of the components of the ginger oil which are lost during the freeze-drying process. This Figure also shows that the 6-, 8- and 10-gingerol and 8-shogaol are effectively concentrated by the freeze-drying process. There are numerous compounds either totally or partially lost during the freeze-drying process that are detectable by this HPLC method. These results show that that freeze-drying (with or without methanol and water) has the same effect. The mass loss from whole SV2 is greater than 70% and the gingerols and shogaols are relatively non-volatile and can be concentrated by freeze-drying.

Code	Weight of SV2 before freeze-drying (g)	Weight of SV2 after freeze-drying (g)	% of SV2 remaining after freeze-drying
B1 (SV2 + water and methanol)	0.9992	0.2784	27.82%
B2 (SV2 + water and methanol)	0.9948	0.2790	28.05%
B3 (SV2 only)	0.9986	0.2926	29.30%
B4 (SV2 only)	1.0100	0.2970	29.41%

Table 7. Recovery data for second freeze-drying trial of SV2.

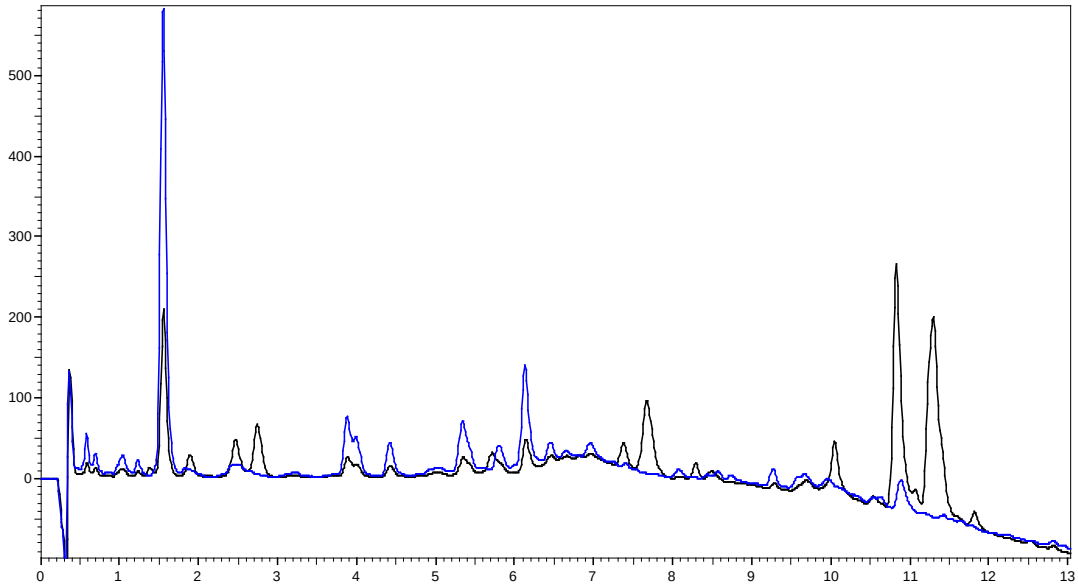

Figure 19. HPLC analysis of: (1) Whole SV2 and (2) Freeze-dried SV2, methanol and water mixture, both made at 1mg/ml in methanol, analysed using the CFT HPLC (SCU-mimic) method (detection at 210nm).

The freeze-drying trials comprehensively showed that diluting the preparative HPLC concentrated SV2 pools with water to reduce the freezing point of the methanol/water mixtures prior to freeze-drying would incur significant losses. The preparative HPLC was used to concentrate each of the pools to a much smaller volume. These concentrates, with a high methanol concentration, were in much smaller volumes and would freeze-dry more quickly. The initial SV2 fractionation produced 5 fractions that were concentrated using this strategy. The results from this experiment are presented in Table 8. These fractions were assessed for *in vitro* anti-inflammatory activity after freeze-drying.

Fraction #	Initial mass of SV2 (g)	Recovered mass of fraction (g)	Recovery after freeze-drying (%)
1	7.25	0.5881	8.1
2	7.25	0.5501	7.6
3	7.25	0.1232	1.7
4	6.00	1.0505	17.5
5	6.00	0.0768	1.3

Table 8. Recovery data for the first set of preparative LC fractionated, concentrated and freeze-dried SV2 fractions.

Preparative LC fractionation of ginger oil (SV2).
Due to the low mass recoveries, a second set of preparative LC fractionations of SV2 were collected (see Figure 20).

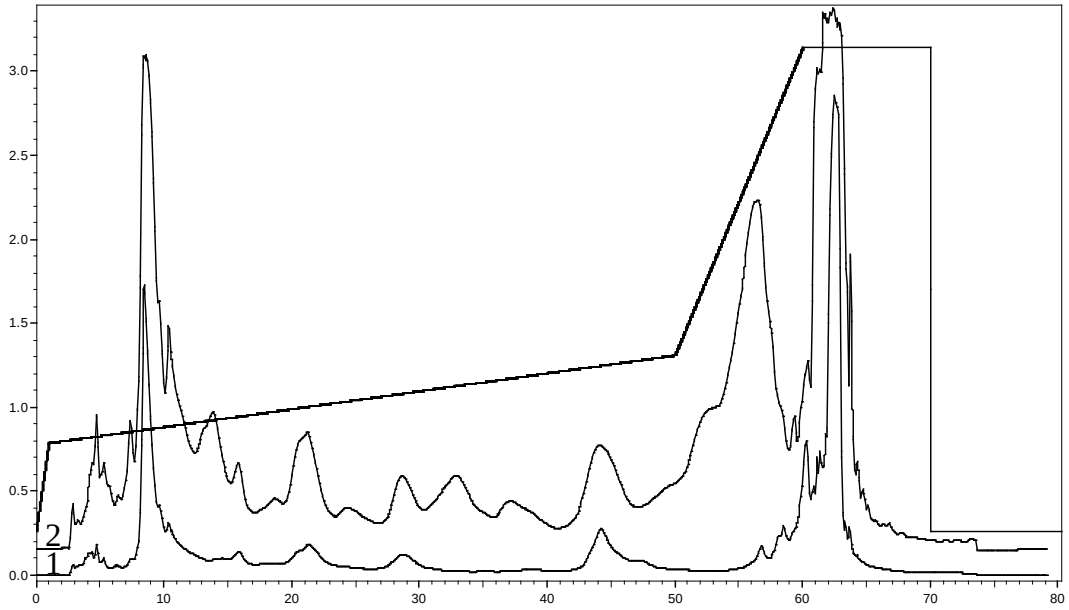

Figure 20. Typical preparative LC fractionation for second set of fractionation runs: (1) 282nm trace and (2) 210nm trace.

The individual preparative LC profiles of each of the 16 replicates were compared and pooled so that similar groups of peaks were kept together in each of four pools. Figures 21, 22 and 23 show the HPLC profiles of the various fractions eluted using preparative HPLC fractionation.

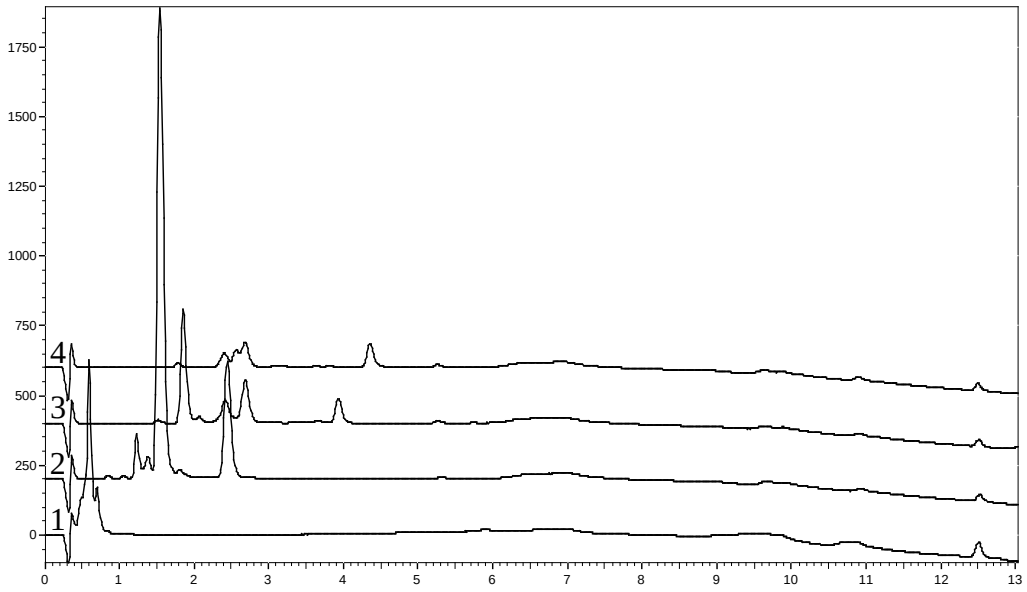

Figure 21. Typical analytical HPLC profiles of fractions from second set of preparative LC fractionations: (1) fraction #5; (2) fraction #10, (3) fraction #15 and (4) fraction #20, analysed using CFT (SCU mimic) HPLC method.

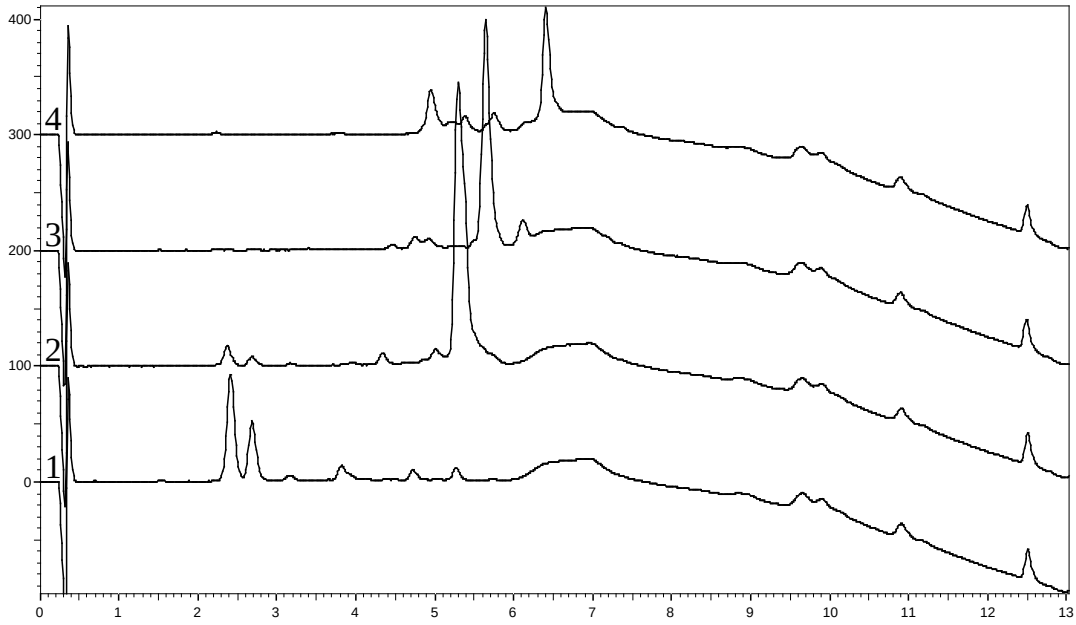

Figure 22. Typical analytical HPLC profiles of fractions from second set of preparative LC fractionations: (1) fraction #25; (2) fraction #30, (3) fraction #35 and (4) fraction #40, analysed using CFT (SCU mimic) HPLC method.

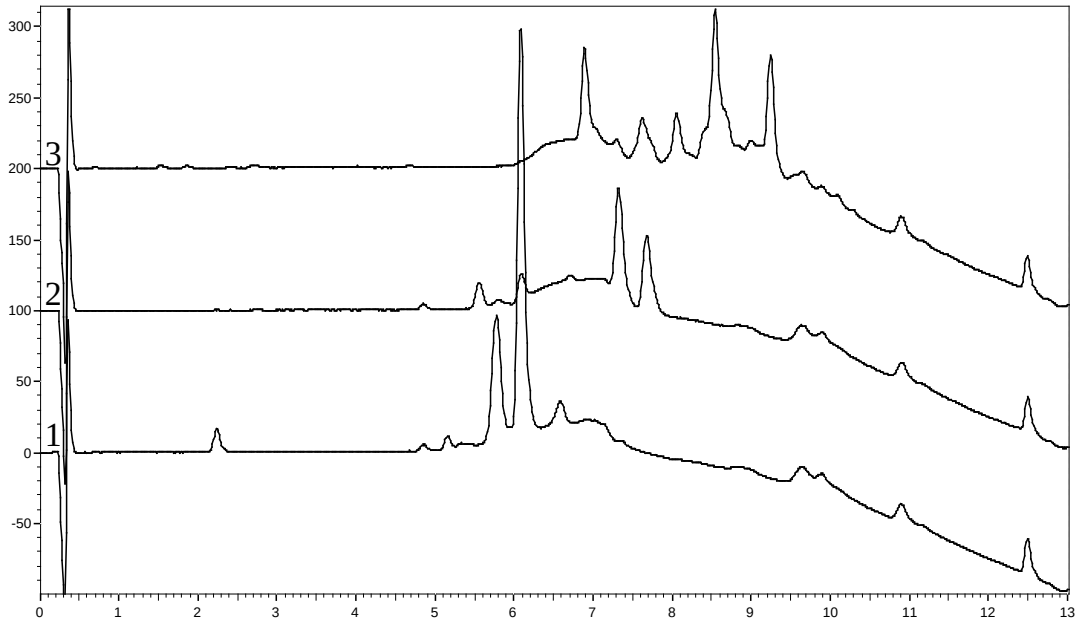

Figure 23. Typical analytical HPLC profiles of fractions from second set of preparative LC fractionations: (1) fraction #45; (2) fraction #50 and (3) fraction #60, analysed using CFT (SCU mimic) HPLC method.

The selection of the best pooling of these fractions from the preparative HPLC was carefully considered and eventually the poolings shown in Figure 24 were used. The analytical profiles of the pools is presented in Figure 25.

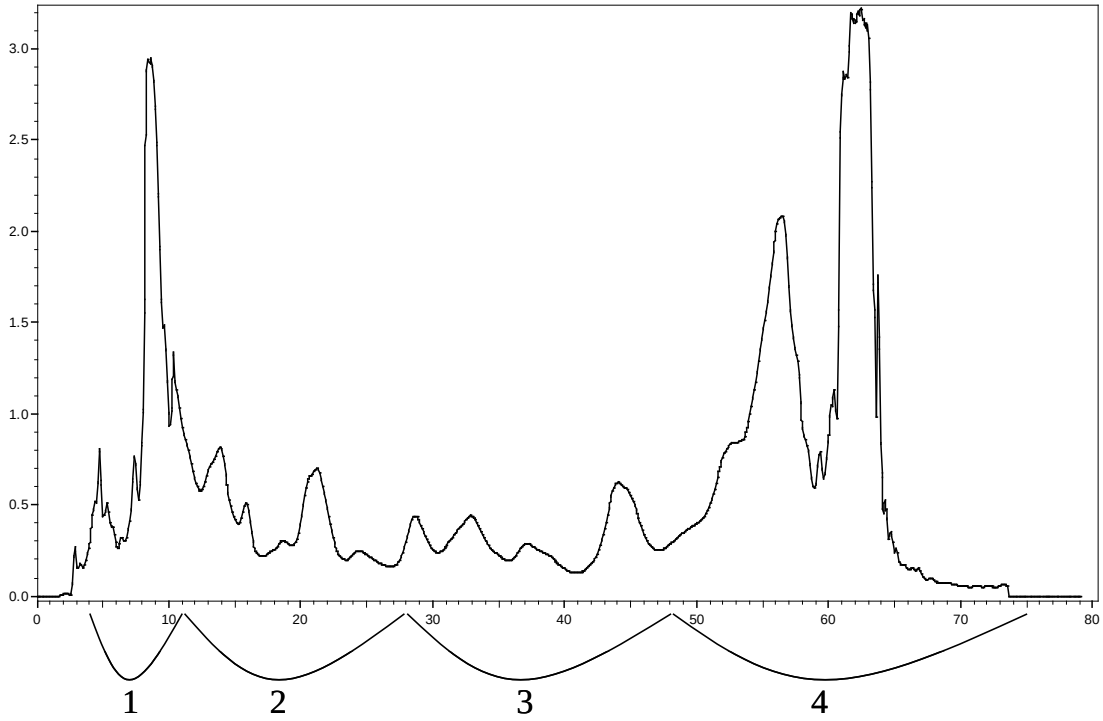

Figure 24. Typical pooling of second set of preparative LC fractionations of SV2. Pool 1, Fractions 4-10; Pool 2, fractions 11-27; Pool 3, fractions 28-47 and Pool 4, fractions 48-74.

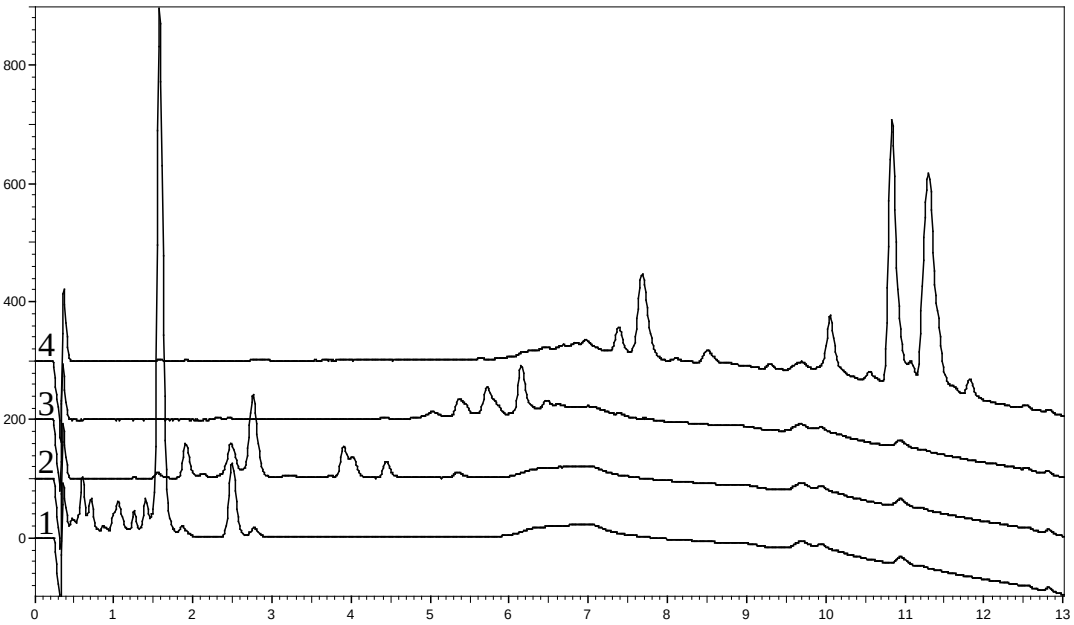

Figure 25. Analytical HPLC of second set of preparative LC fractionations of SV2: (1) Pool #1; (2) Pool #2; (3) Pool #3 and (4) Pool #4, analysed using CFT (SCU mimic) HPLC method.

This Figure shows the logical fractionation from early eluting peaks to later eluting peaks. A small amount of overlap of peaks between some of the pools is able to be explained by poor preparative LC resolution when working with such large loads. We had hoped to do further preparative fractionation of these pools and to recombine common peaks into single pure fractions where possible. Unfortunately, due to limits of the project, we were only able to attempt to purify 6-gingerol from Pool 1 (G1), the other pools were eventually concentrated by preparative LC prior to final drying to remove organic solvents.

A typical HPLC profile of Ginger Oil (SV2) is presented in Figure 26 and it shows the position of 6-, 8- and 10-gingerol and 6-shogaol. Table 9 presents the composition of SV2.

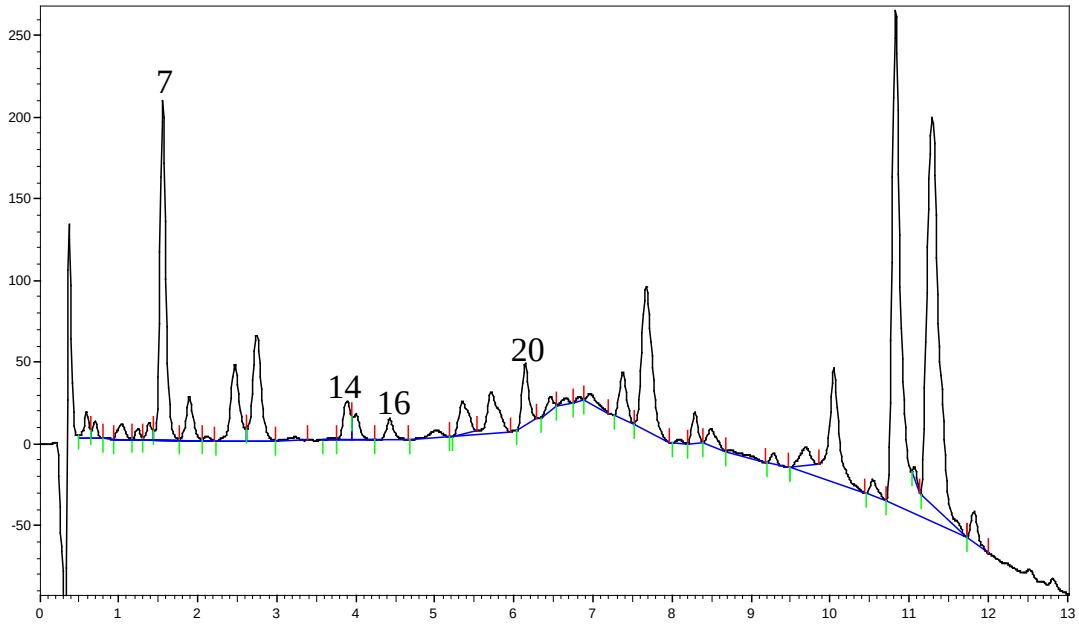

Figure 26. HPLC analysis of 1mg/ml of SV2 in methanol using CFT (SCU-mimic) HPLC method. Peak #7 is 6-gingerol, Peak #14 is 8-gingerol, Peak #16 is 6-shogaol and Peak #20 is 10-gingerol.

Pk #	Peak name	Retention Time	Area	Area Percent
1		0.597	70535	0.7
2		0.704	43885	0.4
3		0.843	2658	0.0
4		1.035	67813	0.6
5		1.237	29513	0.3
6		1.387	50811	0.5
7	6-Gingerol	1.557	1064198	9.8
8		1.899	174207	1.6
9		2.112	14198	0.1
10		2.475	361036	3.3
11		2.741	492945	4.6
12		3.232	22312	0.2
13		3.723	10559	0.1
14	8-Gingerol	3.893	153704	1.4
15		4.000	98497	0.9
16	6-Shogaol	4.427	87626	0.8
17		5.013	47654	0.4
18		5.376	167934	1.6
19		5.717	276713	2.6
20	10-Gingerol	6.144	223585	2.1
21		6.464	44119	0.4
22		6.656	21722	0.2
23		6.827	10924	0.1
24		6.965	51425	0.5
25		7.381	180699	1.7
26		7.669	771210	7.1
27		8.085	15085	0.1
28		8.288	99862	0.9
29		8.491	82822	0.8
30		8.747	33913	0.3
31		9.291	35535	0.3
32		9.707	107810	1.0
33		10.048	736358	6.8
34		10.539	65420	0.6
35		10.827	2532212	23.4
36		11.072	30666	0.3
37		11.296	2420822	22.4
38		11.819	109480	1.0
Totals			10810467	100

Table 9. Report table for HPLC analysis of SV2, prepared at 1mg/ml in methanol, using CFT (SCU-mimic) HPLC method.

From these results, the 6-gingerol peak represents 9.8% of the ginger oil (SV2), but this is probably an overestimation. With a total load of 16 grams, the 6-gingerol contributes 1.56 grams. Our objective with second stage fractionation of Pool #1 was to produce 3 fractions: Fraction #1eluting between 0.3 – 1.45 minutes, Fraction #2 (6-gingerol) eluting between 1.45 – 1.8 minutes, and Fraction #3 eluting between 1.8 – 3.0 minutes by the CFT (SCU-mimic) analytical method.

Several trial preparative LC runs were done to optimise the second stage fractionation method. We used a small proportion of the Pool #1 solution for these trial runs and all of this material was collected and recycled by water dilution, so there were minimal losses of this material. It was necessary to dilute the Pool #1 solution so that it had approximately 30% methanol in it. Complete retention of all compounds to the column was achieved prior to starting the gradient elution of second stage fractionation. A typical preparative LC chromatographic profile is presented in Figure 27.

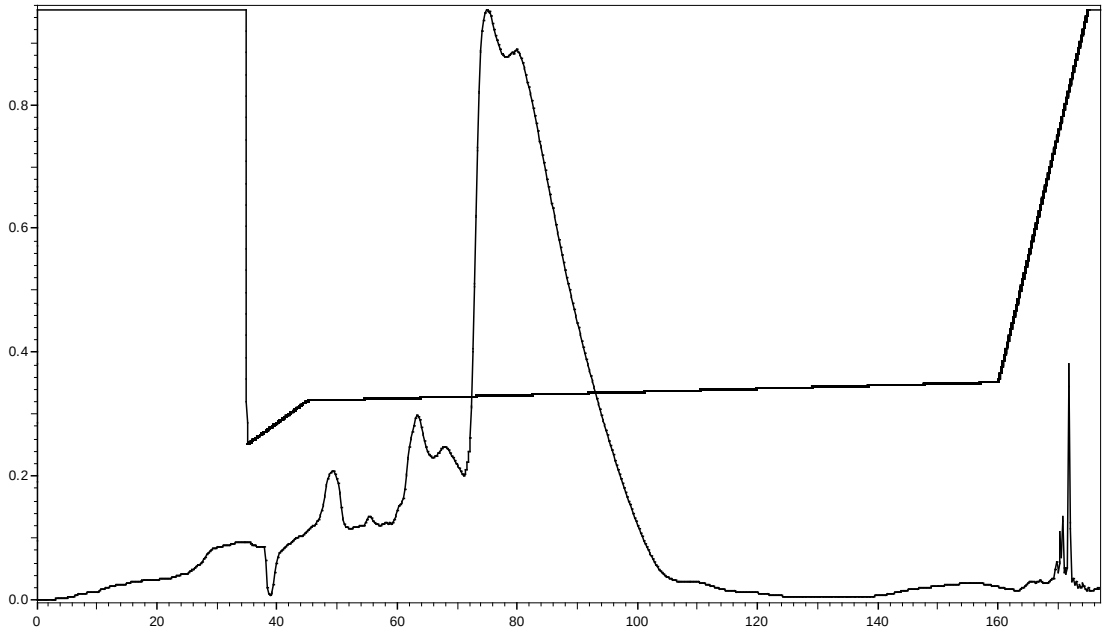

Figure 27. Typical second stage preparative HPLC fractionation of Pool #1 (G1).

The strategy for second stage purification of Pool #1 required balancing the following factors - desired purity of 6-gingerol (to go from 72 to >90%), total mass of Pool #1 compounds for preparative LC fractionation, PLC run times, number of PLC runs to be done, volumes of pooled fractions from 2nd stage purification and further processing or treatment of pools from the second stage purification. Figure 28 shows the fractions which contain the vast majority of the 6-gingerol.

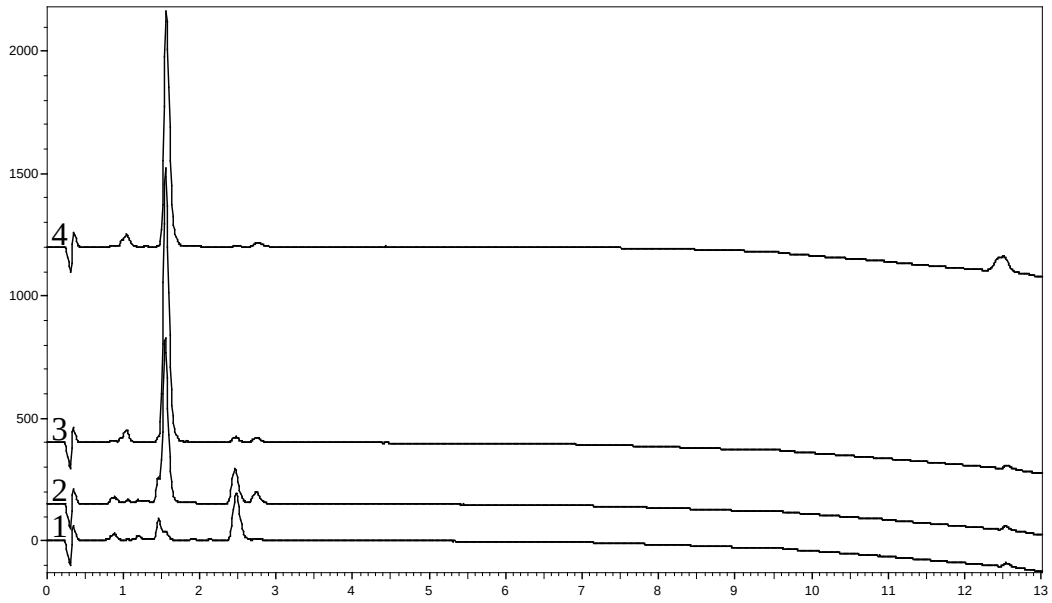

Figure 28. HPLC analysis of individual fractions from second stage preparative HPLC fractionation of Pool #1, Fractions: (1) #70; (2) #71; (3) #75 and (4) #80.

There is a small amount of 6-gingerol (the 1.5 minute peak) in Fraction #70, but it is relatively dilute compared to Fractions #71 to 80 shown on this profile. Since the objective of this fractionation was to produce pure 6-gingerol, Fractions 74-102 were pooled. Figure 29 shows typical pooling for this second stage preparative LC fractionation.

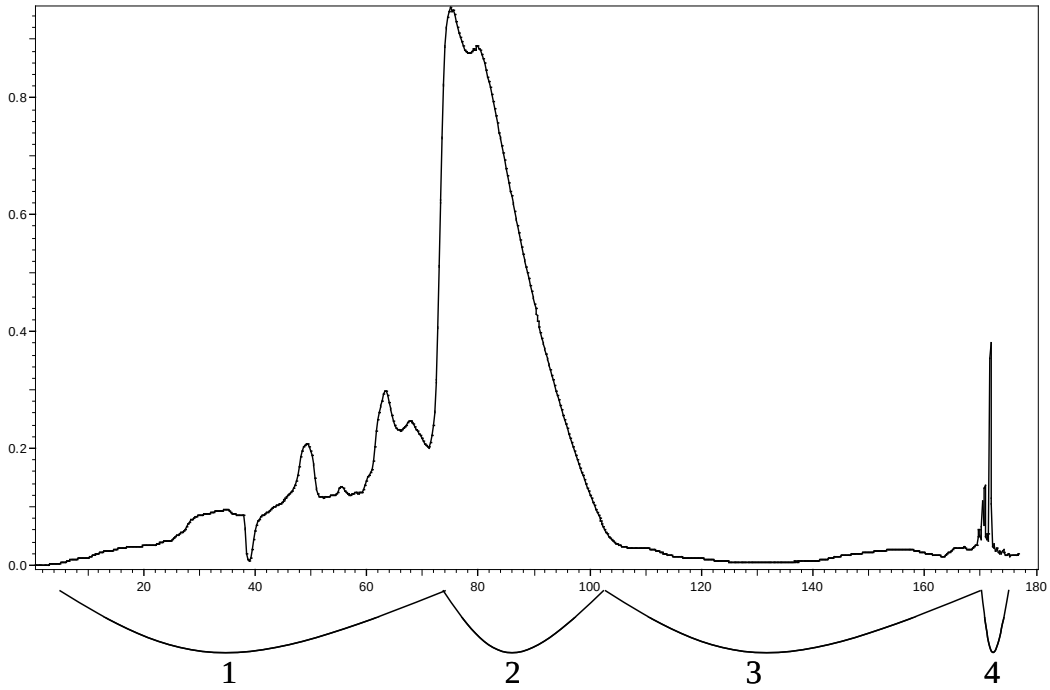

Figure 29. Typical second stage preparative HPLC fractionation of Pool #1 (G1): (1) Pool #1, F#4 to 73; (2) Pool #2, F# 74 to 102; (3) Pool #3, F#103 to 170 and (4) Pool #4, F#171 to 175.

Figure 30 presents the fractions prepared from the second stage fractionation of Pool 2. The 6-gingerol-enriched material in this fraction was over 90% pure.

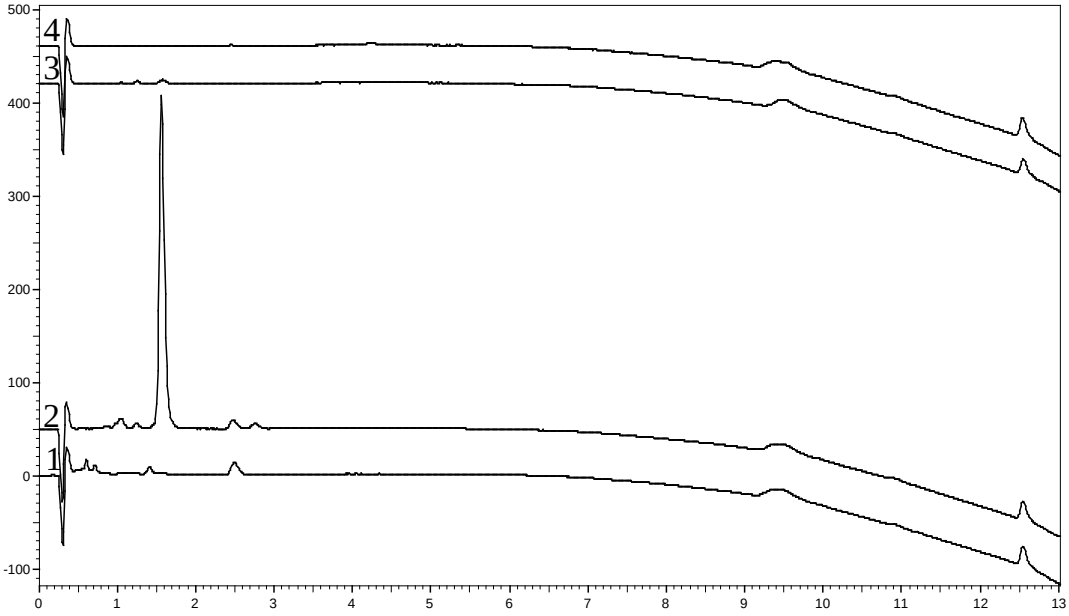

Figure 30. HPLC analysis of pools from second stage preparative HPLC fractionation of pool #1, pools: (1) #1 (pre 6-gingerol, mainly compounds that elute prior between 0.5 and 3 minutes, but with virtually no 6-gingerol); (2) #2 (6-gingerol, 90.4% pure); (3) #3 (post 6-gingerol, three peaks including 6-gingerol that elute between 1 and 2 minutes on this analytical method) and (4) #4 (very late eluting compounds, mainly 6-shogaol at 4.3 minutes).

Preparative LC concentration of 6-gingerol-enriched Pool #1 (Fraction G1).
Since we were unable to completely replicate the fractions produced by the Southern Cross University for bioactivity in the available time, we abandoned any further fractionation runs and focussed our efforts on concentrating those fractions that we had already produced. The 6-gingerol-enriched fraction was diluted with water and loaded onto the preparative HPLC for concentration. Figure 31 shows the composition of the 6-gingerol-enriched fraction prior to concentration.

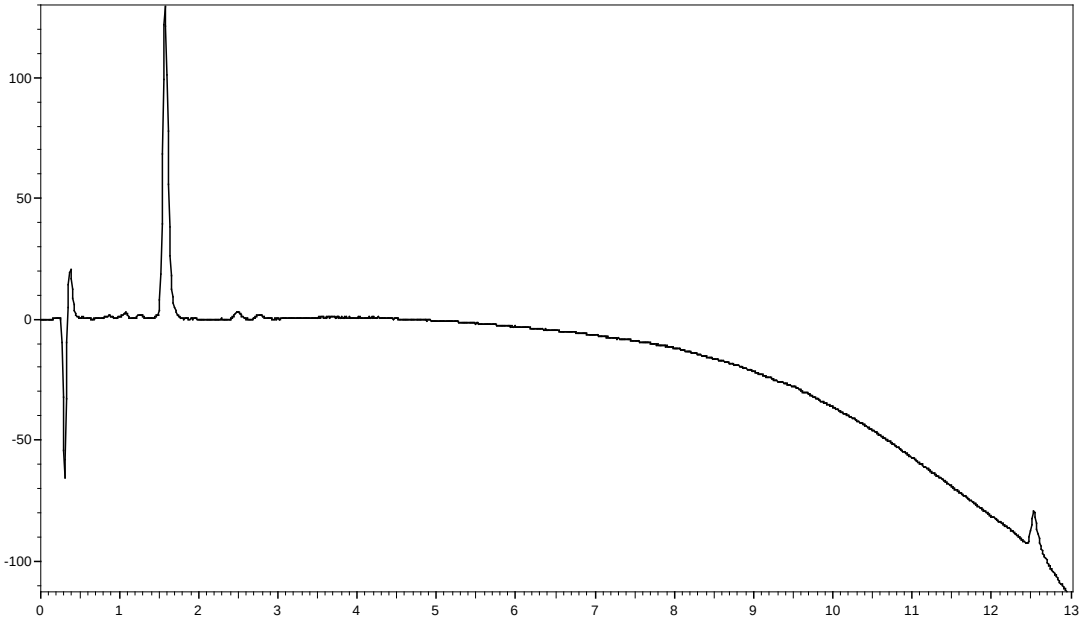

Figure 31. HPLC analysis of water-diluted Pool #1, sub-fraction #2, (G1) prior to preparative LC concentration.

The desorption profile of the 6-gingerol-enriched sub-fraction is presented in Figure 32, while Figure 33 shows the progressive profiles of the material as it is eluted from the preparative HPLC column.

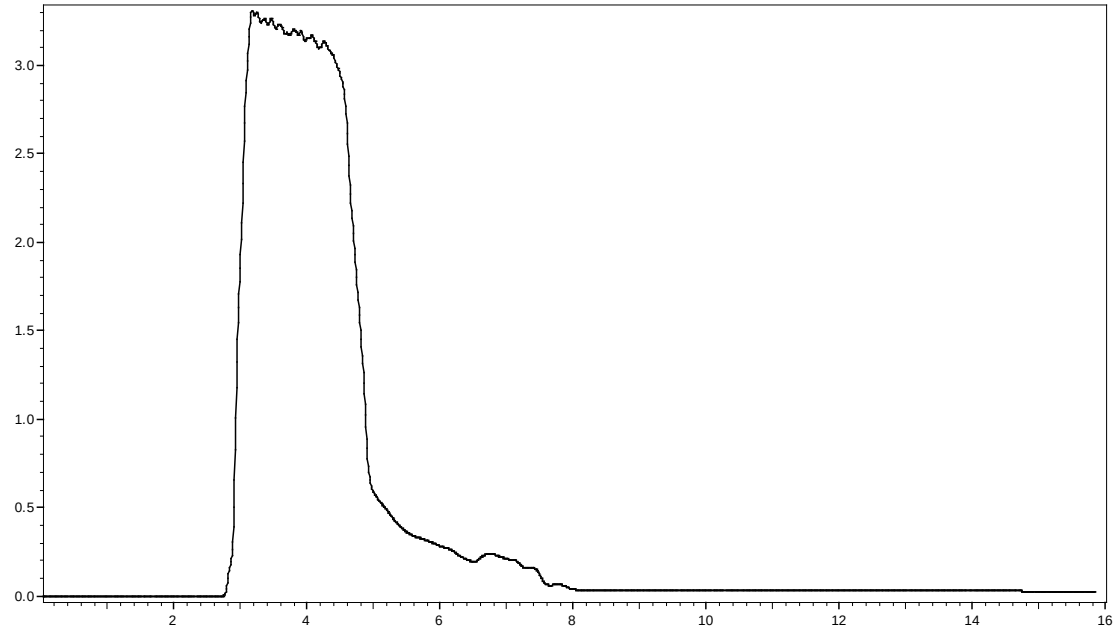

Figure 32. Desorption of Pool #1, sub-fraction #2, (G1) from the preparative HPLC column.

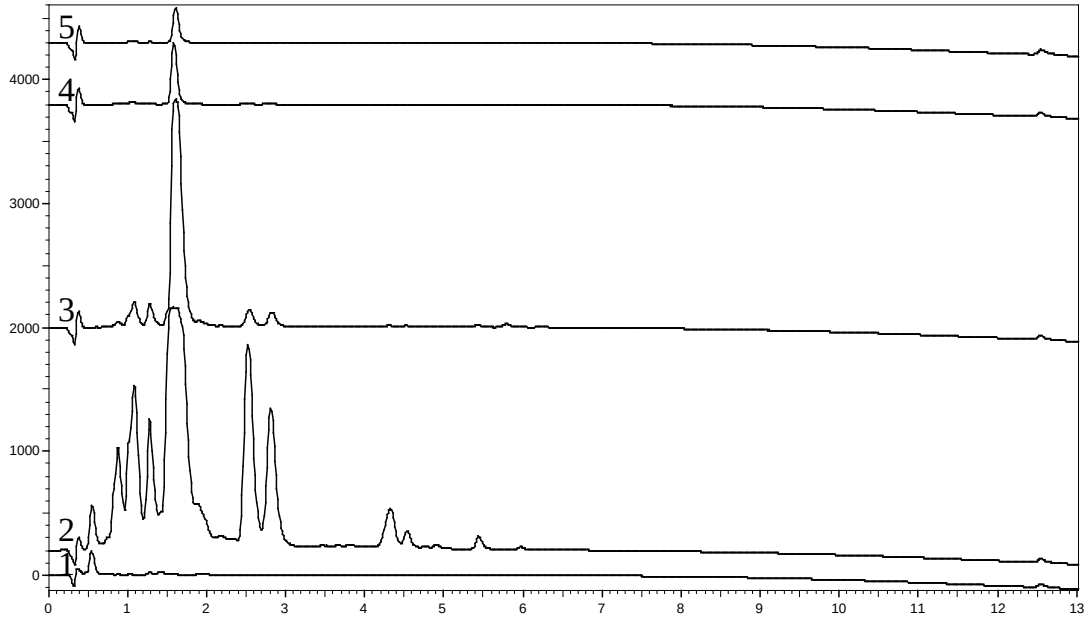

Figure 33. Desorbed fractions from preparative concentration of Pool #1, sub-fraction 2, (G1), Fraction: (1) 4 (3-4min); (2) 5 (4-5min); (3) 6 (5-6min); (4) 7 (6-7min) and (5) 8 (7-8min).

Fraction 4 has virtually no compounds of interest, while Fractions 5, 6, 7 and 8 contain significant amounts of 6-gingerol. This Figure shows the impurities that contaminate the 6-gingerol in this pool (particularly Fraction 5).

Figure 34 shows that the preparative LC concentration method for the G1 (6-gingerol-enriched) fraction resulted in minimal losses of 6-gingerol.

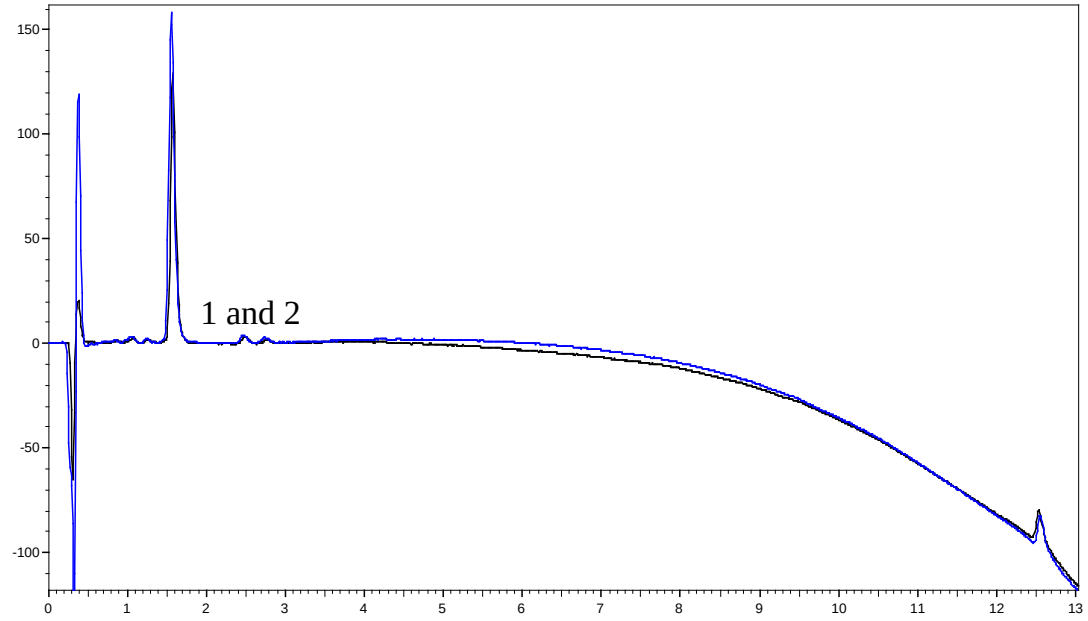

Figure 34. HPLC analysis of: (1) G1 load for preparative LC concentration and (2) G1 concentrate after preparative LC concentration.

Preparative LC concentration of 6-gingerol-depleted Pool #1 (Fraction G2).
A similar concentration process was undertaken for the 6-gingerol-depleted material from Pool 1, sub-fractions 1, 3 and 4. Figure 35 shows the composition of the 6-gingerol-depleted fraction (Pool #1, sub-fractions #1, 3 and 4 combined and water diluted) prior to concentration. Figure 36 presents the desorption profile of the 6-gingerol-depleted fractions, while Figure 37 shows the progressive profiles of the material as it is eluted from the preparative LC column.

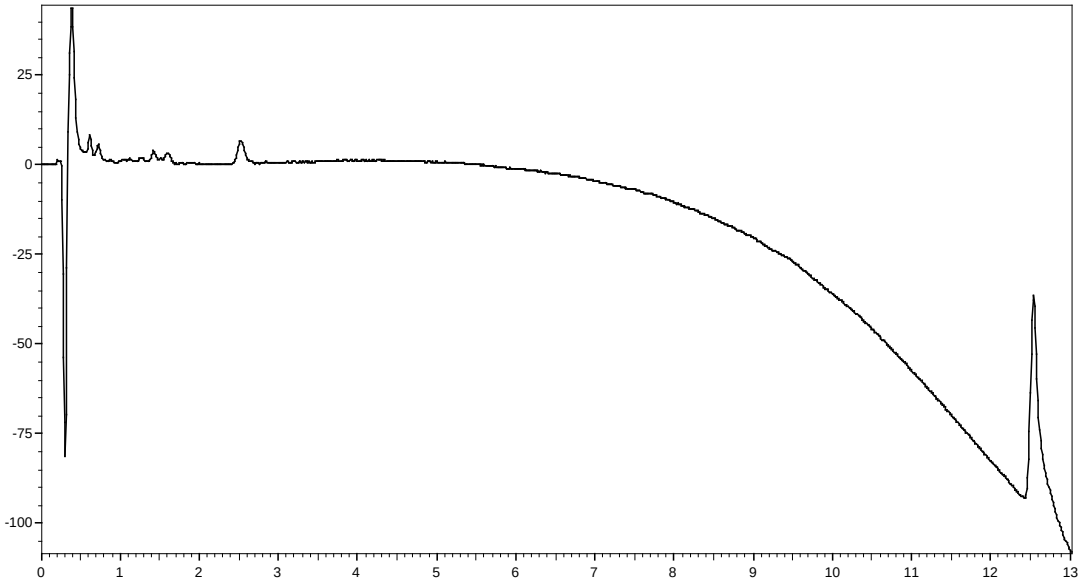

Figure 35. HPLC analysis of water-diluted Pool #1, sub-fractions #1, 3 and 4, (G2) prior to preparative LC concentration.

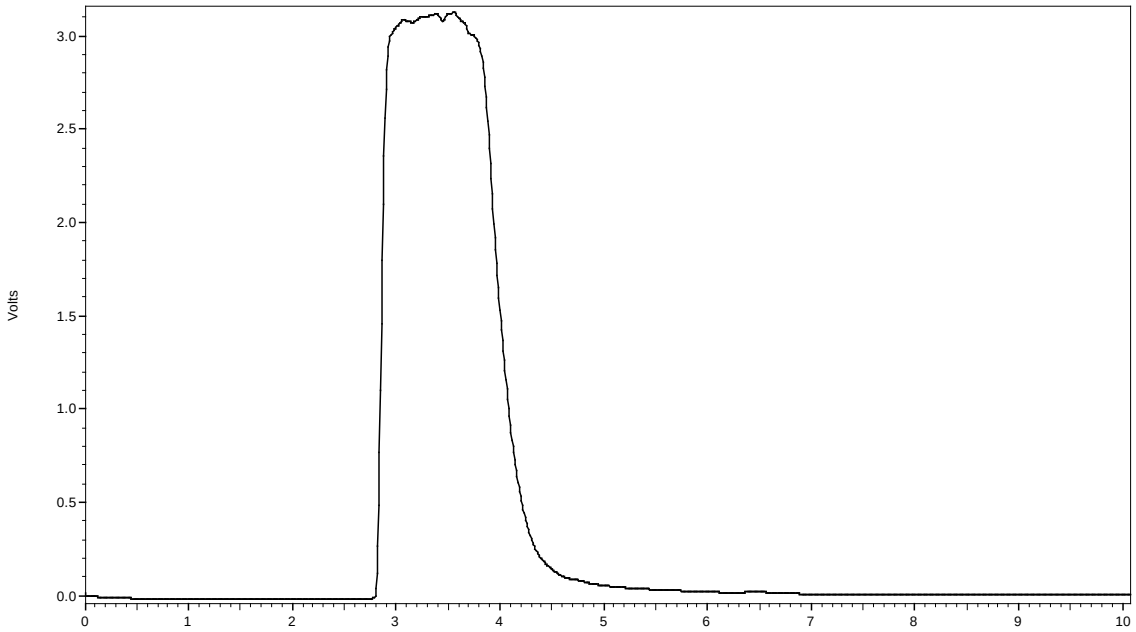

Figure 36. Desorption of G2 (6-gingerol depleted sub-fractions).

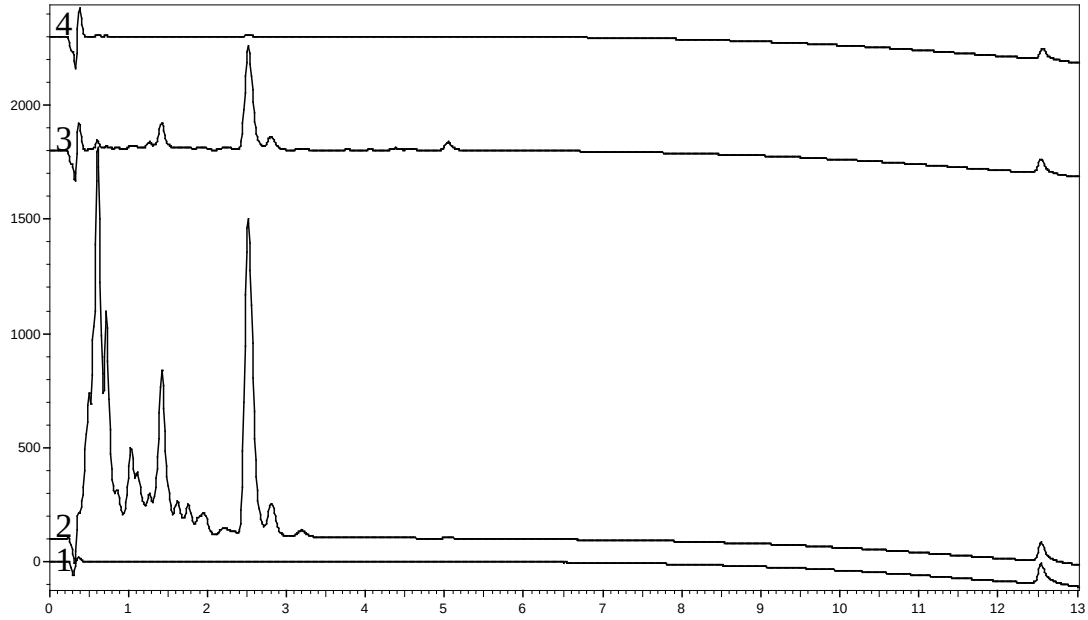

Figure 37. Desorbed fractions from the preparative concentration of the post-6-gingerol G2 Fraction: (1) 4 (2.8-3min); (2) 5 (3-4min); (3) 6 (4-5min) and (4) 7 (5-6min).

This figure shows that Fractions 4 and 7 have virtually none of the compounds of interest, while Fractions 5 and 6 have the majority of the desorbed compounds.

Preparative LC concentration of Fraction G3.
The G3 pool was diluted with water to about 25% methanol and loaded onto the preparative LC column. Figure 38 shows the composition of the G3 fraction prior to concentration. This chromatogram shows that this solution contains 8-gingerol that elutes at 3.9 minutes with 12.9% purity, and 6-shogaol which elutes at 4.4 minutes with 7% purity.

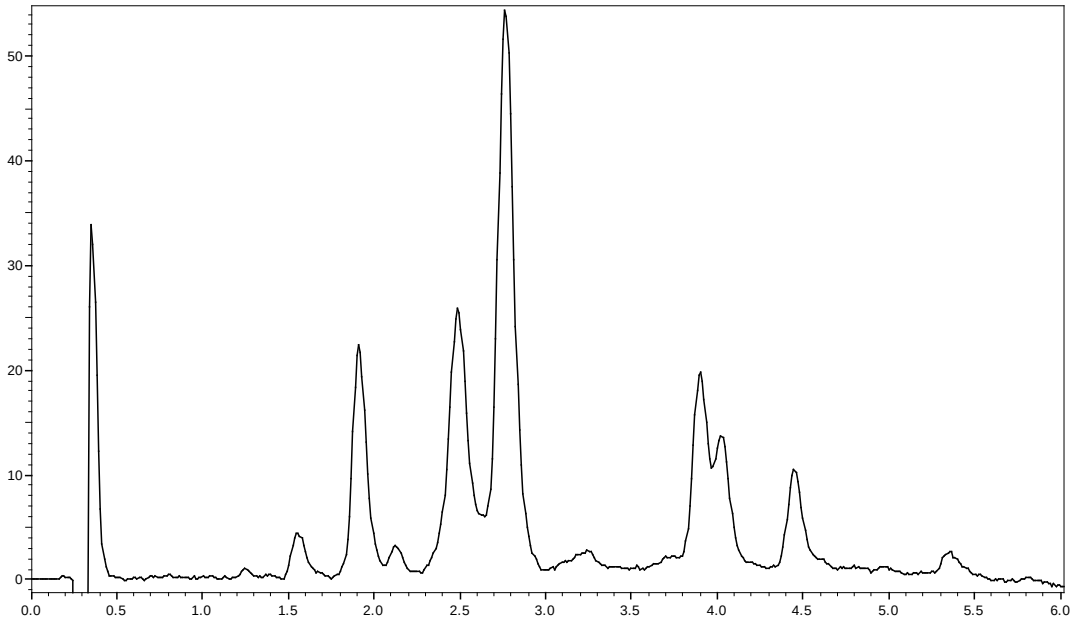

Figure 38. HPLC analysis of G3, prior to preparative LC concentration.

Figure 39 presents the desorption profile of the G3 profile, while Figure 40 shows the progressive profiles of the material as it is elutes from the preparative LC column.

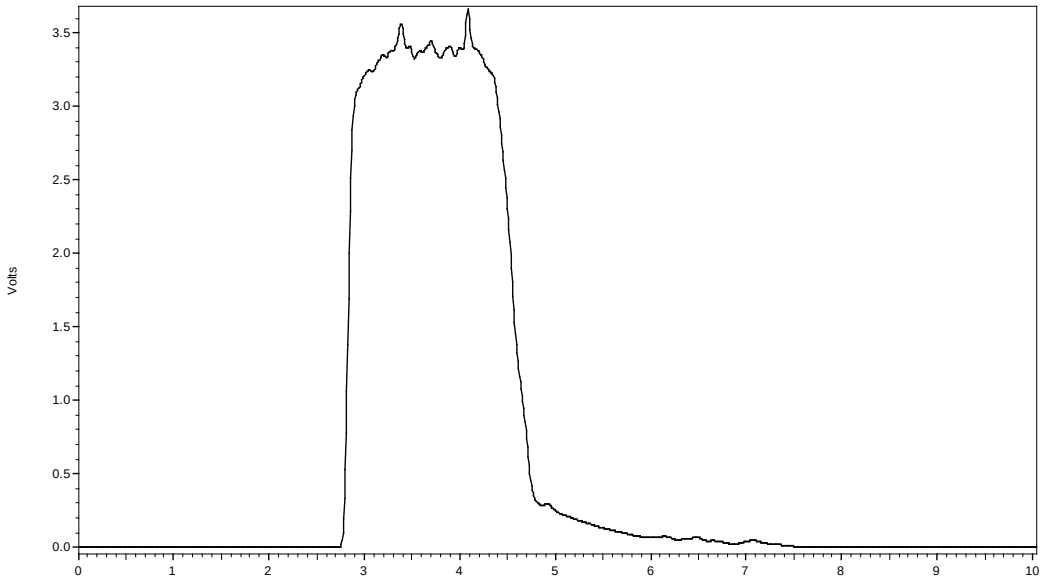

Figure 39. Preparative LC concentration of G3 by desorption of a G3-loaded preparative LC column.

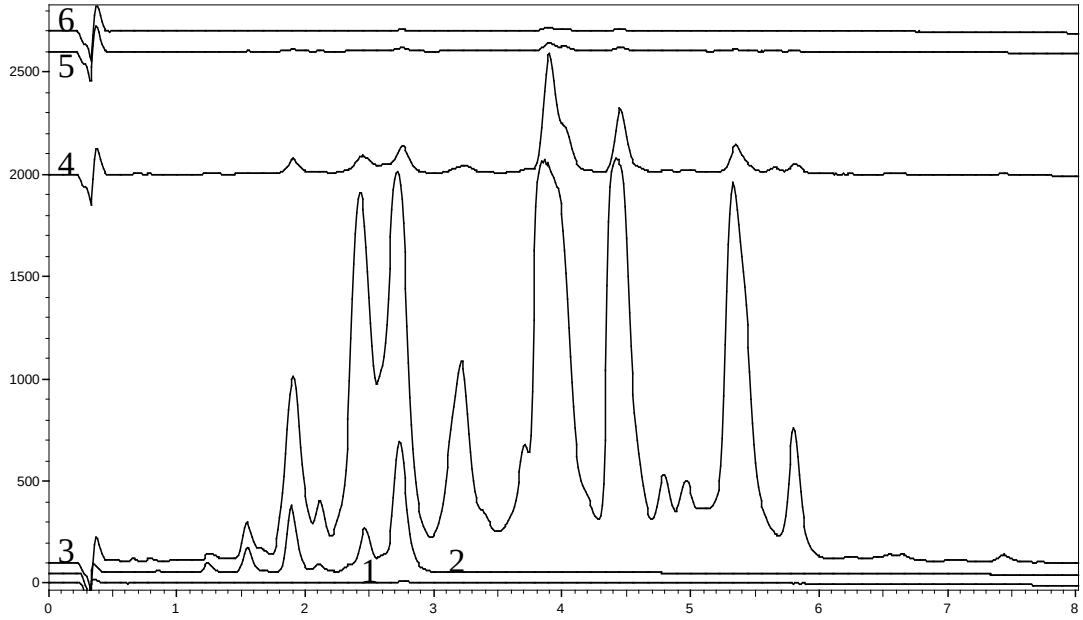

Figure 40. Desorbed fractions from the preparative concentration of G3, Fraction: (1) 3; (2) 4; (3) 5; (4) 6; (5) 7 and (6) 8.

Preparative LC concentration of Fraction G4.
Figure 41 shows the composition of the G4 fraction prior to concentration. This pool contains 10-gingerol, at about 28%, that elutes at about 6.2 minutes. Figure 42 presents the desorption profile of the G4-fraction, while Figure 43 shows the progressive profiles of the material as it is eluted from the preparative LC column.

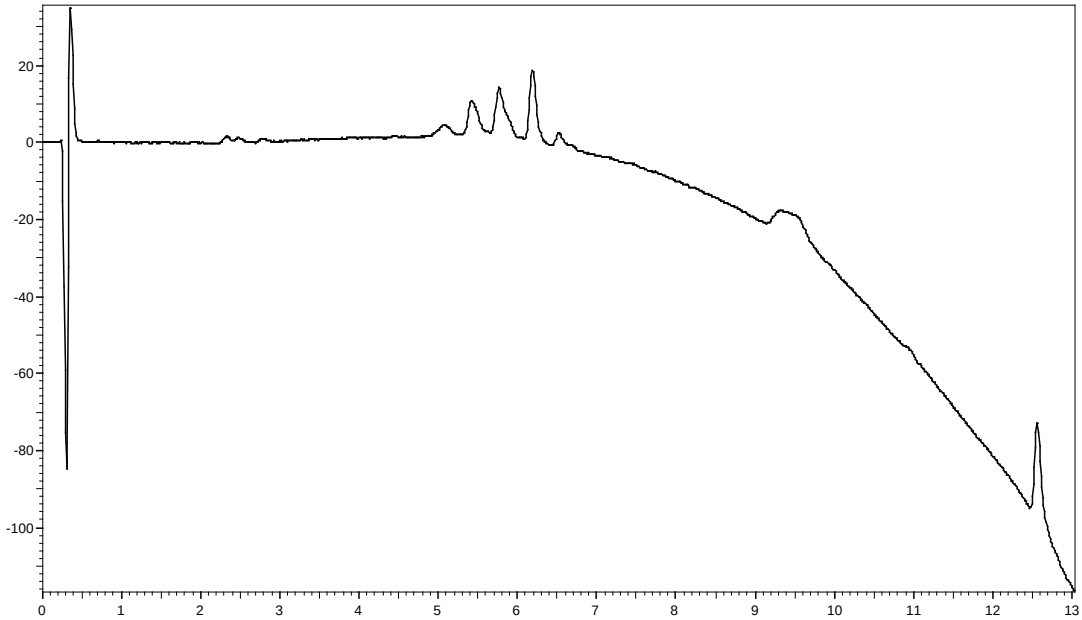

Figure 41. HPLC analysis of the G4 load solution prior to preparative concentration.

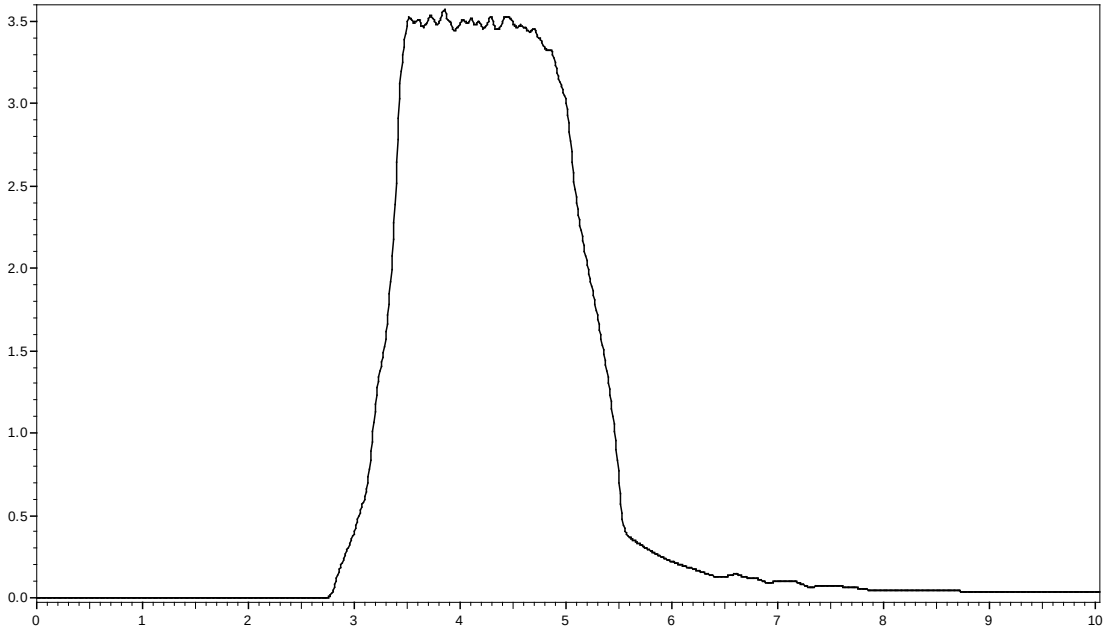

Figure 42. Preparative LC concentration of G4 by desorption of a G4-loaded preparative LC column.

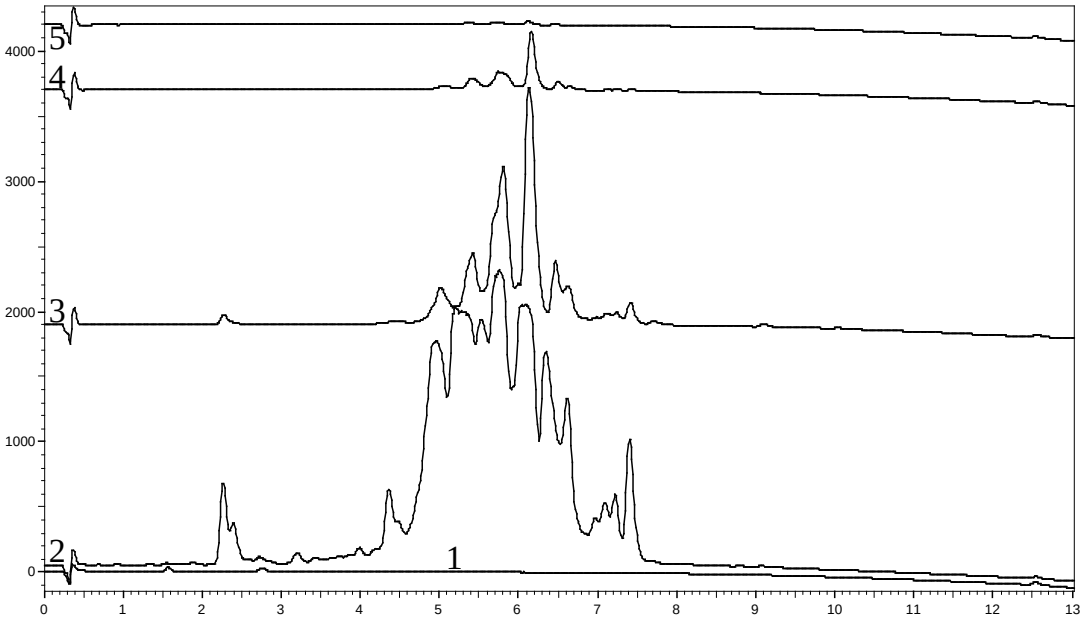

Figure 43. Desorbed fractions from the preparative concentration of G4, Fraction: (1) 4; (2) 5; (3) 6; (4) 7 and (5) 8.

Final concentration of fractions G1 to G5.
The five fractions derived from the ginger oil (SV2) which were ultimately produced by preparative LC fractionation and concentration were subjected to low temperature rotary evaporation to produce the final concentrated oil fractions. These fractions were tested for anti-inflammatory activity. Analytical chromatograms of the final five fractions which were produced in concentrated form (G1-G5) are presented in Figure 44.

The final amount of oil produced from the initial 16 gram load of ginger oil (SV2) was comprised of 5 discrete fractions (see Table 10).

	Final amount (mg)	Proportion of original
G1	705 mg	4.4%
G2	180 mg	1.1%
G3	1525 mg	9.5%
G4	865 mg	5.4%
G5	2420 mg	15.1%
Total	5695 mg	35.6%

Table 10. Final yields fractionation processes.

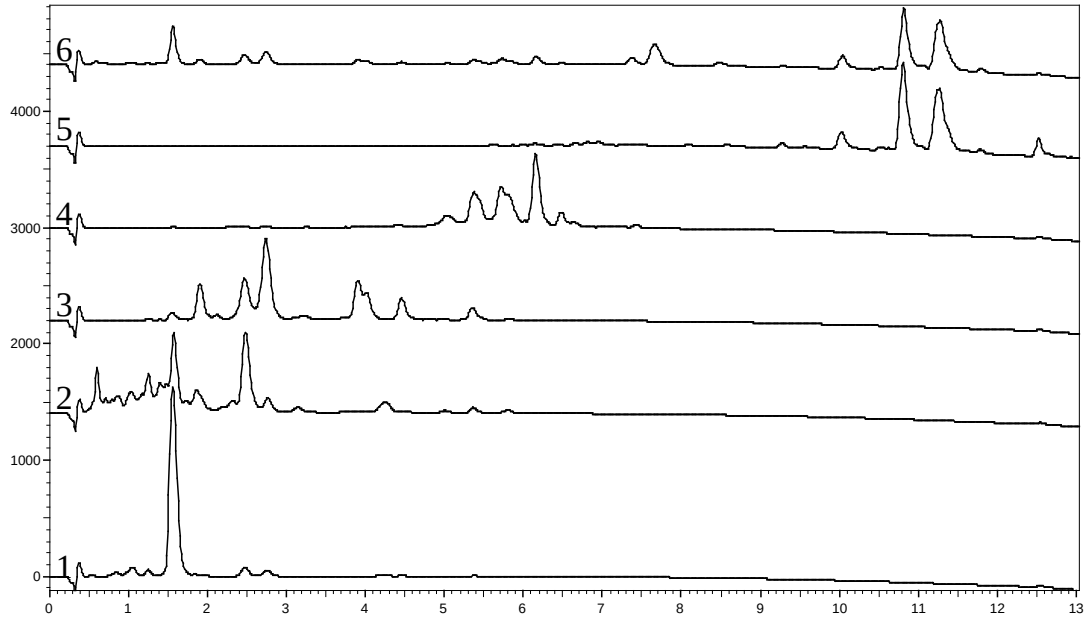

Figure 44. HPLC analyses of final concentrated SV2 fractions, produced by CFT and supplied to Michael Whitehouse for *in vivo* testing: (1) G1; (2) G2; (3) G3; (4) G4; (5) G5 and (6) SV2. Analysed using CFT (SCU mimic) method. All samples prepared at 2mg/mL in methanol.

Evaluation of the bioactivity of ginger extracts

a) In vitro anti-inflammatory activity

Ginger oils (SV1 and SV2) and the fractions produced in this study by preparative HPLC were assessed for anti-inflammatory activity *in vitro* (see Appendices 2 and 3).

In summary, the initial *in vitro* assessment of the two ginger oils (SV1 and SV2) at Southern Cross University found that both oils had significant anti-inflammatory activity in the 3T3 secreted PGE_2 assay system. Both SV1 and SV2 produced greater inhibition of PGE_2 than aspirin or ibuprofen at all but the lowest concentrations (2.5 or 7 μM). IC_{50} values of 5.34 and 5.94 μM were obtained for SV1 and SV2, respectively. The assay measured the dose-dependant inhibition of PGE_2 secretion by a mouse fibroblast cell line. The anti-inflammatory activity of both SV1 and SV2 were shown to be to equivalent to or better than the effect observed when the cells were exposed to commonly used anti-inflammatory drugs - both aspirin (2.5μM) and ibuprofen (7μM) - under the test conditions.

The SV2 ginger oil was fractionated using preparative HPLC at Southern Cross University. All of the known compounds ([6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol) in the ginger oil displayed anti-inflammatory activity in the PGE2 assay. [10]-gingerol had over twice the inhibitory activity of aspirin at 100μM concentration while [6]-gingerol had 1.3 times the inhibitory activity of aspirin. The other fractions from the preparative HPLC had varying degrees of anti-inflammatory activity. While the PGE2 inhibitory activity may be due in part to the presence of [6]- [8]- and [10]-gingerols in some of the fractions, their presence cannot account for the high level of activity detected. Gingerol/shogaol analogs and breakdown products may also be responsible for the anti-inflammatory activity

b) In vivo anti-inflammatory activity

While the resin (SV1) was orally active at 50 mg/kg, the ginger oils (SV1 and SV2) were not readily bioavailable when given orally. This approximate ED_{50} suggests that the ginger oil may be more potent than Ibuprofen (ED_{50} ca. 80 mg/kg), but less potent than Naproxen (ED_{50} ca. 25 mg/kg). By contrast Zinaxin is inactive and even appears to potentiate arthritis development. Collectively, these ginger oils do not appear to be aspirin-like, as they are not gastrotoxic.

The ginger fractions were administered in two different modes (i) a slow-release form from a sub-cutaneous depot (with olive oil) to control a relatively slow-developing chronic inflammation and (ii) a rapidly-delivered format, dispersed in 20 per cent ethanol-Tween 20, given orally to fasted animals with a relatively stable induced fever. Extract G2 (the 6-gingerol-depleted material which eluted early on preparative HPLC) and extract G3 (which contained 8- and 10-gingerol produced significant suppression of arthritis development over the dosing period (days 10-14). After dosing was ceased, there was a significant rebound in symptoms.

CONCLUSIONS

The results of this project lend support to the use of ginger and its extracts for the treatment of medical conditions such as arthritis and rheumatism.

This study investigated the anti-inflammatory activity of two ginger oils produced by supercritical fluid extraction. One was a resinous product while the other was a more typical oil. These samples were initially examined for *in vitro* anti-inflammatory activity using a cell culture model system. The assay examined the ability of the oils to inhibit the secretion of prostaglandin by cultured cells and found that both of the oils were able to produce a greater inhibition of prostaglandin than aspirin or ibuprofen at all but the lowest concentrations. One of these oils was fractionated using small-scale preparative HPLC and the fractions were assessed for their anti-inflammatory activity in the same *in vitro* model system. While all of the fractions produced some anti-inflammatory activity, it was the well-characterised ginger pungents (6-, 8-, and 10-gingerol, and 6-shogaol) which showed the most potent anti-inflammatory activity in the *in vitro* model system. 10-gingerol, for example, had over twice the inhibitory activity of aspirin at 100μM concentration.

Once the anti-inflammatory activity of the ginger oils had been confirmed using the cell culture model, the whole oils were subjected to animal testing. The animal model involved inducing experimental polyarthritis in rats and assessing the effectiveness of various products to retard the development of arthritis. When the ability of the whole oils to slow the development of arthritis was confirmed, one of the oils was subjected to fractionation on a larger scale to produce ginger oil fractions for animal testing. The results of these *in vivo* experiments support findings of the cell studies. Some of the isolated fractions significantly reduced the progression of the inflammatory response in the model system when administered subcutaneously at 75mg/Kg. In some of these studies, when the administration of the ginger oil products was ceased, the symptoms of arthritis returned. The data suggests that 8- and 10-gingerol and 6-shogaol (or a compound or compounds which co-elutes with them) has the greatest potential to control the inflammatory process in the model system used. Interestingly, contrary to literature reports, 6-gingerol (the most abundant pungent in the ginger oil) was not shown to be effective in the control of inflammation. However, 6-gingerol did have an anti-pyretic (fever-reducing) effect at higher dose. In preliminary studies, the ginger oil and most of the fractions did not present as gastroirritants (unlike many of the current medications for inflammatory disease).

The results of this project lend support to the use of ginger and its extracts for the treatment of medical conditions such as arthritis and rheumatism. Further work in the area should involve a more thorough fractionation and characterisation of the major (and minor) components of the ginger oil. A search of the literature revealed that many of the lesser components of ginger (e.g. curcurmene, geraniol, phellandrene, pinene, limonene and zingerone) have ascribed bioactivities, including anti-inflammatory potential. While this study has identified groups of compounds with (and without) anti-inflammatory activity, the isolation, characterisation and assessment of individual components may provide a greater understanding of the mode of action of this widely used traditional medicine.

TECHNOLOGY TRANSFER

An oral presentation by the project leader was made at Buderin Ginger on the 3rd of March 2003 to present the work to the industry partner.

RECOMMENDATIONS

In this investigation, 6-gingerol was isolated in high purity and several of the other major components (e.g. 8- and 10-gingerol and 6-shogaol) were concentrated in less pure fractions. Further work in the area should involve a more thorough fractionation and characterisation of the major (and minor) components of the ginger oil. The identification of compounds in the ginger oil (by LC-MS or GC-MS) would give information on the likely contribution of these compounds to the various bioactivities observed for ginger oil. While this study has identified groups of compounds with (and without) bioactivity, the isolation, characterisation and assessment of individual components may provide a greater understanding of the mode of action of this widely used traditional medicine.

The major focus of this investigation was the assessment of the anti-inflammatory potential of ginger oil in cell and animal model systems. While the greatest potential for the medicinal use of ginger is in the area of inflammation, the bioactivity of ginger oil is much broader than the inhibition of inflammatory processes, and warrants further study.

Potential for isolating important bioactive components and supplementing them back into food or nutraceutical products has considerable promise. The fractions which were identified in this study as having strong anti-inflammatory activity represent only a small proportion of the whole ginger oil. The material which elutes later on chromatography (i.e. the sesquiterpenes) comprises the majority of the ginger oil, but has only a small proportion of the anti-inflammatory activity. If these active components in ginger oil could be carefully extracted, they might represent a valuable natural therapy for one of the major diseases of modern society.

Whole ginger oil (preferably extracted without heat) should be assessed in humans to evaluate its potential use in inflammation (and other conditions). Dose-response studies of the whole oil (and/or fractions) would help to determine the amount of ginger (or ginger oil) required to produce an effect.

The results of this project lend support to the use of ginger and its extracts for the treatment of medical conditions such as arthritis and rheumatism. While there is insufficient data to make these claims on the product label, they could be used to further market ginger products.

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APPENDIX 1.

A Literature Review of the Medicinal Properties of Ginger (*Zingiber officinale* Roscoe).

A Literature Review of the Medicinal Properties of Ginger (Zingiber officinale Roscoe).

By Craig Davis and Alan Wood

CONTENTS

ABBREVIATIONS

^{o}C	degrees Celcius
cm	centimetre
g	gram
GRAS	Generally Regarded as Safe
HDL	high density lipoprotein
Kg	kilogram
LDL	low density lipoprotein
Mg	milligram
USFDA	United States Food and Drug Administration
VLDL	very low density lipoprotein

INTRODUCTION

The *Zingiberaceae* is a large family of perennial herbaceous plants. The family contains approximately 1400 species in 47 genera (Holtum, 1950) which are widely distributed throughout India and tropical Asia, extending to northern Australia. Some of the most common genera of the *Zingiberaceae* include *Aframomum*, *Alpinia*, *Amomum*, *Boesenbergia*, *Costus*, *Curcuma*, *Hedychium*, *Kaempferia* and *Zingiber* (Pancharoen *et al*., 2000).

Ginger (*Zingiber officinale* Roscoe) has thick-lobed, pale yellowish, tuberous rhizomes from which an above-ground stem rises about 1 metre. Native to southern Asia, ginger is widely cultivated in tropical, sub-tropical and temperate regions with abundant rainfall (at least 200 cm per year) (Leung, 1980).

Ginger is among the most frequently and heavily consumed spices throughout the world. The pungent, spicy rhizome has been used in the Indian sub-continent and the orient for at least 25 centuries for culinary and medicinal purposes (Denniff and Whiting, 1976). Ginger is also a common additive in a large number of compounded foods and beverages due to its flavour and pungency (Mascolo *et al.*, 1989). Zingiberaceous plants have been used in traditional or herbal medicine throughout the tropical world and members of the family have attracted continuous phytochemical interest for their biological and pharmaceutical activities (Pancharoen *et al*., 2000). Of the different constituents of ginger, some compounds seem to be responsible for the distinctive taste (Murata *et al*., 1972), while others have specific pharmacological effects (*e.g.* anti-emetic, anti-inflammatory).

THE CHEMISTRY OF GINGER

Zingiberaceous plants are capable of producing a wide range of chemical structures which include monoterpenoids, sesquiterpenoids, diarylheptanoids, arylalkanoids, phenylpropanoids, phenylbutanoids, cyclohexane oxides and flavonoids (Pancharoen *et al*., 2000).

Ginger (*Zingiber officinale*) is one of the best-known and most important spices. It is sold commercially as a fresh rhizome, as a glazed confectionary, as preserved ginger, as a pungent oleoresin extract and as a steam-distilled essential oil. Dried ginger consists of starch (40-60%), protein (10%), lipid (5-10%), fibre (2-5%), inorganic material (6%), residual moisture (10%) and essential oils (1-4%) (Van Beek *et al.*, 1987). The ginger variety, the geographic region, the maturity at harvest, the agroclimatic conditions, the method of analysis, and the market requirements can all influence the composition of different ginger varieties (Mustafa *et al*., 1993).

Ginger is valued both for its aromatic odour and its spicy, pungent taste. Ginger oil consists of a mixture of more than 200 terpenes and some non-terpenoid compounds (Lawrence, 1984a). The extraction and composition of the essential oil and oleoresins from ginger has been extensively studied. Many early chemical investigations of the major pungent principles were undertaken by Thresh (1879) and Nomura (1917a, b). The nature of the complex mixture of relatively unstable phenolic compounds was first elucidated by Connell and Sutherland (1969) who isolated a homologous series of phenolic ketones called gingerols. Purseglove (1972) noted that the characteristic organoleptic properties of ginger could be divided into two classes of constituents – the steam-distillable essential oils (which are responsible for the aroma) and the non-steam-distillable components (which are responsible for the pungency). While essential oils can be used to replace most herbs and spices in terms of aroma, oleoresins produce the subtle roundness of the natural flavour. The volatile constituents give ginger its characteristic pleasant smell, which has been variously described as sweet, warm, rooty, spicy, delicate, green, citrus-like, harsh and camphoraceous (Govindarajan, 1982a). Ginger essential oil and oleoresin products have been used as flavouring agents and additives in the food industry for many years. Ginger oil has also found limited use in the cosmetic, pharmaceutical and perfume industries.

Classes of constituents which have been isolated from ginger (*Zingiber officinale* Roscoe) include gingerols and shogaols (Wu *et al.*, 1998; Kiuchi *et al.*, 1992; Kikuzaki *et al.*, 1992), diarylheptanoids (Endo *et al.*, 1990; Kikuzaki *et al.*, 1991a;b), phenylbutenoids (Matsuda and Jitoe, 1993), flavonoids (Nakatani *et al.*, 1991; Masuda *et al.*, 1991), glycosides and gingesulphonic acids (Yoshikawa *et al.*, 1994), cassumunaquinones (Hildegard *et al.*, 1980) and sesquiterpenes (Akhila and Tewari, 1994; Terhune *et al.*, 1975).

Steam distillation of coarsely ground dry ginger produces ginger oil which is characterised by a high proportion of sesquiterpene hydrocarbons (*e.g.* zingiberol and zingiberone) (Eschenmoser and Schinz, 1950; Kami *et al.*, 1972; Connell, 1971) and a small percentage of monoterpene hydrocarbons and oxygenated compounds (Govindarajan, 1982a). These minor compounds (see Figure 1) include neral, geranial, geraniol, geranyl acetate, linalool, citronyl acetate, α-terpineol, borneol, isoborneol, bornyl acetate, zingiberone, sesquiphenandrene and curcumene

(Herout *et al.*, 1953; Nigam *et al.*, 1964; Connell, 1970; Connell and Sutherland, 1966; Kami *et al.*, 1972; Smith and Robinson, 1981; MacLeod and Pieris, 1984; Nishimura, 2001; Agarwal *et al.*, 2001). A number of constituents not previously identified in ginger oil (2,6-dimethyl hepten-1-ol, α-gurjunene, linalool oxide, isovaleraldehyde, 2-pentanone, cadinol, α- and γ-calacorene, eremophyllene, *t*-muurolol, α-himachallene, α-cubebene, pinanol, α-santalene, geranyl propionate, geranoic acid, α-farnesene, *n*-methyl pyrrole and geranic acid) have recently been reported by Onyenekwe and Hashimoto (1999).

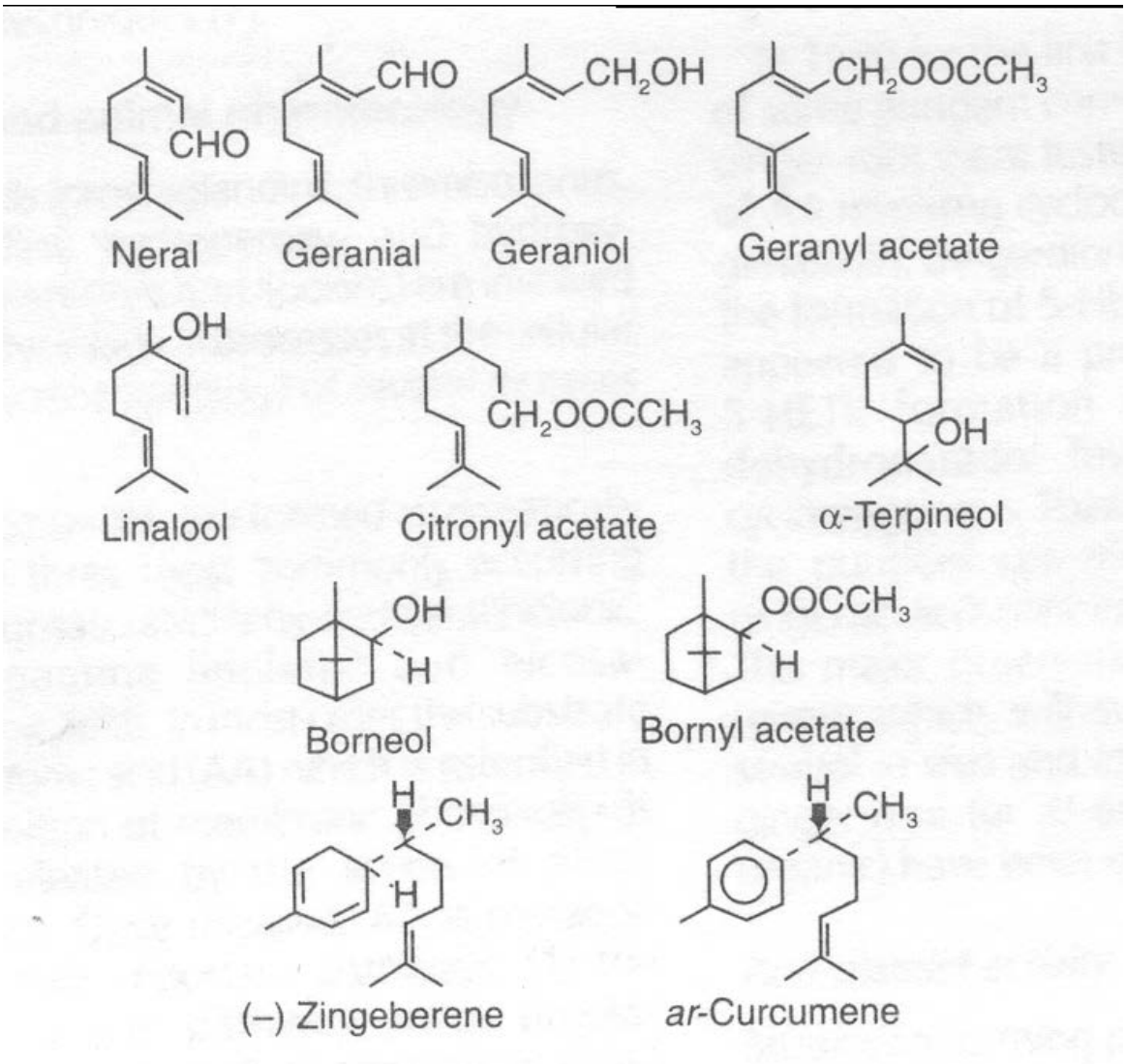


Figure 1. Compounds isolated from ginger volatile oil (from Govindarajan, 1982a)

The pungent principles of ginger have been thoroughly investigated. Ginger oleoresin is generally obtained by solvent extraction of dried unpeeled ginger, since peeled ginger loses much of its essential oil content. The major non-volatile phenolics (the pungent constituents) found in *Z. officinale* are the gingerols (1-(4'-hydroxy-3'-methoxyphenyl)-5-hydroxyalkan-3-one) and their dehydrated products, the shogaols (1-(4'-hydroxy-3'-methoxyphenyl)-5-hydroxyalk-4-en-3-one)

(Balladin *et al.*, 1998). Other non-volatile components of ginger (see Figure 2) include 3-, 4-, 5- and 12-gingerol, 6-paradol, 4-, 6-, 8- and 10-gingerdiol, 6-methyl gingerdiol, 4- and 6-gingerdiacetate, 6-methyl gingerdiacetate and hexahydrocurcumin (Connell and Sutherland, 1969; Connell and McLachlan, 1972; Harvey, 1981; Kikuzaki *et al.*, 1992; Masada *et al.*, 1973; Nomura and Iwamoto, 1928; Mustafa *et al.*, 1993).

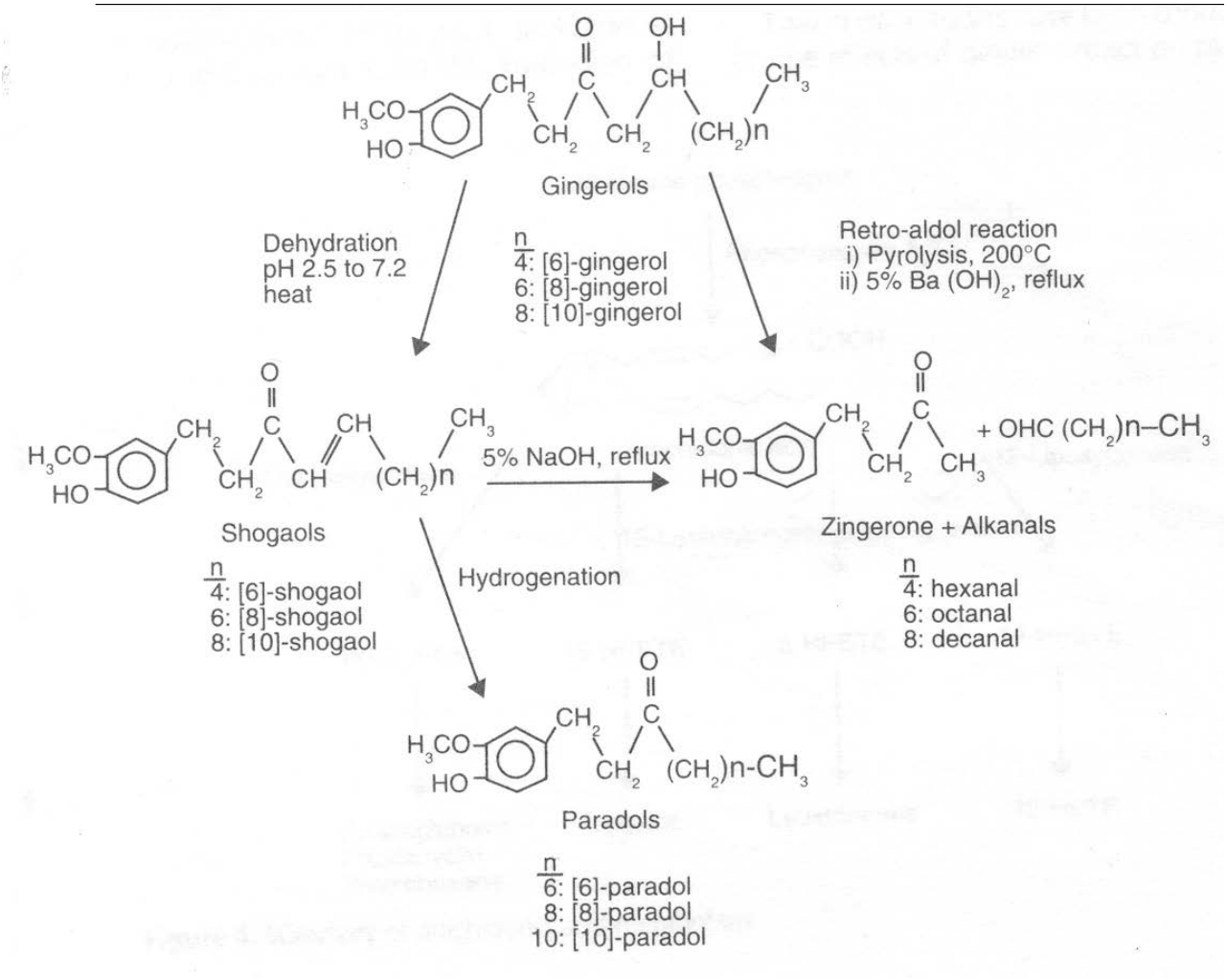
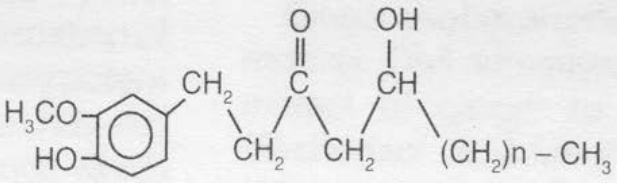


Figure 2. Minor components of ginger, related to 6-gingerol (from Govindarajan, 1982a)

The constituents responsible for the pungent taste of ginger are a homologous series of phenolic ketones known as 4-, 6-, 8-, 10- and 12-gingerol (Kikuzaki *et al.*, 1994). The shogaol series of compounds is virtually absent from fresh ginger, and is derived from the corresponding gingerols during thermal processing or long-term storage (Zhang *et al.*, 1994; Macleod and Pieris, 1984; Sakamura, 1987). Shogaols are gingerol analogues with a 4,5 double bond resulting from the elimination of the 5-hydroxy group (He *et al.*, 1998). The gingerols can also undergo retro-aldol

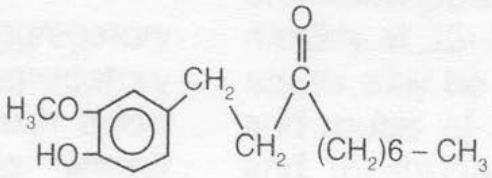
conversion to zingerone and aliphatic aldehydes, and the shogaols can be hydrogenated to paradols (Mustafa *et al*., 1993). Zingerone and shogaol are found in small amounts in fresh ginger and in large amounts in stored ginger (Govindarajan, 1982a). These various conversions (see Figure 3) contribute to the differences in composition between fresh, commercial and stored ginger oleoresins.

1.



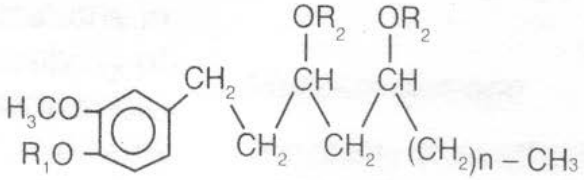
n	
1:	[3]-gingerol
2:	[4]-gingerol
3:	[5]-gingerol
10:	[12]-gingerol

2.



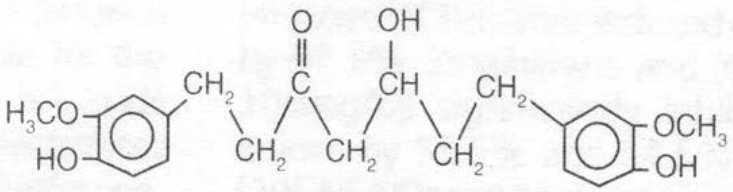
[6] – Paradol

3.



R_1	R_2	n	
H	H	2:	[4]-gingediol
H	H	4:	[6]-gingediol; dihydrogingerol
H	H	6:	[8]-gingediol
H	H	8:	[10]-gingediol
CH_3	H	4:	[6]-methyl gingediol
H	$COCH_3$	2:	[4]-gingediacetate
H	$COCH_3$	4:	[6]-gingediacetate
CH_3	$COCH_3$	4:	[6]-methyl gingediacetate

4.



Hexahydrocurcumin

Figure 3. Conversion of gingerol to shogaol, zingerone and paradol (from Govindarajan, 1982a)

Although gingerols are biologically-active components that may make a significant contribution towards the medicinal applications of ginger, they are thermally-labile. The presence of a β-hydroxy keto group in the structure allows the ready dehydration to the corresponding shogaols. The stability of 6-gingerol at temperatures ranging from 37 to 100^{o}C in aqueous solutions was investigated by Bhattarai *et al.* (2001). 6-gingerol and its major degradation product (6-shogaol) undergo reversible dehydration-hydration transformations. Degradation rates were found to be pH-dependent with greatest stability observed at pH 4.

The biosynthesis of 6-gingerol was investigated by administration of labelled precursors to whole ginger plants (Denniff and Whiting, 1976; Denniff *et al.*, 1980). The hypothetical intermediates (6-dehydrogingerdione, 6-gingerdione and 6-dehydrogingerol) were synthesised and shown to be incorporated into 6-gingerol (Macleod and Whiting, 1979).

When a glycosidically-bound fraction prepared from fresh ginger rhizomes was subjected to enzymatic hydrolysis with glycosidase, the reaction liberated geraniol, 2-heptanol, α-terpineol, nerol, linalool and citronellol, suggesting that these glucosides may be the aroma precursors of fresh ginger (Sekiwa *et al.*, 1999). Further investigations have identified two novel glucosides of 6-gingerdiol in fresh ginger (Sekiwa *et al.*, 2000). The anti-oxidative activities of these compounds were investigated using a linoleic acid model system and shown to have similar anti-oxidant activity.

GINGER BIOACTIVITY

Zingiberaceae is one of the major tropical plant families. Ginger (*Zingiber officinale*) is widely used as a dietary condiment throughout the world because of its fragrant and pungent principles. Many compounds with novel structures and a large number of biologically-active compounds have been identified from these plants. The history and use of ginger is well documented. Ginger has been used since the 4th Century BC (Tyler, 1981), is an ingredient in more than half of all traditional Chinese medicines (Awang, 1992), and has been in medical use in Europe at least since the 12th Century.

Ginger is listed in modern pharmacopoeias and repertories and has a wide range of confirmed pharmacological properties (Weidner and Sigwart, 2000). Its chemistry, pharmacology and pharmacokinetics have been investigated (Emig, 1931; Backon, 1986; Ding *et al.*, 1991). There is a long history of the use of the ginger rhizome in Chinese and Ayurvedic medicine. In western alternative medicine, ginger is used to prevent nausea and motion sickness and to treat inflammatory (rheumatic) conditions. *In vitro* evidence suggests that ginger may also have anti-cancer effects (Surh *et al.*, 1999).

Ginger is widely used in Chinese medicine. Named "Shokyo" (fresh rhizome), "Kanshokyo" (dried rhizome) or "Kankyo" (dried steamed rhizome), these crude drug preparations have different medicinal values and usages. For example, shokyo is used as an anti-emetic, expectorant and anti-tussive while kankyo is used for stomach-ache, chest pain, lower back pain and cough (Suekawa *et al.*, 1984). The Chinese and Indian cultures have traditionally used ginger for diarrhoea, nausea, cholera, bleeding (Leung, 1980), asthma, heart conditions, respiratory disorders (USP, 1998), toothache and rheumatic conditions (Awang, 1992), as a rubefactant, diuretic and stimulant to the gastrointestinal tract (Gujral *et al.*, 1978), as a stomachic, expectorant, anti-asthmatic, haemostatic and cardiotonic (Tang and Eisenbrand, 1992), for digestive disorders, dropsy, neurologia and diabetes (Chopra *et al.*, 1956), as an anti-emetic, anti-inflammatory, anti-pyretic, analgesic, as a treatment for toothache, insomnia, baldness and urinary tract infections (Wilkinson, 2000), for the treatment of cough, stomach-ache, worms, leprosy and skin diseases. Many *Zingiberaceous* plants have been demonstrated to contain compounds with a broad spectrum of biological activities including cytotoxic, molluscicidal, nematocidal, larvicidal anti-malarial, anti-helmintic, anti-hepatotoxic, anti-ulcer, anti-inflammatory, hypothermic, hypolipimenic, hypotensive and spasmolytic activities (Pancharoen *et al.*, 2000), anti-ulcer (Yoshikawa *et al.*, 1992), anti-fungal (Endo *et al.*, 1990), prostaglandin biosynthesis inhibition (Kiuchi *et al.*, 1992), anti-rhinoviral (Denyer *et al.*, 1994), insecticidal (Toshiya and Akiko, 1994), anti-oxidant (Nugroho *et al.*, 1996) hypoglycaemic, anti-microbial, hypocholesterolemic and cytotoxic (Mascolo *et al.*, 1989). Ingestion of these compounds is thought to produce hypoglycaemia and vagal stimulation (and hence cardiac depression) as well as decreases in total serum cholesterol, inhibition of prostaglandin synthesis and inhibition of platelet aggregation (Suekawa *et al.*, 1984; Mascolo *et al.*, 1989; Verma *et al.*, 1993; Ahmed and Sharma, 1997).

The composition (Hirschhorn, 1983) and toxicological properties (Mustafa *et al.*, 1993; Pancho *et al.*, 1989; Perry and Metzger, 1980) of the dried rhizome of *Zingiber officinale* and its extracts may vary greatly. Ginger rhizomes and various

extracts have been described in the literature to be safe and non-toxic (Awang, 1982). The United States Food and Drug Administration (USFDA) has classified the dried rhizome and *Zingiber officinale* extract as “Generally Regarded as Safe” (GRAS) (Anon., 1998).

The dried rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae*), is one of the best known Chinese crude drugs, and it has been investigated extensively in search of its bioactive principles (Tanabe *et al.*, 1991). The constituents of ginger thought to be responsible for these actions are the gingerols (and gingerol analogues), shogaols and several sesquiterpenes (*e.g.* zingiberol and zingiberenol). Gingerols are the constituents responsible for the hot mouthfeel of ginger, including the pungency of the oleoresins (Charles *et al.*, 2000).

In particular, the pungent constituents, which are the principal ingredients of ginger, have been isolated and characterised (Endo *et al.*, 1990, Kikuzaki *et al.*, 1991a, Kikuzaki *et al.*, 1991b). Several bioactive compounds have been identified with anti-cathartic, anti-serotonergic and gastrointestinal motility enhancing effect (Yamahara *et al.*, 1990; Huang *et al.*, 1990; Huang *et al.*, 1991).

The properties of ginger (*Zingiber officinale*) are apparently unknown to 80% of scientists, although used by almost 40% of the world's population as a condiment in food and a remedy for several diseases (Mustafa *et al.*, 1993).

INFLAMMATION

As the average life span of individuals in the industrialised nations increases, the prevalence of chronic diseases such as arthritis has become greater. In the United States alone, the total annual cost attributed to musculoskeletal diseases has been estimated to be $20 billion per year (Kelsey *et al.*, 1978). Arthritis has been called 'the nation's primary crippler' and affects more than one in every seven Americans with one in every three families being touched by the disease. Arthritis comprises more than 100 afflictions which range from rheumatoid arthritis and osteoarthritis (which usually affects and cripples the elderly), to tendinitis (which mostly affects athletes). Patients with chronic, painful diseases often seek alternative therapy (Visser *et al.*, 1992). Interestingly, 94% of arthritis patients try one or more unconventional remedies to treat their affliction during the course of the disease (Brown *et al.*, 1980; Wasner *et al.*, 1980).

Ginger is a pungent, spicy rhizome of the genus *Zingiber*, and is commonly used in cookery and medicine in the Indian subcontinent and the orient. It also has widespread traditional use in conditions associated with pain and inflammation (Atal *et al.*, 1984; Wagner and Hikano, 1985). The effects of prostaglandins in many pathological conditions parallels the anecdotal folklore claims of ginger extracts. While the exact mechanism of action in triggering the biological effects are not fully established, most evidence supports their action on one or more steps of arachidonic acid metabolism.

The active constituents in ginger have also been shown to inhibit the enzymes of arachidonic acid metabolism (Flynn *et al.*, 1986; Kiuchi *et al.*, 1982). The major rate-limiting factor in the generation of prostaglandins and other metabolites of arachidonic acid is the availability of free arachidonic acid. These eicosanoids are generated in response to various physiological, pharmacological or pathological stimuli. Arachidonic acid is released from membrane phospholipids by the action of phospholipases. Arachidonic acid is then converted to prostaglandin endoperoxides by the action of the enzyme, cyclooxygenase. Prostaglandin endoperoxides are subsequently converted into Thromboxane A_2 by the action of another enzyme, thromboxane synthetase, or into prostaglandins (see Figure 4). *In vitro* model systems often use platelet aggregation to assess the effect of various compounds on the prostaglandin pathway. Ginger can increase the production of platelet lipoxygenase products (Rattan, 1988).

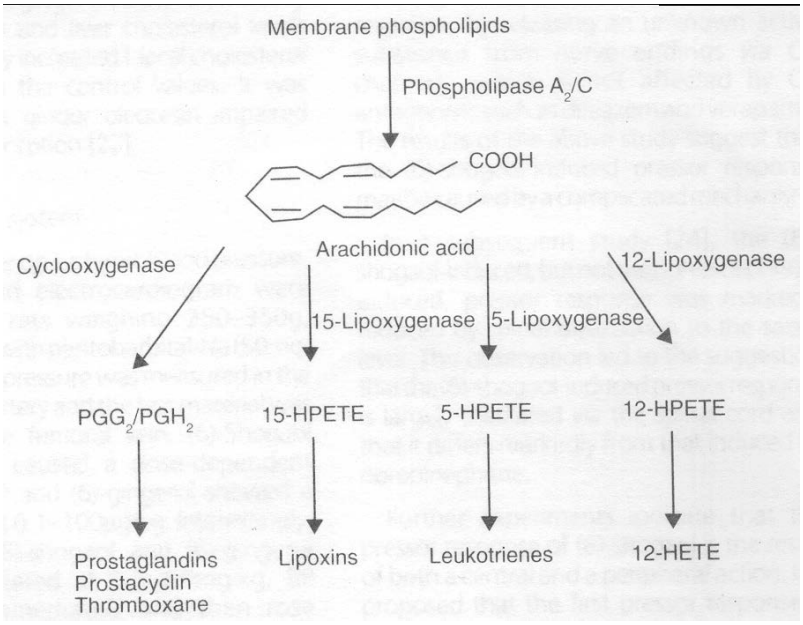


Figure 4. Summary of arachidonic acid metabolism (from Mustafa *et al.*, 1993)
Mechanical, chemical or immunological challenge stimulates cyclooxygenase activity. Living tissue responds to irritation and injury by way of inflammation. Oxygenation of arachidonic acid is increased in inflamed tissues, and prostaglandins and leukotriene levels are elevated (Zurier, 1985). Increased levels of prostaglandins are always observed in inflamed tissues. Prostaglandin E_2 is the predominant product, although Prostaglandin $F_{2\alpha}$, Prostaglandin D_2, Thromboxane B_2 and 6-keto-Prostaglandin $F_{1\alpha}$ have also been detected in inflamed tissues.

Present-day therapy for osteoarthritis is directed at symptoms, since there is no established disease-modifying therapy. Treatment programs involve a combination of non-pharmacological and pharmacological measures, utilising a combination of analgesic, anti-inflammatory and intra-articular programs (Altman and Marcussen, 2001). More than 200 drugs (ranging from non-steroidal anti-inflammatory drugs, corticosteroids, gold salts, disease modifying anti-rheumatic drugs, methotrexate, cyclosporine) have been tested for their anti-inflammatory potential. All of the synthetic drugs are known to produce mild to serious side-effects. For example, the prolonged use of acetaminophen (one of the most widely advocated pain killer in osteoarthritis) can result in serious gastrointestinal damage.
Corticosteroids and non-steroidal anti-inflammatory drugs (which inhibit the cyclooxygenase) are used to treat such disorders. Both types of drugs produce adverse side-effects on prolonged use (Zurier, 1985).

Currently, ginger is one of the most popular herbal medications for rheumatic diseases. While the beneficial effects of ginger have been widely reported (Srivastava and Mustafa, 1989a), only a few controlled studies have been performed. Ginger has been reported to have a suppressive effect in arthritic rats (Weidner, 1997) and chemical substances found in ginger have been shown to have an anti-inflammatory potential (Kiuchi *et al.*, 1992). Ginger is described in Ayurvedic and Tibb systems of medicine to be useful in inflammation and rheumatism (Srivastava and Mustafa, 1992). Ginger has been postulated to interfere with the

cyclooxygenase and lipoxygenase enzymes of the prostaglandin and leukotriene biosynthetic pathways (Srivastava and Mustafa, 1989b).

Mascolo *et al.* (1989) found potential anti-inflammatory, antipyretic and hypoglycaemic properties in extracts from *Zingiber officinale*. The severity of pedal oedema was reduced (to be comparable with acetylsalicilic acid) in an animal model system. In acute inflammation, prostaglandins (and other arachidonate metabolites) interact with substrates (such as histamine and bradykinin) to augment vascular permeability (Williams *et al.*, 1983) and produce oedema (Moncada *et al.*, 1973). The theory which best explains the anti-inflammatory activity of aspirin-like drugs is based on the discovery that the drugs inhibit prostaglandin biosynthesis through their interaction with prostaglandin synthetase (Vane, 1971).

Alternative medicine is used extensively by patients with, for example, chronic osteoarthritic pain. The effect of an extract of ginger (one of the most popular herbal medications) has been tested in a controlled setting (Bliddal *et al.*, 2000). Ginger extracts were compared with the most relevant clinical alternative (Ibuprofen) in a randomised, placebo-controlled, cross-over study of osteoarthritis.

Oral consumption of dried, powdered ginger for between 3 months and 2.5 years by patients with rheumatoid arthritis, osteoarthritis or muscular discomfort has been shown to result in relief of pain and swelling (Srivastava and Mustafa, 1989a; Srivastava and Mustafa, 1992). The investigators of these studies suggested that the ameliorative effects of ginger could be related to the inhibition of prostaglandin and leukotriene biosynthesis. In other studies, ginger oil (obtained by steam distillation of dried ginger) has been shown to be an inhibitor of both cyclooxygenase and lipoxygenase activities (Kiuchi *et al.*, 1982; Flynn *et al.*, 1986). The oral administration of ginger oil to rats for 26 days has been shown to significantly reduce paw and joint swelling (Sharma *et al.*, 1994), suggesting that ginger oil possesses anti-inflammatory properties. Pungent components of ginger inhibit cyclooxygenase and lipoxygenase activity in the arachidonic acid metabolic pathway and thereby probably reduce inflammation and relieve pain in rheumatic disorders and migraine headache (Mustafa and Srivastava, 1990). Consumption of ginger has also been shown to reduce plasma thromboxane B_2 levels in humans.

Ginger contains a number of compounds (*e.g.* 6-gingerol) which have been shown to be able to inhibit prostaglandin synthetase *in vitro* (Kiuchi *et al.*, 1982). Four other components (6-dehydrogingerdione, 10-dehydrogingerdione, 6-gingerdione and 10-gingerdione) were found to be more potent than indomethacin as prostaglandin inhibitors. A ginger extract has also been shown to inhibit the production of thromboxane and prostaglandin in a dose-dependent manner (Srivastava, 1984a, Srivastava, 1984b). Hydroxy-methoxy-phenyl compounds are dual inhibitors of cyclooxygenase and 5-lipoxygenase (Bliddal *et al.*, 2000). In a carrageenan-induced paw swelling assay (Mascolo *et al.*, 1989) and several enzyme assays (Kiuchi *et al.*, 1992), extracts of *Zingiber officinale* were shown to potently inhibit the inflammatory processes (comparable to non-steroidal anti-inflammatory drugs).

Seventeen pungent oleoresin principles of ginger (*Zingiber officinale*, Roscoe) and synthetic analogues were evaluated for inhibition of cyclooxygenase-2 enzyme activity in the intact cell. These compounds exhibited a concentration- and

structure-dependent inhibition of the enzyme. *In vitro* cyclooxygenase enzyme activity was strongly inhibited by 8-paradol and 8-shogaol, and by two synthetic ginger analogues. Analysis of these phenolic compounds revealed three important structural features that affect cyclooxygenase inhibition: (i) the lipophilicity of the alkyl side-chain, (ii) the substitution of hydroxy and carbonyl groups on the side-chain, and (iii) the substitution of hydroxy and methoxy groups on the aromatic moiety (Tjendraputra *et al.*, 2001).

6-gingerol exhibits diverse pharmacological activities, including inhibition of cyclooxygenase and lipoxygenase activities (Kiuchi *et al.*, 1982; Kiuchi *et al.*, 1992; Flynn *et al.*, 1986). 6-gingerol and four gingerdione derivatives (6- and 10-dehydrogingerdione and 6- and 10-gingerdione) were isolated from ginger rhizome and shown to be potent inhibitors of prostaglandin biosynthesis. Some were more potent inhibitors than indomethacin which is known to be one of the strongest inhibitors (Kiuchi *et al.*, 1982).

Ginger is well-known as a crude drug with several pharmacological functions. An aqueous extract of ginger has been shown to inhibit the biosynthesis of thromboxane and prostaglandin (Srivastava *et al.*, 1984a, Srivastava *et al.*, 1984b; Srivastava, 1986). The inhibitory principles have been reported to be gingerol analogues (Kiuchi *et al.*, 1982). Inhibitors of platelet aggregation have been reported to require *o*-methoxyphenol-components (*e.g.* as found in the gingerol analogues) for their activity (Kawakishi *et al.*, 1994).

Besides producing cyclooxygenase products (prostanoids), it has been found that human rheumatoid and osteoarthritic synovium can generate 5-lipoxygenase products. Leukotrienes C_4, D_4 and E_4 were identified in the synovial membranes from rheumatoid and osteoarthritic patients (Kopicky *et al.*, 1985). The level of Leukotriene B_4 in the fluids of rheumatoid arthritis patients is slightly in excess of that in the fluids from osteoarthritis patients. Interestingly, the levels of Leukotriene B_4 have been found to be increased six-fold in the synovial fluids from patients with gout compared to the Leukotriene B_4 levels in corresponding fluids obtained from patients with rheumatoid arthritis and osteoarthritis (Rae *et al.*, 1982).

Monohydroxy lipoxygenase products show weak chemokinetic and chemotactic properties both *in vitro* and *in vivo* in human and rabbit polymorphonuclear leucocytes (Palmer *et al.*, 1980). The dihydroxy product, Leukotriene B_4, on the contrary, shows powerful effects on the polymorphonuclear leucocytes. It produced degranulation of polymorphonuclear leucocytes of several species *in vitro* and accumulation of these cells *in vivo* (Bray, 1983; Higgs *et al.*, 1981). Klickstein *et al.* (1980) showed that synovial fluid of patients with rheumatoid arthritis and spondyloarthritis contained higher levels of Leukotriene B_4 and 5-HETE (from which leukotrienes are derived).

During the last 45 years many chemical investigations have been carried out on the constituents of the essential oil (Van Beek *et al.*, 1987; Chen and Ho, 1988). All together more than 200 different volatile compounds have been identified in essential oil wherein the pharmacological activity is confined (Lawrence, 1984a). The essential oil contains mixture of various terpenes as well as some other non-terpenoid compounds. It is likely that crude ginger powder intake brings about

amelioration of symptoms by, for example, interfering with the production and release of products of lipid membranes (eicosanoids, reactive oxygen), peptides and proteins (lysosomal enzymes, growth factors, lymphokines, bradykinin), and amino acids (histamine, serotonin). Ginger has been proposed to inhibit both the cyclooxygenase (Srivastava, 1986; Kiuchi *et al.*, 1982) and lipoxygenase products (Flynn *et al.*, 1986; Suckawa *et al.*, 1986). Ginger may be a dual inhibitor of eicosanoid synthesis and has also been reported to contain anti-histaminic and anti-oxidant factors (Duke and Ayensu, 1985).

Non-steroidal anti-inflammatory drugs have three major actions, all of which are related to inhibition of cyclooxygenase resulting in decreased formation of prostanoids. Firstly, an anti-inflammatory action, achieved by reduced production of vasodilator prostaglandins (Prostaglandin E_2, Prostaglandin I_2), means less vasodilation and indirectly less oedema. Secondly, an analgesic effect is achieved by reduced prostaglandin production (less sensitisation of nociceptic nerve endings to the inflammatory mediators bradykinin and 5-hydroxytryptamine). Thirdly, an anti-pyretic effect is probably due to a decrease in the mediator Prostaglandin E_2 generated in response to inflammatory pyrogens, such as Interleukin-1. Since ginger inhibits prostanoid synthesis and also products of 5-lipoxygenase, its ameliorative effects in arthritis and muscular discomforts could be related to reduced formation of prostanoids and leukotrienes. Hence, a decrease in the carrageenan-induced oedema formation in the rat's paw after 3 hours of ginger extract administration has been demonstrated and the potency of the extract in the acute inflammation test appears to be comparable to that exhibited by acetyl salicylic acid reported in the same study (Mascolo *et al.*, 1989).

No adverse reactions were observed by patients who have consumed amounts of ginger normally available through food (between 1 and 2 grams per day) for periods ranging from 3 months to 2.5 years. Most patients observed relief of symptoms within 1-3 months. Compelled by the nature of the disease, most of them are continuing with ginger. Some of the patients who stopped taking their daily doses suffered a return of symptoms with weeks to 2 months. Relief was again achieved with the resumption of ginger intake.

PLATELET ACTIVATION INHIBITORS

Gingerols represent a potential new class of platelet activation inhibitors The ability of a series of synthetic gingerols and related phenyl-alkanol analogues to inhibit human platelet activation was compared to aspirin. Arachidonic acid-induced platelet serotonin release and *in vitro* aggregation were used as markers of platelet activation inhibition. Gingerols and related analogues inhibited the arachidonic acid-induced platelet release reaction in a dose range similar to aspirin. The mechanism underlying inhibition of this reaction and cell aggregation may be via an effect on cyclooxygenase activity in platelets because representative gingerols and related analogues potently inhibited cyclooxygenase activity in rat basophilic leukemia cells. These results may provide a basis for the design of more potent synthetic gingerol analogues, with potencies similar to aspirin, as platelet activation inhibitors with potential value in cardiovascular disease (Koo *et al*., 2001).

Analgesia

6-shogaol (1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one) is one of the pungent components of the steamed (but not raw) rhizome of ginger (Connell and Sutherland, 1969). Both 6-shogaol and the chemically-related capsaicin are pungent components which produce similar *in vivo* and *in vitro* effects (Onogi *et al.*, 1992). 6-paradol (1-(4'-hydroxy-3'-methoxyphenyl)-3-decanone), which can be obtained from gingerol by successive dehydration and hydrogenation (Lee and Surh, 1998), is known to have analgesic activity (Lee, 1991).

Anti-Nausea

Another prevalent use of *Zingiber officinale* is as an anti-emetic (Mustafa *et al.*, 1993; Kawai *et al.*, 1994). The mechanism underlying ginger's purported anti-emetic activity is unknown, but there is speculation of a direct effect on the gastrointestinal tract. A mechanism involving the central nervous system cannot be ruled out, since several ginger components antagonise serotonin type 3 receptors, but this has not been clearly demonstrated (Micklefield *et al.*, 1999; Lumb, 1993a; Srivastava, 1984a).

6-gingerol and 6-shogaol are implicated in ginger's anti-nauseant properties as they are found to suppress gastric contraction and increase both gastrointestinal motility and spontaneous peristaltic activity (Suekawa *et al.*, 1984). This effect then reduces the gastrointestinal feedback to central chemoreceptors, thus reducing the feeling of nausea. Several clinical trials have established the efficacy of *Zingiber officinale* in vertigo (Grontved and Hentzer, 1986), in pregnant women (hyperemesis gravidarum) (Fisher-Rasmussen *et al.*, 1990; Murphy, 1998), in post-operative nausea (Phillips *et al.*, 1993b; Visalyaputra *et al.*, 1998; Bone *et al.*, 1990; Arfeen *et al.*, 1995), and in nausea associated with chemotherapy (Sharma *et al.*, 1997).

Several pharmaceutical and natural health companies (*e.g.* Blackmores and Bullivants) have produced products with powdered or dried ginger rhizome for use in arthritis and as anti-nauseants for use in the treatment of motion or morning sickness. The recommended dose for alleviation of morning sickness is 500 to 1000 mg/day (Fischer-Rasmussen *et al.*, 1990; Murphy, 1998).

Ginger is widely used as a condiment and therapeutic agent in many countries (Stuart, 1979). In Saudi Arabian traditional medicine, ginger is used as an anti-emetic, stomachic, carminative (Ageel *et al.*, 1987). In Chinese medicine, it is employed in colic and in atonic dyspepsia and is used as a stimulant (Keys, 1985). Mowrey and Clayson (1982) found that ginger is highly effective in motion sickness and significantly reduces gastrointestinal distress in human subjects.

ANTI-OXIDANT ACTIVITY

Spices and vegetables posses anti-oxidant activity that can be applied for preservation of lipids and reduce lipid peroxidation in biological systems. In addition to imparting flavour to the food, dietary spices possess potential health benefits (*i.e.* anti-oxidant activity) by inhibiting the lipid peroxidation (Shobana and Naidu, 2000). Ginger is consumed world-wide as a spice and flavouring agent. Limited *in vitro* studies have shown that water and organic solvent extracts of ginger possess anti-oxidant properties (Kiuchi *et al.*, 1992; Krishnakantha and Lokesh, 1993; Reddy and Lokesh, 1992; Jitoe *et al.*, 1992). Zingerone, a compound isolated from ginger, has been shown to scavenge superoxide anions (Krishnakantha and Lokesh, 1993), and to inhibit lipid peroxidation (Reddy and Lokesh, 1992). The anti-oxidant activity of ginger extract on enzymatic lipid peroxidation was dose-dependent (inhibition of fatty acid oxidation in the presence of soybean lipoxygenase). The anti-oxidant activity was retained even after boiling for 30 minutes at 100^{o}C indicating that the active constituents were resistant to thermal denaturation.

Dichloromethane and methanol extracts of *Zingiber* genera were screened for anti-oxidant activity. All the extracts showed strong anti-oxidant activity comparable with or higher that of α-tocopherol (Habsah *et al.*, 2000).

Anti-ulcer and digestive activity

An anti-ulcer principle (6-gingesulfonic acid) and three new monoacyldigalactosylglycerols (gingerglycolipids A, B and C) were isolated from the water-soluble fraction of the methanolic extract of dried ginger rhizome (Yoshikawa *et al.*, 1992). 6-Gingesulfonic acid had a greater anti-ulcer activity than 6-gingerol and 6-shogaol. Five cyclic diarylheptanoids were also isolated from the dichloromethane extract of ginger rhizome and their structures were elucidated by spectroscopic and chemical methods (Kikuzaki and Nakatani, 1996).

The pungent principles (6- and 10-gingerol) present in the acetone extract of ginger were found to increase bile secretion and were also mainly responsible for the cholagogic effect of ginger (Yamahara *et al.*, 1985). Ginger and ginger extracts, including zingiberene (the major constituent of the acetone extract of ginger) and 6-gingerol, significantly inhibited HCl/ethanol-induced gastric lesions in rats (Kasahara *et al.*, 1983; Yamahara *et al.*, 1988; Sertie *et al.*, 1992). This suggests that zingiberene and 6-gingerol are the important constituents in the stomachic medications containing ginger (Yamahara *et al.*, 1988). The pungent principles of ginger (6-shogaol and 6-, 8- and 10-gingerol) were reported to enhance gastrointestinal motility (Yamahara *et al.*, 1990). A number of sesquiterpenes (β-sesquiphellandrene, β-bisabolene, *ar*-curcumene and α-zingiberene), 6-gingerol and 6-shogaol have been identified in Taiwanese ginger as having anti-ulcer effects in rats (Yamahara *et al.*, 1988).

An increase in bile secretion and an anti-emetic action by an acetone extract of ginger and 6-shogaol given orally to mice has been shown to accelerate gastrointestinal movement. 6-shogaol given intravenously inhibits such movement. Galanolactone antagonised 5-hydroxytryptamine receptors which might explain the anti-emetic and gastrointestinal movement enhancing effects. Zingiberone and 6-shogaol were also reported to protect against gastric mucosal lesions (Mustafa *et al*., 1993).

Zingiber officinale is a potent inhibitor of thromboxane synthetase (Backon, 1986), and the inhibition of thromboxane is important in the prevention of peptic ulcer (Gilbert *et al.*, 1983; Bennett, 1983).

Some of the chemical compounds from ginger may prove to have anti-inflammatory, anti-emetic, cardiotonic and gastroprotective properties in humans without side effects (Mustafa *et al*., 1993).

GASTROINTESTINAL ACTIVITY

The effect of a ginger rhizome extract was studied on fasting and post-prandial gastroduodenal motility with stationary manometry in 12 healthy volunteers. Oral ginger improves gastroduodenal motility in the fasting state and after a standard test meal (Micklefield *et al.*, 1999).

ANTI-CANCER ACTIVITY

Recently, considerable attention has been focused on identifying naturally-occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. A wide array of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anti-carcinogenic and anti-mutagenic activities. The majority of these naturally occurring phenolics retain anti-oxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of hot chili pepper, protects against experimentally-induced mutagenesis and tumourigenesis. It also induces apoptosis in various immortalised or malignant cell lines. Curcumin, a yellow ingredient from turmeric (*Curcuma longa* L., *Zingiberaceae*), has been extensively investigated for its cancer chemopreventive potential. Yakuchinone A [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanone] and yakuchinone B [1-(4'-hydroxy-3'-methoxyphenyl)-7phenylhept-1-en-3-one] present in *Alpinia oxyphylla* Miquel (*Zingiberaceae*) have inhibitory effects on phorbol ester-induced inflammation and skin carcinogenesis in mice, and oxidative stress *in vitro*. These diarylheptanoids suppress phorbol ester-induced activation of ornithine decarboxylase and production of tumour necrosis factor-α or interleukin-1α. They also nullified the phorbol ester-stimulated induction of activator protein 1 in cultured human promyelocytic leukemia (HL-60) cells. In addition, both yakuchinone A and B induced apoptotic death in HL-60 cells. Ginger (*Zingiber officinale* Roscoe, *Zingiberaceae*) contains pungent ingredients (e.g. 6-gingerol and 6-paradol) which also have anti-tumour promotional and anti-proliferative effects. Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), a phytoalexin found in grapes and other dietary and medicinal plants, and (-)-epigallocatechin gallate, a major anti-oxidative green tea polyphenol, exert striking inhibitory effects on diverse cellular events associated with multi-stage carcinogenesis. In addition, these compounds have the ability to suppress proliferation of human cancer cells via induction of apoptosis (Surh, 1999).

Zingiberaceae rhizomes commonly used in the Malaysian traditional medicine were screened for anti-tumour promoter activity using the short-term assay of inhibition of 12-O-tetradecanoyl phorbol-13-acetate-induced Epstein-Barr virus early antigen in Raji cells. The rhizome extracts from *Z. officinale* exhibited viral activation inhibitory activity but had no cytotoxicity effect in Raji cells. These naturally-occurring non-toxic compounds which inhibit the viral activation and are extracted from *Zingiberaceae* species used in Malaysian traditional medicine may contribute to the development of cancer prevention methods at the tumour-promoting stage (Vimala *et al.*, 1999). Ginger contains mutagenic (gingerol and shogaol) and anti-mutagenic (Zingiberone) compounds. Ginger extracts exhibit cytotoxic effects in cultured plant cells, but it is not known whether ginger can suppress tumour growth in experimental animals and humans (Mustafa *et al.*, 1993). Many spices, including plants of the ginger family, possess anti-carcinogenic activity. However, the molecular mechanisms by which they exert their anti-tumourigenic effects are unknown. Activator protein 1 has a critical role in tumour promotion, and blocking of this tumour promoter-induced activation inhibits neoplastic transformation. Epidermal growth factor induces cell transformation and activator protein 1 activity. Two structurally related compounds of the ginger family (6-gingerol and 6-paradol) block epidermal growth factor-induced cell transformation and activator protein 1 activation, but act by different mechanisms (Bode *et al.*, 2001).

ANTI-MICROBIAL ACTIVITY

Dichloromethane and methanol extracts of *Zingiber* genera were screened for anti-microbial activity. The anti-microbial activity of most of the extracts was anti-bacterial (Habsah *et al.*, 2000).

The composition of essential oils from the rhizomes of *Zingiber officinale* Roscoe were studied. Essential oil of *Z. officinale* was in accordance with the literature data. The essential oil of *Z. officinale* showed anti-microbial activity against all Gram-positive and Gram-negative bacteria tested, as well as against yeasts and filamentous fungi, using the agar diffusion method (Martins *et al.*, 2001).

ANTI-CHOLESTEROL ACTIVITY

Oxidative modification of LDL is thought to play a key role in the pathogenesis of atherosclerosis. Consumption of nutrients rich in phenolic anti-oxidants has been shown to be associated with attenuation of development of atherosclerosis. The dietary consumption of ginger extract by apolipoprotein E-deficient mice significantly attenuates the development of atherosclerotic lesions and reduces plasma and LDL cholesterol levels and their susceptibility to oxidation and aggregation (Fuhrman *et al.*, 2000).

The effect of administering ginger, one of the commonly consumed spices, was studied in a model system using high-fat-fed rats. Ginger was administered daily by intragastric intubation. There was a significant decrease in the levels of cholesterol, phospholipids, and free fatty acids in the tissues (liver, intestine, kidney and aorta) and serum of the ginger-treated rats. Supplementation of the control and high-fat-fed rats with ginger increased the concentration of HDL and decreased the concentration of LDL and VLDL in the serum as compared with the levels in the rats not receiving the supplement. Thus, dietary intake of ginger was found to reduce the risk of atherosclerosis markedly by virtue of its hypolipidemic and anti-atherogenic effects (Murugaiah *et al.*, 1999).

OTHER ACTIVITIES

6-shogaol is known to reduce blood pressure by both a central and a peripheral action. 8-shogaol has a cardiotonic action via enhancement of the Ca-ATPase in the sarcoplasmic reticulum (Mustafa *et al*., 1993).

Gingerol and shogaol also exhibited potent molluscicidal activity on *Biomphalara glabrata* (Adewunmi et al., 1990).

A series of gingerols, shogaols and numerous diarylheptanoids and related analoges have been tested for their anti-hepatotoxic actions using CCl_4- and GalN-induced cytotoxicity in primary cultured rat hepatocytes (Hikino *et al.*, 1985). Gingerols, shogaols and diarylheptanoids exert anti-hepatotoxic actions with the length of the linear chain in the gingerols and shogaols and the presence of hydroxyls on the phenyl ring in diarylheptanoids being important for the activity (Hikino *et al.*, 1985).

Crude aqueous extracts of ginger were gavaged and ginger oil was administered by intraperitoneal injection to male mice. Chromosome damage was studied in a preparation made from bone marrow cells following colchicine injection to all mice and examination of the cells after pre-treatment in hypotonic solution, fixation, air-drying and staining in Giemsa solution. Attention is drawn to the weakness of the clastogenic activity expressed by the ginger extract. In comparison, ginger oil gave a higher frequency of chromosomal aberrations, and the extract may contain substances that suppress clastogenesis in the bone marrow cells of mice (Mukhopadhyay and Mukherjee, 2000).

A few common spices or their active principles were examined for their possible influence on digestive enzymes of pancreas in experimental rat. Animals were maintained on a diet containing ginger for 8 weeks. Dietary ginger enhanced pancreatic lipase activity, and stimulated pancreatic amylase, trypsin and chymotrypsin. This stimulatory influence of ginger on the pancreatic enzymes was not observed when their intake as restricted to a single oral dose. The positive influences on the pancreatic digestive enzymes exerted by a good number of spices consumed in diet could be a factor contributing to the well-recognised digestive stimulant action of spices (Platel and Srinivasan, 2000).

In acute toxicity tests, mice tolerated ginger extract as gavage of up to 2.5 g/kg with no mortality or side-effects during a 7-day trial period. However, increase in doses to 3.0-3.5 g/kg the extract resulted in 10-30% mortality. Under similar experimental conditions, acetyl salicylic acid (600 mg/kg) produced mortality in 25% of animals, stomach ulcers in 40% of animals and discomfort and/or hypothermia in 60% of animals (Mascolo *et al.*, 1989). While there is no mention of adverse effects of ginger in the literature (Grontved *et al.*, 1988). However, as only one-time consumption of ginger produced a persistent effect on the function of blood platelets (Dorso *et al.*, 1980). Hence, the consumption of ginger should be maintained at a half gram of powdered ginger daily.

Ginger might act by another mechanism too. Since for the formation of prostaglandins, a peroxide tone seems mandatory (Kiuchi *et al.*, 1982), ginger might reduce the level of lipid peroxides which are regulated by lipoxygenase and membrane NADH oxidase activities, the latter producing hydrogen peroxide which

has been shown to stimulate cyclooxygenase activity (Hemler *et al.*, 1979). In addition, oxidase-generated H,O_2 an] O2- can initiate membrane lipid oxidation generating peroxidised lipids in amounts sufficient to stimulate cyclooxygenase activity. This suggests that prostaglandin production by phagocytic cells at inflammatory sites or during immune responses might be regulated by oxidants and lipid peroxides generated by them.

Choice of extraction method

Extraction procedures of the rhizome aim to give the highest yield of extract at the maximum extraction rate, minimum energy consumption and least residual solvent whilst achieving a high-quality flavour in both essential oil and oleoresin. Traditional azeotropic ethanol and steam distillations are high energy-consuming processes and do not give high yields of ginger oil. Typical yields are 0.2 to 2.7%. Steam distillation leads to high levels of monoterpene hydrocarbons and low amounts of volatile components, possibly the result of thermal degradation (Chen and Ho, 1987). Liquid extraction can require large volumes of often toxic, flammable and expensive organic solvents which can be difficult to remove from extracts. High-pressure liquid carbon dioxide extraction has several advantages over steam distillation (*e.g.* better yield, shorter extraction time, lower energy consumption and better sensory properties of the extract).

The rapid and successful separation of selected compounds from a plant extract containing a myriad of substances can present a serious problem. It is therefore important to undertake a crude separation of the plant components before more sophisticated procedures are employed. Such separations may include extraction using selected solvents, partition into two immiscible solvents (liquid-liquid extraction) and vacuum or flash chromatography (Zarate *et al.*, 1992). Attempts have been made to isolate gingerols from a crude extract of ginger using liquid-liquid extraction (Shoji *et al.*, 1982; Farthing and O'Neill, 1990).

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APPENDIX 2.

Analysis of Samples (SV1 and SV2) for Anti-inflammatory Activity

Introduction

This assay was run to determine the effectiveness of the supplied samples SV1 and SV2 to inhibit Prostaglandin E_2 (PGE_2) production in a mouse 3T3 fibroblast cell line.

The anti-inflammatory assay performed by our laboratory measures the level of PGE_2 in stimulated mouse fibroblast 3T3 cell line after treatment with the two ginger oils. The use of a stimulated cell line rather than using isolated enzyme systems gives much more useful data.

PGE_2 is formed in a variety of cells from PGH_2, which itself is synthesised from arachiodonic acid by the enzyme prostaglandin synthetase. The assay measures secreted levels of PGE_2 from the supernatant of the fibroblast cells and indicates the overall anti-inflammatory activity of an extract/compound on this cell line.

Materials

3T3 Swiss Albino fibroblast cells were obtained from the European Collection of Cell Cultures; cell culture medium components were obtained from Invitrogen; calcium ionophore A23187, DMSO and acetylsalicylic acid (aspirin) were obtained from Sigma. Nunc Nunclon^{TM} Delta Surface tissue culture plates were obtained from Medos. The Prostaglandin E_2 EIA Kit- Monoclonal was obtained from Cayman Chemical, Ann Arbour, MI.

Methods

Extract Handling

The two (2) extracts (SV1 and SV2) were provided on the 10-01-02. The samples were dissolved in DMSO at 10 mg/mL and 2 x 5-fold serial dilutions were prepared in DMSO.

Tissue Culture

A confluent 175cm^2 flask of 3T3 Swiss Albino fibroblast cells was detached from the flask using 0.25% trypsin/EDTA and 1/8 of the cells were resuspended in 14mL media (DMEM without phenol red + 2mM glutamine + 10% foetal bovine serum). Cells were plated out (100 µL/well) into 96-well tissue culture plates. The cells were grown overnight at 37^{o}C, 5%CO_2.

Assay

The samples were tested at final concentrations of 600, 200, 67, 22, 7 and 2.5 µg/mL. One µL of the diluted samples was added to the cells in the wells. The solvent control was 1 µL DMSO, while the positive control was aspirin (5 mM, 1 µL) giving a final concentration of 50 µM.

The cells were incubated for 3 hrs in the presence of the samples. Calcium ionophore A23187 (5mM, 1 µL) in DMSO was added to the wells, and incubation continued for 15min. The plate was then centrifuged (1500*g*, 3 min) and the culture supernatant was removed. The supernatant was diluted by serial dilutions 1:500 and 1:1000 in EIA assay buffer (Cayman

Chemical). The supernatants were assayed for prostaglandin E_2 using the Prostaglandin E_2 EIA Kit- Monoclonal, according to the kit protocol. Each sample was assayed at two dilutions in duplicate.

Results and Discussion

Table 1 shows significant activity was observed both samples provided. The inhibition of the PGE_2 response to aspirin (500 μM) was around 36.3 % of the stimulated control value while the ibuprofen (25 μM) caused an inhibition of around 30.6 %. Both SV1 and SV2 produced greater inhibition of PGE_2 than aspirin or ibuprofen at all but the lowest concentrations (2.5 or 7 μM). Figures 1 and 2 show PGE_2 inhibition curves for SV1 and SV2 respectively. IC_{50} values of 5.34 and 5.94 μM were obtained for SV1 and SV2 respectively. SV1 and SV2 produced dose dependant inhibition of secreted PGE_2 from the mouse fibroblast cell line equivalent to or better than both aspirin and ibuprofen under the test conditions.

Sample	**Concentration (μg/mL)**	**%A23187**	**x/Aspirin**	**x/Ibuprofen**
Aspirin	500μM	39.155	1.077	1.281
Aspirin	500μM	33.521	0.922	1.096
Ibuprofen	25μM	32.676	0.899	1.069
Ibuprofen	25μM	28.451	0.783	0.931
SV1	600	34.085	0.938	1.115
SV1	600	29.859	0.822	0.977
SV1	200	34.366	0.946	1.124
SV1	200	27.887	0.767	0.912
SV1	67	29.578	0.814	0.967
SV1	67	27.324	0.752	0.894
SV1	22	29.577	0.814	0.968
SV1	22	27.042	0.744	0.885
SV1	7	36.338	0.999	1.189
SV1	7	35.211	0.969	1.152
SV1	2.5	78.028	2.147	2.552
SV1	2.5	64.507	1.775	2.110
SV2	600	25.634	0.705	0.838
SV2	600	27.606	0.760	0.903
SV2	200	24.789	0.682	0.811
SV2	200	26.761	0.736	0.875
SV2	67	24.789	0.682	0.811
SV2	67	26.479	0.729	0.866
SV2	22	25.070	0.690	0.820
SV2	22	28.169	0.775	0.921
SV2	7	41.690	1.147	1.364
SV2	7	46.479	1.279	1.520
SV2	2.5	69.859	1.922	2.285
SV2	2.5	76.056	2.093	2.488

Table 1 Percent inhibition of secreted PGE_2 from *3T3 cells exposed to samples SV1 and SV2.*

Figure 1 Percent inhibition of PGE_2 in 3T3 cells after exposure SV1

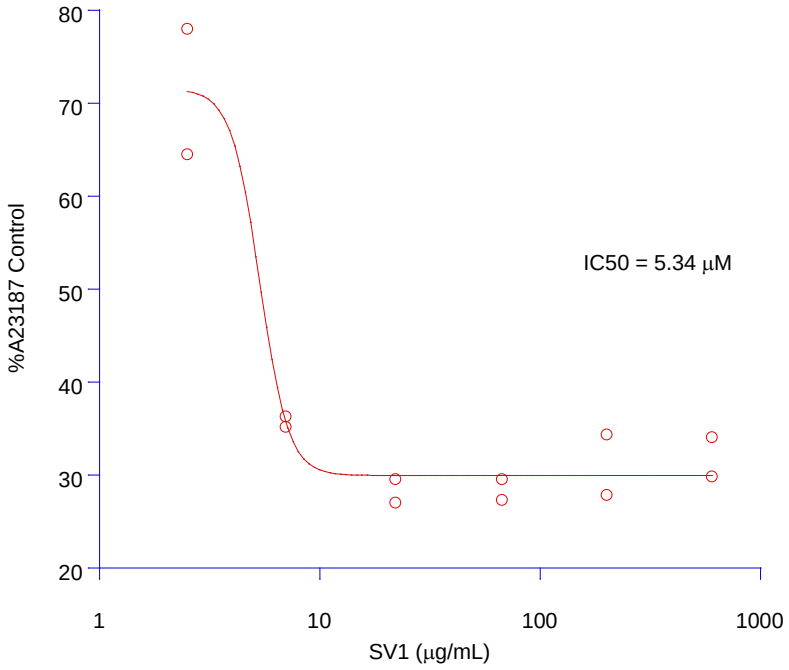
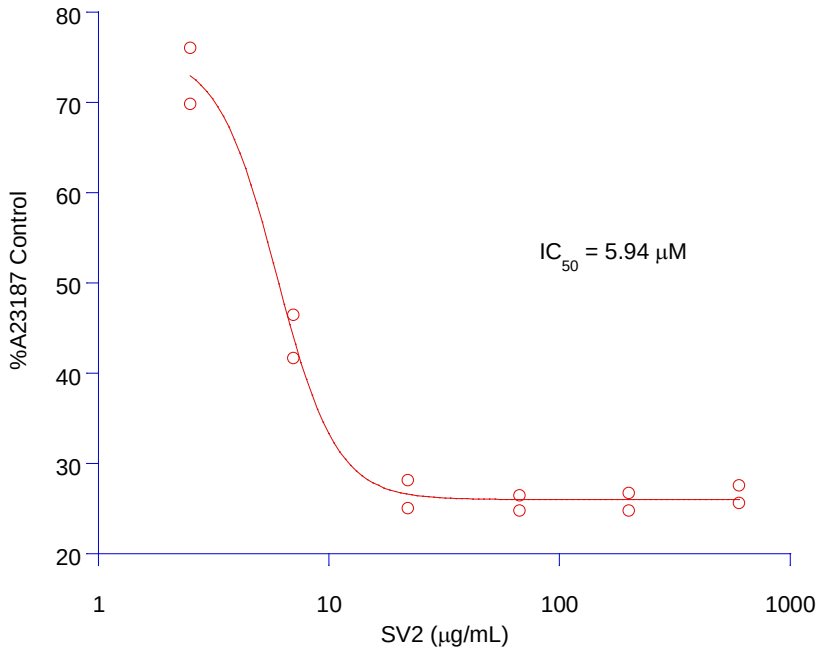


Figure 2 Percent inhibition of PGE_2 in 3T3 cells after exposure SV2



Recommendations

The two oils analysed have very good anti-inflammatory activity in the 3T3 secreted PGE_2 assay system. It might be of interest to fractionate and further analyse these oils for the presence and activity of the gingerol and other related phenylalkanol derivatives.

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APPENDIX 3.

Report on the PGE2 Anti-inflammatory Activity of fractionated SFE ginger oil SV2.

APPENDIX 4

Concerning ginger products as potential anti-inflammatory agents

M Whitehouse

Mid-March 2002

This report consists of 4 pages and 4 tables.

Summary

Products were examined for:

(a) (Chronic) anti-inflammatory activity in rats developing adjuvant-induced polyarthritis;
(b) Anti-pyretic activity; and
(c) Steroid-synergising activity.

The sliced and spent gingers were not significantly active in the anti-arthritic test. The SFE-extracted oil SV-2 was minimally active in all three tests, when given orally, but was active given subcutaneously (s.c.) in (a) and (b). The Resin fraction was effective orally in (a) and (b) and much more potent given s.c. in (b) and (c). The Steam Distillate (SD) was only active in (a) given s.c. The SV-2 and SD were not gastrotoxic: the Resin was untestable, not being freely dispersible in aqueous media alone.

Materials

Zynax and Zinaxin were purchased in Australia and overseas. Other OTC ginger products were either bought in local health food stores or donated by reputable herbalists from their practise; being used within their stated shelf-lives.

Other preparations were provided by Dr Davis.

Oils were dissolved in olive oil for long term dosing or in 25% ethanol - 0.04% Tween-20 for short term studies.

Methods

1. The first-line screen was the experimental polyarthritis in female Wistar rats, induced by inoculating an arthritigenic adjuvant into the tailbase on day 0. When the first signs of arthritis were apparent (day 10), animals were dosed orally once daily for four days and the signs of arthritis re-determined on day 14. A number of herbal products and most NSAIDs tested have proved effective in retarding arthritis development.

2. The next screen was for Antipyretic activity by giving test agents to female Wistar rats developing yeast-induced fever; either a) prophylactically before inoculating the yeast

or b) therapeutically after the fever was fully established, eight hours later. These tests confirm aspirin-like activity.

3. A test for corticosteroid-like activity is based on prevention of the chronic (fibrotic) inflammation caused by inoculating rear paws of Wistar rats with 0.5 mg Zymosan (a purified yeast cell wall preparation). Test materials are given three hours later i.e. when the early histamine-induced oedemic response has peaked. The residual paw inflammation is then measured 18 hours later – and daily thereafter if needs be.

This test is more efficiently used to locate steroid-synergising activity by co-dosing with a low, marginally effective, dose of steroid (e.g. 2.5 mg/kg prednisone or 0.1 mg/kg dexamethasone).

4. Since aspirin is gastro-irritant, especially in fevered or arthritic rats, some of the ginger oils were given orally to fasted disease-stressed animals either a) singly to look for intrinsic gastrotoxic activity or b) in combination with Ibuprofen, an OTC NSAID, to see if the preparations might show gastroprotectant activities.

Results

Anti-inflammatory activity in polyarthritic rats (Table 1)

This table gives the most recent interesting results. A fuller retrospective report will be provided if required. The most obvious result is that these oils are not readily bioavailable i.e. effective drugs, given orally. The Resin is perhaps an exception, as it still seems to be orally active at 50 mg/kg. This dose may be an approximate ED_{50} which certainly means it is more potent than Ibuprofen (ED_{50} ca. 80 mg/kg) but less potent than Naproxen (ED_{50} ca. 25 mg/kg). By contrast Zinaxin is inactive and even potentiates arthritis development.

It would be interesting to test some subfractions of the Resin to see by how much the activity can be concentrated.

Anti-pyretic activity (Table 2)

The SV-2 oil was quite potent given subcutaneously, in contrast to the steam distillate. The Resin showed some activity given orally.

Steroid-synergising activity (Table 3)

Only the reference drug, Lyprinol (given orally), and the Resin fraction given subcutaneously were active. How much this latter activity is due to counter-irritation is hard to tell without conducting a dose-response study.

Gastrotoxicity (Table 4)

None was encountered. The Resin could not be tested adequately as it was not freely dispersible in 100% aqueous media (with minimal emulsification). The 25% ethanol vehicle used for the short-term drug studies is inappropriate here as it of itself can be gastric irritant.

Comments

1. Ginger products have been tested at the PA Hospital intermittently since 1994. A first published report is attached (Inflammopharmacology 1999). That dealt only with products available OTC.

2. The data in tables 1,2, and 3 seem to provide a 'watershed' i.e. you now have some active fractions in contrast to some previous preparations (ethanolic extracts, etc.) and all the OTC products surveyed above.

3. Collectively these data indicate ginger oils are not aspirin-like, not being gastrotoxic, but may still have the potential to be pseudo-NSAID(s) – particularly if some way can be found to either enhance their absorption from the GI tract or diminish their first pass (hepatic, intestinal) detoxification. Perhaps we should be looking at a parenteral delivery system. Realistically, this means a transdermal formulation.

4. What is needed now is some realistic investment of time, effort and dollars to go beyond these probing experiments. To begin with, we should repeat:

(i) the arthritis studies to (a) confirm the ED_{50} for the Resin p.o. – so there is a realistic reference point from which to evaluate subfractions; and (b) locate approximate ED_{50}s for all the oils given subcutaneously (this would be helpful for future evaluation of transdermal preparations – see below).

(ii) The steroid-synergising experiment to locate an ED_{50} for the Resin given s.c. – to provide an independent reference point.

(iii) Do a small study (30-40 rats) of the three oils in various transdermal formulations e.g. olive oil plus or minus a penetration enhancer (e.g. cineole), ethanol-propylene glycol, or even DMSO.

(iv) Look at possible gastroprotectant activities of the oils when co-administered with gastro-irritant NSAIDs e.g. 50 mg/kg Ibuprofen.

(v) Test for acute anti-inflammatory activity, using the carrageenan-induced paw oedema model, with or without Misoprostol as a synergist. This is the standard 'quickie' test for 'run-of-the-mill' NSAIDs.

5. Estimated costs of such further work (Phase 2) would include about 70 animals ($1300) and a labour component of between $2-3000; i.e. a minimum of $4000.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF GINGER EXTRACTS IN RATS DEVELPOPING POLYARTHRITIS

Mean changes in arthritic signs (days 10-14) after test materials administered on days 10 through 13 (n=4 rats/group).

Test product*	Route	Dose mg/Kg	Rear paw increase	Fore paw inflam.	Δ Wt (gm)	Arth. Score	Percentage inhibition		
							Rear	Fore	Arth. Score
None	-	-	0.95 mm	2.5+	+08	2.5+	-	-	-
Spiced ginger	p.o.	300	0.91 mm	2.8+	-08	2.3+	04	-12	08
Spent ginger	p.o.	300	0.63 mm	2.5+	+10	1.5+	34	0	40
None	-	-	0.60 mm	2.2+	+06	2.0+	-	-	-
GE-SV2	p.o.	100	0.48 mm	2.0+	+11	1.6+	20	09	20
GE-SV2	s.c.	100	0.01 mm	0.0+	+06	0.5+	98	55	75
GE-SV1	p.o.	100	0.20 mm	0.2+	+19	0.4+	67	91	80
GE-SD	p.o.	100	0.36 mm	2.4+	+09	1.3+	40	-09	35
GE-SD	s.c.	100	0.02 mm	0.5+	+13	0.5+	97	77	75
None	-	-	0.79 mm	2.5+	+07	1.8+	-	-	-
GE-SV1	p.o.	50	0.45 mm	0.0+	+03	0.9+	43	60	50

(E.986/1026/38)

GE = ginger extracts; p.o. = oral; s.c. = subcutaneous; SD = steam distillate

Comments: 1. Missing here are s.c. in olive oil (delivery vehicle) and s.c. GE – SV1.
2. Uncertain counter-irritancy effect from s.c. delivery.

TABLE 2: ANTIPYRETIC ACTIVITY

Test materials given prophylactically i.e. 45 minutes before yeast (1.5 g/kg s.c.).
Rectal temperatures recorded 5.5 hours later. Untreated rats had a fever = 1.7±0.2°C.

Dose		**Δ temperature (°C) after administering**	
Test materials	**Mg/kg**	**p.o.**	**s.c.**
GE-SV2	300	0	-1.9
GE-Res	300	-0.5	-0.7
GE-SD	300	0	+0.3
SFE-Fzg*	300	0	
Zinaxin	600	+0.1	-0.1
Zingiber Complex	116**	+0.5	
Paracetamol	300	-1.6	

(E.1035/42/43)

*An oil from SFE extraction – Finzelberg, Germany.
**As gingerols; product from Golden Glow, Qld.
Rats = 2/group. GE products administered in 25% ethanol – Tween 20.

TABLE 3: LOOKING FOR STEROID-SYNERGISING ACTIVITY

Rats dosed with Prednisone = 2.5 mg/kg alone or with test materials 3 hrs after injecting Zymosan (to initiate chronic, fibrotic, inflammation) in each rear paw of Wistar rats.

Treatment	**Route**	**Mg/kg**	**% inhibition**
Prednisone (P) only	p.o.	-	13%
P + GE-SV2	p.o.	100	19
	s.c.	100	08
P + GE-Res	p.o.	100	11
	s.c.	100	70
P + GE-SD	p.o.	100	13
	s.c.	100	-01
Olive oil alone	s.c.	2 ml	-07
Lyprinol	p.o.	20	56

(E.1034/6)

Rats = 2/group. GE products administered in olive oil.

TABLE 4: ASSESSING GASTROTOXICITY IN FASTED, DISEASE-STRESSED DARK AGOUTI OR WISTAR RATS

Product (150 mg/kg)	**Wistar**	**Dk Agouti**
GE-SD	02 (E)	0
GE-SV2	0	0
SFE/Fzg.	0	03
Zinaxin	0 (E)	0 (E)
Aspirin	80	68

(E.1045)

Rats = 2/group.

APPENDIX 5.

Concerning the latest ginger oil fractions as potential anti-inflammatory agents

M Whitehouse

Mid-January 2003

This report consists of 2 pages and 2 tables (Nos. 5 and 6).

Summary

Only samples G-2 and G-3 showed anti-inflammatory activity. Only sample SV-1 showed anti-pyretic activity.

Materials

Fractions 2 and 4 were dated 7.10.02. Samples G1-G5 dated 19.12.02 and the original SV-1?/02.

For testing as anti-inflammatory agents these fractions were diluted into olive oil = 30 mg/ml and administered at a dose of 2.5 ml/kg subcutaneously i.e. 75 mg ginger extract/kg. For anti-pyretic/gastrotoxicity studies, the fractions were first diluted in ethanol and then dispersed into water with the addition of 0.04 per cent (v/v) Tween-20; the final concentration being 15 mg/ml ginger extract in 20 per cent (v/v) ethanol-Tween. The dose administered orally was 10 ml/kg i.e. 150 mg/kg.

Methods

As outlined in the March 2002 report. 1) For evaluating anti-inflammatory activity in animals developing adjuvant-induced polyarthritis, olive oil solutions/dispersions were administered once daily s.c. on days 10-13. 2) For evaluating anti-pyretic/gastro-irritant activity in animals with pre-established fever, aqueous ethanolic dispersions were administered orally once only.

Restrictions on the animal supply meant that only three animals per group could be used to generate the data in Table 5B. For Table 5A, four animals per group were used.

Results

Table 5A gives the results of testing two of the first fractions delivered. Table 5B records data from the most recent delivery (January 2003).

Fractions 2 and 4 were not active.

Extract G2 = the scarcest and extract G3 significantly suppressed arthritis development over the dosing time/period (days 10-14). After ceasing dosing, there was a significant rebound in symptoms. Fraction 3 caused some weight loss at this dose = 75 mg/kg given s.c. in olive oil.

Table 6 shows that only SV-1 at the higher dose of 150 mg/kg, given orally with 20 per cent ethanol, had any rapid effect on the fever. For comparison, two pure phenols = paracetamol and two hydroxy-benzyl alcohol (saligenin from Willowbark) were strong anti-pyretics in this assay. Unfortunately, there was insufficient G-2 to run this test.

Inspection of the rat's stomach at the conclusion of observing any changes in body temperature (CA.3hours) showed that G-3 might have been gastro-irritant, eliciting extra inflow of gastric juice into the stomach. Only animals dosed with SV-1 showed any signs of mucosal erythema or bleeding.

Comments

The ginger fractions were administered in two different modes deemed most suitable for the pathological circumstances namely (i) a slow-release form from a s.c. depot (with olive oil) to control a relatively slow-developing chronic inflammation and (ii) a rapidly-delivered format, dispersed in 20 per cent ethanol-Tween 20, given orally to fasted animals with a relatively stable induced fever.

The oral route gives the ginger components maximum initial exposure to gut and liver detoxicant enzymes. The s.c. route was used to hopefully reduce this problem.

Proposals for further work

1. To study SV-1 in all the other routine assays.

2. To re-investigate G-3 for anti-inflammatory activity at lower doses both when given alone (for monotherapy) or as an adjunct to low dose aspirin or sodium salicylate i.e. combination therapy.

3. As further animals become available, study the other fractions originally prepared from SV-2 (additional to Nos. 2 and 4) included in Table 5A.

TABLE 5: ANTI-INFLAMMATORY ACTIVITY OF GINGER EXTRACTS IN RATS DEVELPOPING POLYARTHRITIS

Test materials given once daily subcutaneously.
Mean changes in arthritic signs (days 10-14) after test materials administered on days 10 through 13.

Test product*	Dose/Kg	Rear paw increase (mm)	Fore paw inflam.	Δ Wt (gm)	Arth. Score	Percentage inhibition		
						Rear	Fore	Arth. Score
Olive oil	2mL	0.52	3.1+	+16	1.9+	-	-	-
Fraction 2	75mg	0.45	1.9+	+27	1.1+	13%	0%	43%
Fraction 4		0.40	2.2+	+12	1.5+	24%	(-15%)	22%
Olive oil	2mL	0.61	2.1+	+11	1.4+	-	-	-
G-1	75mg	0.59	1.5+	+12	11.5+	04%	29%	(-07%)
G-2	75mg	0.08	0.2+	+13	0.4+	87%	91%	72%
G-3	75mg	0.00	1.2+	-01	0.5+	100%	43%	65%
G-4	75mg	0.45	2.0+	+15	1.6+	27%	05%	(-14%)
G-5	75mg	0.32	1.5+	+06	1.4+	48%	29%	0%

(E.1123/1148)

* all test products were suspended in olive oil.

Comment: Fractions G-2 and G-3 show promise.

TABLE 6: ANTIPYRETIC ACTIVITY

Test materials given therapeutically after fever was established. Rectal temperatures recorded 1, 2 and 3 hours later. Untreated rats had a fever = 1.7 ± 0.2°C.

Dose		**Δ temperature (°C) after 2 hours**
Test materials	**Per kg**	**p.o.**
20% ethanol (E)	10 ml	-0.5
G-1 in 20% E	150 mg	-0.8
G-2 in 20% E	150 mg	-0.7
G-4 in 20% E	150 mg	-0.5
G-5 in 20% E	150 mg	-0.2
SV-1 in 20% E	150 mg	-1.1
Paracetamol	150 mg	-1.4
Saligenin	200 mg	-2.5

(E.1154)

APPENDIX 6.

LCMS data for the SFE ginger product.

APPENDIX 7.

Additional Literature Search of Compounds in ginger with reported bioactivities.

Geraniol CAS# 106-24-1 (Guaniol, Lemonol, Nerol, Neryl Alcohol, Geraniol alcohol, Geranyl alcohol, 3,7-Dimethyl 2,6-octadien-1-ol).

Aggarwal, K. K., Ateeque Ahmad, et al. (2000). "Antimicrobial activity spectra of Pelargonium graveolens L. and Cymbopogon winterianus Jowitt oil constituents and acyl derivatives." Journal of Medicinal and Aromatic Plant Sciences 22(1B): 544-548. The essential oils of citronella Java (Cymbopogon winterianus) and rose geranium (Pelargonium graveolens) were partitioned into different fractions under high vacuum in a packed fractionating column for their separation into pure constituents such as citronellal, d-citronellol [(+)-citronellol], l-citronellol [(+)- citronellol] and geraniol. The formate derivatives of geraniol, l- and d-citronellol could be prepared with optimum yield and confirmed through GC. The spectra of the antimicrobial activities of the essential oils and their constituents in relation to optical isomers and their derivatives were analysed. Differential antimicrobial activities were studied through in vitro bioassays against 12 bacterial (Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, Streptococcus mutans, Yersinia enterocolitica, Salmonella typhi, Escherichia coli and Mycobacterium smegmatis) and 7 fungal (Microsporum gypseum, Aspergillus niger, A. flavus, Trichophyton rubrum, Sporothrix schenckii, Candida albicans AI and C. albicans) strains. The distinct activity patterns indicated structure-function relationships for the optical isomers.

Apfelbach, R. and E. Weiler (1985). "Olfactory deprivation enhances normal spine loss in the olfactory bulb of developing ferrets." Neurosci Lett 62(2): 169-73. Ferrets show a sensitive phase in their postnatal development during which they can become imprinted to food odors. At the same time the number of granule cell spines in the olfactory bulb reaches a maximum, declining significantly thereafter. In ferrets, exposed continuously to saturated levels of geraniol odor in the cage environment, the normal decline in spine number (occurring between day 60 and 90) is significantly enhanced. No such effects were observed during earlier ontogenetic phases. This late postnatal phase is further associated with a marked and significant decrease in total brain weight. The significance of these events to olfactory imprinting and plasticity in the developing brain is discussed.

Ashida, Y., A. Matsushima, et al. (2002). "Geraniol-inducible glutathione S-transferase in cultured soybean cells." Biosci Biotechnol Biochem 66(1): 168-70. When the cultured cells of Glycine max (soybean) were treated with 5 mM geraniol as a chemical stress, an mRNA level was elevated in a rapid but transient increase. The mRNA was cloned and sequenced, and found to correspond to the mRNA encoding glutathione S-transferase (GST). The GST mRNA level and GST activity were elevated to maxima at 4-6 h and 8

h, respectively, after treatment of the cultures with geraniol. These indicate that GST is one of the geraniol-responsive factors in soybean cells.

Baek, S. H., Y. O. Kim, et al. (1998). “Boron trifluoride etherate on silica-A modified Lewis acid reagent (VII). Antitumor activity of cannabigerol against human oral epitheloid carcinoma cells.” Arch Pharm Res 21(3): 353-6. Geraniol (1), olivetol (2), cannabinoids (3 and 4) and 5-fluorouracil (5) were tested for their growth inhibitory effects against human oral epitheloid carcinoma cell lines (KB) and NIH 3T3 fibroblasts using two different 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and sulforhodamine B protein (SRB) assay. Cannabigerol (3) exhibited the highest growth-inhibitory activity against the cancer cell lines.

Baker, F. C., B. Mauchamp, et al. (1983). “Farnesol and farnesal dehydrogenase(s) in corpora allata of the tobacco hornworm moth, Manduca sexta.” J Lipid Res 24(12): 1586-94. The metabolism of [3H]farnesol was studied in cell-free preparations of corpora allata from the tobacco hornworm, Manduca sexta, to assess the role of this presumed biosynthetic precursor of juvenile hormone (JH) III. A reversed-phase ion-pair liquid chromatographic (RP-IPC) procedure was devised to separate farnesol from several potential intermediates in its presumed metabolism to JH III: farnesal, farnesoic acid, 10,11-epoxyfarnesoic acid, and methyl farnesoate. Following incubation of (2E,6E)-[1,5,9-3H]farnesol with homogenates of corpora allata from fifth instar larvae or adult female M. sexta, and analysis by RP-IPC, the major radiolabeled products corresponded to farnesoic acid, farnesal, and a polar product(s) presumably derived from the tritium on C-1 of farnesol. Inclusion of NAD+ in the incubations conducted with crude homogenates resulted in enhanced [3H]farnesol metabolism, decreased accumulation of [3H]farnesal, and increased levels of [3H]farnesoic acid. Substitution of NADP+ for NAD+ was ineffective, suggesting that farnesol and/or farnesal dehydrogenase were NAD+-dependent enzymes. Pellet fractions obtained by differential centrifugation of crude homogenates exhibited both farnesol and farnesal dehydrogenase activity but only the latter was clearly stimulated by addition of NAD+. The alcohol/aldehyde dehydrogenase(s) showed some substrate specificity for the 2E isomer; nerol and (2Z,6E)-farnesol were barely metabolized under conditions in which either geraniol or (2E,6E)-farnesol were rapidly oxidized. The identity of the [3H]farnesal zone obtained from RP-IPC was further established by normal-phase liquid chromatography and by gas-liquid chromatography-mass spectrometry.

Bamba, F. L. and J. Wepierre (1993). “Role of the appendageal pathway in the percutaneous absorption of pyridostigmine bromide in various vehicles.” Eur J Drug Metab Pharmacokinet 18(4): 339-48. We studied the percutaneous absorption of [14C]-labelled pyridostigmine bromide mixed into various vehicles through normal and appendage-free scar rat skin, in vitro during 72 h. At the end of the experiment, the percentages of the drug absorbed were higher for nerol 8% in ethanol (respectively 78.4 +/- 3.6% and 72.8 +/- 4.5% on normal and scar skin) and azone 5% in ethanol-propylene glycol (90:10) (respectively 76.4 +/- 4.4% and 57.2 +/- 7.1% on normal and scar skin). Propylene glycol 10% in ethanol inhibits pyridostigmine absorption: 9.9 +/- 2.6% and 2.2 +/- 1.2% vs 14.7 +/- 3.8% and 5.5 +/- 5.1% with ethanol on control and scar skin. The

transappendageal pathway seems to be less important for nerol (55% to 82% of the absorption routes between 4 h and 32 h) and azone (60% to 79% of the absorption routes until 32 h) than for propylene glycol (63% to 96% of the absorption pathways during the whole experiment), dimethylsulfoxide (about 78% during the first 32 h) and ethanol (more than 50% during most of the time). These results show that it is possible to increase or decrease the percutaneous absorption, as well as to modulate the relative importance of the transepidermal route and the transfollicular pathway.

Bansal, N., D. A. Nyquist, et al. (1992). "Protein-linked isoprenoid lipids in dexamethasone-treated human lymphoid lines in culture." Biochem Cell Biol 70(6): 489-95. Accumulation of isoprenoids was studied in two cell lines derived from acute T-cell leukemia: CEM-C7 cells, whose growth is inhibited by the glucocorticoid dexamethasone, and CEM-C1 cells, which are resistant to this steroid. Isoprenoids were measured by growing the cells in serum-free medium in the presence of lovastatin, which blocks synthesis of mevalonate, and then labeling with exogenous [3H]mevalonolactone. In both cell lines, isoprenoids associated with proteins were detected in cytoplasm, nucleus, and chromatin, and in the chromatin residue that remains after extraction of histone and nonhistone proteins. Differences in labeling were detected after treatment with dexamethasone in the CEM-C7 line, showing a decrease in the cytoplasmic fraction with a corresponding increase in both the nuclear and chromatin fractions as compared with untreated cells. No change was seen in the CEM-C1 line. In both cell lines, 25-30% of the incorporated label was released by treatment with acid or alkali. However, the majority of the label required treatment with methyl iodide for the release of organic-soluble tritiated products. After extraction with chloroform, the lipid fractions contained farnesol, geraniol, dolichols, and possibly nerolidol.

Banthorpe, D. V., B. M. Modawi, et al. (1978). "Redox interconversions of geraniol and nerol in higher plants." Phytochemistry 17(7): 1115-1118. The use of 14C, 3H-labelled precursors showed that for plant feedings carried out in winter, isothujone (trans-thujan-3-one) was formed in Tanacetum vulgare from nerol (3,7-dimethyl-octa-cis-2,6-dien-1-ol) without loss of hydrogen from C-1 of the precursor. In contrast, formation from geraniol (the corresponding trans-isomer) involved stereospecific loss of the pro-(1S) hydrogen. This suggests that geraniol and nerol were interconverted by a redox system. However, anomalous results were obtained from similar studies at other seasons with T. vulgare, and on the biosynthesis of alpha - and beta -pinenes (pin-3-ene and pin-2-(10)-ene) in Pinus pinaster, 1,8-cineole (1,8- oxidomethane) in Mentha piperita and Eucalyptus globulus, and of carvone (menth-6,8(9)-dien-2-one) in M. spicata.

Banthorpe, D. V., G. A. Bucknall, et al. (1976). "Biosynthesis of geraniol and nerol in cell-free extracts of Tanacetum vulgare." Phytochemistry 15(1): 91-100. Cell-free extracts from tansy leaves synthesized geraniol and nerol (3,7-dimethylocta-trans-2-ene-1-ol and its cis isomer) in up to 11.9 and 2.4% total yields from IPP-(4-14C) and MVA-(2-14C), respectively. Optimum preparations were obtained from plant material just before flowering.

Banthorpe, D. V., O. Ekundayo, et al. (1978). “Evidence for geraniol as an obligatory precursor of isothujone.” Phytochemistry 17(7): 1111-1114. The results of in vivo (leaves) and in vitro studies on Tanacetum vulgare are reported. They indicated that the precursor mevalonic acid-[14C, 3H] was converted in the sequence geraniol 'right arrow' nerol 'right arrow' isothujone.

Banthorpe, D. V., S. E. Barrow, et al. (1983). “Seasonally-dependent oxidative metabolism of terpenes in Tanacetum vulgare.” Zeitschrift fur Pflanzenphysiologie 111(2): 175-177. In a 5-year experiment cell-free extracts were prepared from destalked leaves collected at intervals from plants of a vegetatively-propagated clone. The potted plants were kept in a cold greenhouse from October to March, then cut back and placed outdoors. The extracts converted labelled isopentenyl pyrophosphate and geraniol into epoxidized and hydroxylated products. Enzyme activity was greatest in winter and, at its maximum, varied by up to 33-fold in different years in a manner which was correlated qualitatively with the weather. Enzyme levels were consistently high in severe winters. Such correlations support a previous suggestion [see HcA 48, 10099] that in T. vulgare plants these enzyme systems convert lower terpenes into water solubles before the onset of leaf abscission and necrosis of aerial parts.

Baraldi, P. G., S. Manfredini, et al. (1992). “Geiparvarin analogues. 3. Synthesis and cytostatic activity of 3(2H)-furanone and 4,5-dihydro-3(2H)-furanone congeners of geiparvarin, containing a geraniol-like fragment in the side chain.” J Med Chem 35(10): 1877-82. Continuing our study on the structural features of geiparvarin (1), responsible for cytostatic activity, a series of 4,5-dihydro-3(2H)-furanones 10a-f and of 3(2H)-furanones 11a-f as well as 2",3"-dihydrogeiparvarin (14) have been designed and synthesized. Their cytostatic activity was evaluated against proliferation of murine (L1210, FM3A) and human (Raji, Molt/4F, and MT4) tumor cells. Modifications in the region of the olefinic double bond by introduction of the characteristic alkenyl side chain of ascofuranone (compounds 10a-f and 11a-f) markedly decreased the cytostatic activity as compared to geiparvarin itself, but this effect does not seem to be correlated to the presence of the furanone moiety linked to the alkenyl chain or to the ability to afford Michael type adducts. Replacement of the coumarin portion by other aromatic rings did not alter the cytostatic activity. The essential inactivity of 2",3"-dihydrogeiparvarin (14) points to the importance of the 3(2H)-furanone ring system in the cytostatic activity; consequently, this moiety may be considered as the determinant pharmacophore for antitumor activity, while the side chain plays a rather modulatory role.

Barany, E. and M. Loden (2000). “Content of fragrance mix ingredients and customer complaints of cosmetic products.” Am J Contact Dermat 11(2): 74-9. BACKGROUND: In relation to the wide use of cosmetics, serious adverse effects are rare. Occasionally, unwanted effects such as contact dermatitis are reported. Allergic reactions to cosmetics are often caused by fragrances. OBJECTIVE: The aim was to investigate the content of fragrance mix (FM) ingredients in cosmetic products of the brand ACO HUD (Stockholm, Sweden) and the frequency of customer skin complaints about fragranced and unfragranced products over 4.5 years. METHOD: Content of FM ingredients in the fragrances used was both analyzed and requested from the suppliers. Customer

complaints were those reported to the company. RESULTS: Between 1 and 7 of FM ingredients were present in levels of less than 0.1 to 770 ppm. The ingredients, in order of frequency, were geraniol, eugenol, hydroxycitronellal, alpha-amyl cinnamic aldehyde, isoeugenol, cinnamic alcohol, and oak moss. Cinnamic aldehyde was not found. No significant difference was found either between the frequency of complaints about products with and without fragrance (P = .21) or in a paired comparison of 17 formulas marketed with and without fragrance (P = .24). CONCLUSION: The study suggests that the investigated fragranced products had a low content of FM ingredients, which might explain the absence of a higher frequency of adversities. Furthermore, it appears that under such circumstances fragrances may be used without introducing an increased rate of spontaneous complaints of skin reactions.

Bard, M., M. R. Albrecht, et al. (1988). “Geraniol interferes with membrane functions in strains of Candida and Saccharomyces.” Lipids 23(6): 534-538. Geraniol, an olefinic terpene, inhibited growth of 3 C. albicans and 1 S. cerevisiae isolates. Geraniol enhanced the rate of potassium leakage out of whole cells and also was shown by fluorescence polarization, to increase C. albicans membrane fluidity. Biophysical studies using differential scanning calorimetry, fluorescence polarization and osmotic swelling of phospholipid vesicles demonstrated that geraniol decreased the phase-transition temperature of dipalmitoylphosphatidylcholine vesicles, affected fluidity throughout the bilayer, particularly the central portion of the bilayers, and caused an increase in bilayer permeability to erythritol. It is suggested that geraniol may have potential use as an antifungal agent.

Beuerman, R. W. (1975). “Slow potentials of the turtle olfactory bulb in response to odor stimulation of the nose.” Brain Res 97(1): 61-78. Odor stimulation of the nose in the box turtle and the gopher tortoise produced a characteristic series of slow potentials in the olfactory bulb which were referred to as the odor evoked response. When recorded with direct coupling, the odor evoked response had 3 components: wave I, a short duration monophasic event; wave II, a long duration variation in the DC potential; and wave III, an oscillatory potential superimposed on wave II. Waves I and II were negative at bulbar surfaces receiving olfactory input and positive deep within the bulb. This series of potentials could be evoked by 3 methods of odor stimulation: (1) large puffs delivered from odorant test bottles, (2) small puffs delivered from a syringe and (3) continuous flow with concentration and nasal flow rate parameters controlled by an olfactometer. When the odor evoked response was recorded at a bulbar locus, these potentials were seen in response to each stimulation and the amplitudes of each wave were reproducible with the same stimulus. The amplitudes of the 3 waves were compared in the gopher tortoise and differed with the 3 odorants tested--high purity geraniol, technical grade geraniol and amyl acetate. Odorant concentration also directly affected the response amplitudes of all 3 wave components. The amplitudes of waves I and III markedly decreased with closely spaced stimulations recovering to near the initial values when the interstimulus interval was increased severalfold. This series of sensory evoked potentials is considered to reflect the processing of odor information from the olfactory receptors by the olfactory bulb.

Beytia, E., P. Valenzuela, et al. (1969). “Terpene biosynthesis: formation of nerol, geraniol, and other prenols by an enzyme system from Pinus radiata seedlings.” Arch Biochem Biophys 129(1): 346-56. Binder, G., T. v. d. Berg, et al. (1996). “Regeneration of plants and production of volatiles from callus cultures of Melissa officinalis L. 3. Effect of exogenous growth regulators on essential oil composition.” Angewandte Botanik 70(5/6): 181-184. Regenerates of lemon balm (M. officinalis) were established as an in vitro-system to study the effects of exogenous growth regulators (NAA, BAP [benzyladenine] and ABA) on morphology and essential oil composition. Long term cultivation resulted only in changes of minor essential oil constituents; citronellal, citronellol, nerol and geraniol contents were elevated, whereas geranyl acetate content was reduced. The essential oil composition of plants growing in the presence of NAA exhibited similar changes in citronellal, citronellol and geranyl acetate, i.e. like plants at an advanced stage of development. Supplementation of the medium with a high BAP concentration induced plants to accumulate >10 % alloaromadendrene, a sesquiterpene hydrocarbon found only as a trace compound in control plants and not described before for the leaf essential oil of naturally grown M. officinalis. ABA had a slight effect on the production of some minor compounds, and showed a synergistic effect in combination with BAP.

Boyd, E. M. and E. P. Sheppard (1970). “The effect of inhalation of citral and geraniol on the output and composition of respiratory tract fluid.” Arch Int Pharmacodyn Ther 188(1): 5-13. Buchbauer, G., L. Jirovetz, et al. (1993). “Fragrance compounds and essential oils with sedative effects upon inhalation.” Journal of Pharmaceutical Sciences 82(6): 660-664. In experiments with female 6- to 8-week-old Swiss mice [see also Planta Medica (1987) 53, 315-318], a total of 44 fragrance compounds and essential oils, obtained from Dragoco Company (Vienna, Austria) and known to possess sedative properties, were screened for their potential aromatherapeutic value when administered by inhalation. The motility of untreated mice was compared with that of mice exposed to a specific compound after no pretreatment or after a caffeine-induced overagitation treatment. Compared with the motility of untreated mice (100%) that of mice exposed to lavender [Lavandula sp.] oil, neroli [Citrus aurantium] oil, linalool, linalyl acetate, citronellal, benzaldehyde, 2-phenylethyl acetate, alpha-terpineol and sandalwood [Santalum album] oil was decreased by 78.4, 65.3, 73.0, 69.1, 49.8, 43.7, 45.0, 45.0 and 40.0%, respectively. In contrast, an increased motility was observed after exposure to geraniol, isoborneol, isoeugenol, orange [Citrus sp.] terpenes and thymol. The sedative effect of lavender oil, isoeugenol, linalool, maltol, carvone and linalyl acetate counteracted caffeine-induced overagitation; overagitation was amplified by anthranilic acid methyl ester, farnesol, lime (Tilia sp.) blossom oil and nerol inhalation. Serum samples, taken shortly after the inhalation treatment, were analysed by GC-MS, GC-fourier transform infrared and GC-flame inonization techniques in order to identify active constituents. A total of 21 substances were identified at concentrations of up to 0.1 ng/ml serum. Correlations of the aroma detection thresholds and sedative properties associated with these substances indicated that there might be a direct pharmacological interaction of fragrance molecules with body tissues rather than a reflective interaction caused by a pleasant feeling.

Buckley, D. A., S. H. Wakelin, et al. (2000). “The frequency of fragrance allergy in a patch-test population over a 17-year period.” Br J Dermatol 142(2): 279-83. Fragrances are widely encountered in our daily environment and are known to be a common cause of allergic contact dermatitis. We have reviewed our patch test data from 1980 to 1996 to establish whether the pattern of fragrance allergy has changed with time. During this period, 25,545 patients (10,450 male, 15,005 female) were patch tested with the European standard series. The mean annual frequency of positive reactions to the fragrance mix was 8.5% in females (range 6.1-10.9) and 6.7% in males (range 5.1-12.9). Females were 1.3 times more likely to be allergic to fragrance ($P < 0.001$, 95% confidence interval, CI 1.17-1.41). Males with fragrance allergy were older than females by 5.6 years (mean age 48.2 vs. 42.6 years; $P < 0.001$, 95% CI 3.9-7.3). The incidence of a concomitant positive patch test to balsam of Peru in fragrance-sensitive patients showed wide variation, suggesting that it is not a reliable marker of fragrance allergy. There was a positive correlation between the isomers isoeugenol and eugenol. Oak moss remained the most common overall allergen throughout the study, positive in 38.3% of females and 35.6% of males who were tested to the constituents of the fragrance mix. During the period of the study the incidence of positive tests to oak moss increased by 5% yearly ($P = 0.001$, 95% CI 2.2-8.7). The frequency of allergic reactions to eugenol and geraniol remained relatively constant. Isoeugenol and alpha-amyl cinnamic aldehyde sensitivity increased and hydroxycitronellal showed a slow decline. There was a striking reduction in the frequency of sensitivity to cinnamic aldehyde (by 18% yearly; $P < 0.001$, 95% CI 14.3-21.0) and cinnamic alcohol (by 9% yearly; $P < 0.001$, 95% CI 5.2-12.9); these are now uncommon fragrance allergens. These data show temporal trends which may reflect the frequency of population exposure to individual fragrances.

Burke, Y. D., M. J. Stark, et al. (1997). “Inhibition of pancreatic cancer growth by the dietary isoprenoids farnesol and geraniol.” Lipids 32(2): 151-6. Fruits and vegetables have protective effects against many human cancers, including pancreatic cancer. Isoprenoids are one class of phytochemicals which have antitumor activity, but little is known about their effects on cancer of the pancreas. We tested the hypothesis that isoprenoids would inhibit the growth of pancreatic tumor cells. Significant (60-90%) inhibition of the anchorage-independent growth of human MIA PaCa2 pancreatic tumor cells was attained with 25 microM farnesol, 25 microM geranylgeraniol, 100 microM perillyl amine, 100 microM geraniol, or 300 microM perillyl alcohol. We then tested the relative in vivo antitumor activities of dietary farnesol, geraniol, and perillyl alcohol against transplanted PC-1 hamster pancreatic adenocarcinomas. Syrian Golden hamsters fed geraniol or farnesol at 20 g/kg diet exhibited complete inhibition of PC-1 pancreatic tumor growth. Both farnesol and geraniol were more potent than perillyl alcohol, which inhibited tumor growth by 50% at 40 g/kg diet. Neither body weights nor plasma cholesterol levels of animals consuming isoprenoid diets were significantly different from those of pair-fed controls. Thus, farnesol, geraniol, and perillyl alcohol suppress pancreatic tumor growth without significantly affecting blood cholesterol levels. These dietary isoprenoids warrant further investigation for pancreatic cancer prevention and treatment.

Cain, W. S., F. T. Schiet, et al. (1995). “Comparison of models of odor interaction.” Chem Senses 20(6): 625-37. Subjects rated the overall perceived intensity of concentrations of the odorants cineole, geraniol, hexyl salicylate, and linalyl acetate smelled alone and in binary mixtures. The subjects also rated intensity of specified constituents (e.g. amount of cineole in cineole, and in mixtures of cineole and linalyl acetate). The intensity of the stronger component alone offered a close description of perceived intensity. In addition to the Stronger Component model, two other psychological models (Vector and U model) and two psychophysical models (UPL2 and Equiratio Mixture model) offered descriptions ranging from fair to very good. Psychological models gave better fits, but lack explanatory power. Some results indicated that weaker odors add more potently than stronger odors, an outcome incompatible with these models. The psychophysical models, based on the additivity of single components, generally overestimated perceived intensity. Judgments of individual qualities gave only slight encouragement to any expectation of differences in masking or maskability among odorants. The results highlight the need to test particular critical hypotheses regarding how people perceive mixtures.

Calnan, C. D., E. Cronin, et al. (1981). “Allergy to phenyl salicylate.” Contact Dermatitis 7(4): 208-11. Six cases of contact dermatitis from a lip salve are described. Five were allergic to phenyl salicylate and one to geraniol in the fragrance. The dermatitis spread in a ring around the mouth. Phenyl salicylate has been removed from the formulation.

Cardullo, A. C., A. M. Ruszkowski, et al. (1989). “Allergic contact dermatitis resulting from sensitivity to citrus peel, geraniol, and citral.” J Am Acad Dermatol 21(2 Pt 2): 395-7. A bartender with hand dermatitis had allergic contact sensitivity to the skin of lemon, lime, and orange but not to their juices. Although most reported cases of citrus peel allergy are due to d-limonene, for our patient, reactions to patch tests for geraniol and citral, two minor components of citrus peel oil, were positive, whereas those for d-limonene were negative. Contact allergy to citrus peel oil should be considered in patients with hand dermatitis who are occupationally exposed to citrus fruits.

Carnesecchi, S., K. Langley, et al. (2002). “Geraniol, a component of plant essential oils, sensitizes human colonic cancer cells to 5-Fluorouracil treatment.” J Pharmacol Exp Ther 301(2): 625-30. Differentiation of human colonic cancer cells at confluency has been correlated to their increased resistance to chemotherapeutic agents. The aim of this study was to determine whether blocking Caco-2 cell differentiation could sensitize the cells to 5-fluorouracil (5-FU) treatment. We show that in cells at confluency, geraniol (400 microM) prevented the formation of brush-border membranes and inhibited the expression of intestinal hydrolases (sucrase, lactase, alkaline phosphatase). The antiproliferative effect of geraniol (400 microM) together with 5-FU (5 microM) was twice that of 5-FU alone. The cytotoxicity induced by 5-FU was enhanced in the presence of geraniol, as shown by a 50% increase of lactate dehydrogenase release in the culture medium. These effects are related to enhanced intracellular accumulation of 5-FU in the presence of geraniol as shown by a 2-fold increase in intracellular 5-[6-(3)H]FU (1.5 microCi/ml). It is concluded that geraniol sensitizes colonic cancer cells to 5-FU treatment, by increasing the cytotoxicity of the drug, and that this results from the

facilitated transport of 5-FU and the blockade of the morphological and functional differentiation of the cancer cells.

Carnesecchi, S., Y. Schneider, et al. (2001). “Geraniol, a component of plant essential oils, inhibits growth and polyamine biosynthesis in human colon cancer cells.” J Pharmacol Exp Ther 298(1): 197-200. Geraniol and other monoterpenes found in essential oils of fruits and herbs have been suggested to represent a new class of agents for cancer chemoprevention. As a first step in clarifying the mode of action of geraniol on colon carcinogenesis, we studied its effects on the growth of a human colon cancer cell line (Caco-2). Geraniol (400 microM) caused a 70% inhibition of cell growth, with cells accumulating in the S transition phase of the cell cycle, and concomitant inhibition of DNA synthesis. No signs of cytotoxicity or apoptosis were detected. Geraniol caused a 50% decrease of ornithine decarboxylase activity, a key enzyme of polyamine biosynthesis, which is enhanced in cancer growth. This led to a 40% reduction of the intracellular pool of putrescine. Geraniol also activated the intracellular catabolism of polyamines, indicated by enhanced polyamine acetylation. These observations indicate that polyamine metabolism is presumably a target in the antiproliferative properties of geraniol.

Carriere, F., G. Gil, et al. (1989). “Biotransformation of geraniol by photoautotrophic, photomixotrophic and heterotrophic plant cell suspensions.” Phytochemistry 28(4): 1087-1090. Cell suspension cultures of 4 plant species (Euphorbia characias, Nicotiana tabacum, Catharanthus roseus and Glycine max) were maintained under different carbon and energy supply regimes, i.e. photoautotrophy, photomixotrophy and heterotrophy, and were assayed for their capacity to biotransform geraniol. The reactions of interconversion of geraniol into nerol, or oxidation of alcohols into their respective aldehydes, were mainly determined by the species, regardless of the modes of culture. A rapid metabolism of monoterpenic alcohols to unidentified compounds was observed. In one cell suspension (G. max), a high biotransformation activity (40-60%) into neral and geranial was detected.

Chadha, A. and K. M. Madyastha (1984). “Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes.” Xenobiotica 14(5): 365-74. Metabolites isolated from the urine of rats after oral administration of geraniol (I) were: geranic acid (II), 3-hydroxy-citronellic acid (III), 8-hydroxy-geraniol (IV), 8-carboxy-geraniol (V) and Hildebrandt acid (VI). Metabolites isolated from urine of rats after oral administration of linalool (VII) were 8-hydroxy-linalool (VIII) and 8-carboxy-linalool (IX). After three days of feeding rats with either geraniol or linalool, liver-microsomal cytochrome P-450 was increased. Both NADH- and NADPH-cytochrome c reductase activities were not significantly changed during the six days of treatment. Oral administration of these two terpenoids did not affect any of the lung-microsomal parameters measured.

Chagonda, L. S., C. Makanda, et al. (2000). “The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe.” Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils

from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Chang, Y. C. and H. I. Maibach (1997). “Pseudo flautist's lip: allergic contact cheilitis from geraniol.” Contact Dermatitis 37(1): 39. Charles, D. J. and J. E. Simon (1993). “Changes in essential oil content and composition with leaf development in Ocimum gratissimum L.” Acta Horticulturae(344): 421-427. O. gratissimum has important medicinal, antimicrobial and anthelmintic properties. The leaves from a mature plant of a geraniol chemotype were analysed for essential oil content. The relative percentage of geraniol in the oil increased from 51.6% in the young leaves to 73.3% in mature leaves, then decreased to 64.2% during senescence. The sesquiterpene content was 42.9, 20.2 and 26.9% in young, mature and senescing leaves, respectively. Most of the 17 constituents identified in the oil were present at all stages of leaf development. It is thought that this chemotype might have potential for the perfume and flavour industries.

Chaumont, J. P. and D. Leger (1992). “[Campaign against allergenic moulds in dwellings. Inhibitor properties of essential oil of Geranium 'Bourbon', citronellol, geraniol and citral].” Ann Pharm Fr 50(3): 156-66. Many fungal airborne spora show allergenic effects. Indoor (dwelling, work rooms, hospital chambers) can be disinfected by elimination of living particles. We have undertaken experiments in more and more spacious bulks for evaluation of the antifungal effects of vapours of essential oils and some volatiles compounds. Results show that the Mucorales and Geotrichum resist strongly. On the contrary, the Cladosporium strains, some Aspergillus and Penicillium, Trichothecium roseum are the most sensitive, specially towards the citral vapours. Experiments in hospital can be undertaken.

Chinou, I. B., V. Roussis, et al. (1996). “Chemical and biological studies on two Helichrysum species of Greek origin.” Planta Medica 62(4): 377-379. The essential oils obtained from the aerial parts of H. amorginum and H. italicum (collected from Amorgos, Greece) were analysed by GC and GC-MS. From the 25 identified constituents representing 89.98 and 82.06% of the 2 oils respectively, geraniol, geranyl acetate, neryl acetate and nerolidol were the major components. The essential oils and geraniol, geranyl acetate and neryl acetate exhibited antibacterial activity against Staphylococcus aureus, S.

epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae and Escherichia coli.

Chinou, I. B., V. Roussis, et al. (1997). “Chemical and antibacterial studies of two Helichrysum species of Greek origin.” Planta Med 63(2): 181-3. The chemical composition of the essential oils obtained from the aerial parts of Helichrysum stoechas ssp. barrelieri and H. taenari was analysed by GC and GC/MS. From the thirty-nine identified constituents representing the 73.87% and 87.41% of the two oils, respectively, beta-elemene, beta-caryophyllene, geraniol, and camphene were the major components. Furthermore, it was found that the oils exhibited significant antibacterial activity against six Gram (+/-) bacteria.

Chirkina, N. N. and E. A. Osipova (1974). “The antimicrobial properties of essential oil of Helichrysum italicum grown in the Crimea.” Nauchnye Doklady Vysshei Shkoly, Biologicheskie Nauki 17(1): 86-89. Attributed to the presence of nerol, geraniol, eugenol and carbonyl compounds.

Chiruvolu, S., H. E. Warriner, et al. (1994). “A phase of liposomes with entangled tubular vesicles.” Science 266(5188): 1222-5. An equilibrium phase belonging to the family of bilayer liposomes in ternary mixtures of dimyristoylphosphatidylcholine (DMPC), water, and geraniol (a biological alcohol derived from oil-soluble vitamins that acts as a cosurfactant) has been identified. Electron and optical microscopy reveal the phase, labeled Ltv, to be composed of highly entangled tubular vesicles. In situ x-ray diffraction confirms that the tubule walls are multilamellar with the lipids in the chain-melted state. Macroscopic observations show that the Ltv phase coexists with the well-known L4 phase of spherical vesicles and a bulk L alpha phase. However, the defining characteristic of the Ltv phase is the Weissenberg rod climbing effect under shear, which results from its polymer-like entangled microstructure.

Chmiel, A. (2001). “Industrial biotechnology of medicinal plants and problems with its commercialization.” Annales Universitatis Mariae Curie-Sklodowska. Sectio EEE, Horticultura 9(Supplementum): 1-18. The present state of both plant-derived medicines and biotechnology of medicinal plants is discussed. An example of ginseng shows that traditional wild medicinal plants, provided they have been properly investigated chemically and pharmacologically, can be fully accepted by modern Western medicine. For such plants, even if they disappear in their natural environment, land field cultivation can be developed and their cells can be cultivated in industrial installations. At present, the largest plant cell bioreactors have a volume of 20 to 75 m3. For many biological products, it is possible to produce them in a bioreactor in much greater concentrations of the pharmacologically active components (for example, over 20% of rosmarinic acid in dry cell mass of Coleus blumei) than when they are present in genuine plant material (approx equal to 1% for rosmarinic acid). Other examples of high-yielding biotechnologies are biosynthesis of berberine, geraniol, ginsenosides, indole alkaloids, nicotine, peroxidase, sanquinarin and shikonin. Biotechnologies of all those plant metabolites have been developed at an industrial scale. However, only a few of them are industrially produced at present. A key factor for medicinal plant biotechnology is purely

economical, i.e. relationships between the selling price, market size and production costs of products. Only those biotechnological processes which ensure high yields of expensive products for the market can be realized in long-term industrial production.

Choi, H. S., H. S. Song, et al. (2000). “Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl.” J Agric Food Chem 48(9): 4156-61. Thirty-four kinds of citrus essential oils and their components were investigated for radical-scavenging activities by the HPLC method using 1,1-diphenyl-2-picrylhydrazyl (DPPH). To examine the oils' relative radical-scavenging activities compared with that of a standard antioxidant, Trolox was employed. All of the essential oils were found to have scavenging effects on DPPH in the range of 17. 7-64.0%. The radical-scavenging activities of 31 kinds of citrus essential oils were comparable with or stronger than that of Trolox ($p < 0.05$). The oils of Ichang lemon (64.0%, 172.2 mg of Trolox equiv/mL), Tahiti lime (63.2%, 170.2 mg of Trolox equiv/mL), and Eureka lemon (61.8%, 166.2 mg of Trolox equiv/mL) were stronger radical scavengers than other citrus oils. Citrus volatile components such as geraniol (87.7%, 235.9 mg of Trolox equiv/mL), terpinolene (87.4%, 235.2 mg of Trolox equiv/mL), and gamma-terpinene (84.7%, 227.9 mg of Trolox equiv/mL) showed marked scavenging activities on DPPH ($p < 0.05$).

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Colli, S., S. Eligini, et al. (1997). “Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis.” Arterioscler Thromb Vasc Biol 17(2): 265-72. We examined the effect of fluvastatin, the first entirely synthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor that is structurally different from other vastatins, on tissue factor (TF) expression in human macrophages spontaneously differentiated in culture from blood monocytes. Fluvastatin decreased TF activity in a dose-dependent manner (1 to 5 mumol/L) in both unstimulated and lipopolysaccharide-stimulated macrophages, and this reduction paralleled the decrease in immunologically recognized TF protein. The same results were obtained with another lipophilic vastatin, simvastatin, but not with hydrophilic pravastatin. The reduction in TF expression was also observed in macrophages enriched in cholesterol after exposure to 50 micrograms/mL acetylated low density lipoprotein. The inhibitory effect of fluvastatin on TF activity and antigen was fully reversible by coincubation with 100 mumol/L mevalonate or 10 mumol/L all-trans-geranylgeraniol but not with dolichol, farnesol, or

geraniol. Suppression of TF antigen and activity was accompanied by a diminution in TF mRNA levels, which was completely prevented by mevalonate. Furthermore, fluvastatin impaired bacterial lipopolysaccharide-induced binding of c-Rel/p65 heterodimers to a kappa B site in the TF promoter, indicating that this drug influences induction of the TF gene. We conclude that lipophilic vastatins inhibit TF expression in macrophages, and because this effect is prevented by mevalonate and geranylgeraniol, a geranylgeranylated protein plays a crucial role in the regulation of TF biosynthesis. The suppression of TF in macrophages by vastatins indicates a potential mechanism by which these drugs interfere with the formation and progression of atherosclerotic plaque as well as thrombotic events in hyperlipidemic patients.

Collu, G., H. H. J. Bink, et al. (1999). “Determination of the activity of the cytochrome p450 enzyme geraniol 10-hydroxylase in plants by high-performance liquid chromatography.” Phytochemical Analysis 10(6): 314-318. The cytochrome P450 enzyme geraniol 10-hydroxylase plays an important role in the biosynthesis of pharmaceutically important alkaloids in Catharanthus roseus. An HPLC assay was developed for this enzyme based on the UV detection of the product 10-hydroxygeraniol after its separation from the substrate geraniol on a reversed-phase C-18 column. This system can be used for the UV detection of nerol, which is also a substrate for geraniol 10-hydroxylase, and its product 10- hydroxynerol. The presence of interfering enzymes which epoxidize rather than hydroxylate geraniol and nerol could also be detected. The developed HPLC assay was validated with respect to the incubation time (linear up to 45 min) and the amount of protein added per incubation (linear up to 400 micro g of protein). The HPLC assay will be a useful tool during the purification of geraniol 10-hydroxylase from cell suspension cultures of C. roseus.

Collu, G., N. Unver, et al. (2001). “Geraniol 10-hydroxylase, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis.” FEBS Lett 508(2): 215-20. Geraniol 10-hydroxylase (G10H) is a cytochrome P450 monooxygenase involved in the biosynthesis of iridoid monoterpenoids and several classes of monoterpenoid alkaloids found in a diverse range of plant species. Catharanthus roseus (Madagascar periwinkle) contains monoterpenoid indole alkaloids, several of which are pharmaceutically important. Vinblastine and vincristine, for example, find widespread use as anti-cancer drugs. G10H is thought to play a key regulatory role in terpenoid indole alkaloid biosynthesis. We purified G10H from C. roseus cells. Using degenerate PCR primers based on amino acid sequence information we cloned the corresponding cDNA. The encoded CYP76B6 protein has G10H activity when expressed in C. roseus and yeast cells. The stress hormone methyljasmonate strongly induced G10h gene expression coordinately with other terpenoid indole alkaloid biosynthesis genes in a C. roseus cell culture.

Cometto-Muniz, J. E., W. S. Cain, et al. (1998). “Trigeminal and olfactory chemosensory impact of selected terpenes.” Pharmacol Biochem Behav 60(3): 765-70. In Experiment 1, four normosmics and four anosmics (three congenital, one idiopathic) provided odor and nasal pungency thresholds, respectively, for the following terpenes: delta3-carene, p-cymene, linalool, 1.8-cineole, and geraniol, plus the structurally related compound

cumene. Additionally, all subjects provided nasal localization (i.e., right/left) and eye irritation thresholis. Trigeminally mediated thresholds (i.e., nasal pungency, nasal localization, and eye irritation) lay about three orders of magnitude above odor thresholds, which ranged between 0.1 and 1.7 ppm. The results implied uniform chemesthetic sensitivity across tasks and sites of impact. In Experiment 2, normosmics and anosmics provided odor and nasal pungency thresholds, respectively, for three pairs of isomeric terpenes: alpha- and gamma-terpinene, alpha- and beta-pinene, and R(+)- and S(-)-limonene. Odor thresholds ranged between 1.4 and 19 ppm, that is, about an order of magnitude higher than those of the previous terpenes, with no substantial differences between odor thresholds of members of a pair. Regarding chemesthetic impact, only alpha-terpinene evoked nasal pungency. The overall outcome suggests comparable trigeminal chemosensitivity between nose and eyes and between normosmics and anosmics, as shown before for homologous n-alcohols. It also lends support to a previously derived solvation model of the chemesthetic potency of airborne substances, and indicates the likely importance of certain molecular-size restrictions for effective trigeminal impact.

Contin, A., G. Collu, et al. (1999). “The effects of phenobarbital and ketoconazole on the alkaloid biosynthesis in Catharanthus roseus cell suspension cultures.” Plant Physiology and Biochemistry (Paris) 37(2): 139-144. The cytochrome P-450 enzyme geraniol 10-hydroxylase plays an important role in the biosynthesis of terpenoid indole alkaloids in suspension cultures of C. roseus. Enzyme activity was induced by the treatment of cells with phenobarbital, and inhibited by treatment with ketoconazole. Alkaloid accumulation increased after phenobarbital treatment whereas it decreased after ketoconazole treatment. Phenobarbital and ketoconazole did not affect the in vivo conversion rate of loganin to secologanin, a reaction proposed to be catalysed by a cytochrome P450 enzyme.

Correll, C. C., L. Ng, et al. (1994). “Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase.” J Biol Chem 269(26): 17390-3. The degradation of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is highly regulated and is dependent on both a sterol and non-sterol derivative of mevalonic acid (MVA). We recently proposed that the non-sterol component is derived from farnesyl diphosphate (FPP), presqualene pyrophosphate, or squalene (Correll, C. C. and Edwards, P. A. (1994) J. Biol. Chem. 269, 633-638). In the current study, we have used digitonin-permeabilized cells to further define this MVA-derived non-sterol component required for the regulated degradation of HMG-CoA reductase. The addition of either FPP or farnesol to digitonin-permeabilized cells resulted in a rapid and dose-dependent degradation of HMG-CoA reductase. The effect of FPP, but not farnesol, was blocked by the phosphatase inhibitor sodium fluoride. The enhanced degradation of HMG-CoA reductase in permeabilized cells specifically required farnesol, since the addition of any of the structurally related isoprenoids geraniol, geranyl diphosphate, geranylgeranyl diphosphate, nerolidol, or all-cis-farnesol, or of the non-sterol squalene to the permeabilized cells did not stimulate enzyme degradation. The present studies demonstrate for the first time that the accelerated degradation of HMG-

CoA reductase can be initiated in vitro. Further, since farnesol is shown to be specifically required for the enhanced degradation of the enzyme in vitro, we propose that this isoprenoid alcohol is important in this process in intact cells.

Corsini, A., M. Mazzotti, et al. (1993). "Relationship between mevalonate pathway and arterial myocyte proliferation: in vitro studies with inhibitors of HMG-CoA reductase." Atherosclerosis 101(1): 117-25. The role of mevalonate and its products (isoprenoids) in the control of cellular proliferation was examined by investigating the effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (vastatins) on growth and on cholesterol biosynthesis of cultured arterial myocytes (SMC). Simvastatin (S) and fluvastatin (F), but not pravastatin (P), decreased the rate of growth of rat vascular SMC. The inhibition, evaluated as cell number, was dose-dependent with IC50 values of 2.8 and 2.2 microM for S and F, respectively; P (1-500 microM) was inactive. The inhibition of cell growth induced by 3.5 microM S (70% decrease) was prevented completely by the addition of 100 microM mevalonate, partially (70-85%) by the addition of 10 microM geraniol, 10 microM farnesol and 5 microM geranylgeraniol, but not by the addition of squalene, confirming the specific role of isoprenoid metabolites in regulating cell proliferation. All the tested vastatins inhibited the incorporation of [14C]acetate into cholesterol but P had 800 times lower potency than S and F. Similar results were obtained in SMC from human femoral artery. At least 80% inhibition of cholesterol synthesis was necessary to induce a decrease in SMC proliferation. To further investigate the relationship between cholesterol synthesis and cell growth, two enantiomers of F were investigated. The enantiomer more active on HMG-CoA reductase was 70- and 1.6-fold more potent on arterial myocyte proliferation than its antipode and the racemic mixture, respectively.

Crowell, P. L. (1999). "Prevention and therapy of cancer by dietary monoterpenes." J Nutr 129(3): 775S-778S. Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity. For example, d-limonene, which comprises >90% of orange peel oil, has chemopreventive activity against rodent mammary, skin, liver, lung and forestomach cancers. Similarly, other dietary monoterpenes have chemopreventive activity against rat mammary, lung and forestomach cancers when fed during the initiation phase. In addition, perillyl alcohol has promotion phase chemopreventive activity against rat liver cancer, and geraniol has in vivo antitumor activity against murine leukemia cells. Perillyl alcohol and d-limonene also have chemotherapeutic activity against rodent mammary and pancreatic tumors. As a result, their cancer chemotherapeutic activities are under evaluation in Phase I clinical trials. Several mechanisms of action may account for the antitumor activities of monoterpenes. The blocking chemopreventive effects of limonene and other monoterpenes during the initiation phase of mammary carcinogenesis are likely due to the induction of Phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification. The post-initiation phase, tumor suppressive chemopreventive activity of monoterpenes may be due to the induction of apoptosis and/or to inhibition of the post-translational isoprenylation of cell growth-regulating proteins. Chemotherapy of chemically induced mammary tumors with monoterpenes results in tumor redifferentiation concomitant with

increased expression of the mannose-6-phosphate/insulin-like growth factor II receptor and transforming growth factor beta1. Thus, monoterpenes would appear to act through multiple mechanisms in the chemoprevention and chemotherapy of cancer.

Cruz, T., M. M. Cabo, et al. (1993). "Chemical composition and antimicrobial activity of the essential oils of different samples of Thymus baeticus Boiss." Phytotherapy Research 7(1): 92-94. The aerial parts of female and hermaphrodite plants were collected during the flowering period from 2 sites in Granada, Lanjaron and Ugijar. The components of the essential oils were determined by GC (data tabulated). No phenolic components were detected in any of the samples. There were marked quantitative differences in oil composition between the 2 geographic sources. The main constituent of the essential oil of hermaphrodite plants from Ugijar was 1,8-cineole (21.4%), and these plants also contained limonene (4.5%), camphor (0.5%), and bornyl acetate (0.7%) which were not detected in the oil from female plants from this area. The main constituent of the essential oil obtained from female plants from Ugijar was terpinen-4- ol (22.8%). The main constituents of the essential oil of hermaphrodite plants from Lanjaron were geraniol (20.7%) and 1,8- cineole (15.8); the main constituent of the essential oil of female plants from Lanjaron was 1,8-cineole (20.9%). In assays against 8 species of human pathogenic bacteria, and 1 yeast (Candida albicans), all the oils showed considerable activity against most of the strains. Only Salmonella typhimurium and Staphylococcus aureus were unaffected by any oil at the highest concentration tested, 175 mug/ml. Antimicrobial activity was greatest in oils with a high geraniol content.

Dagnino, D., J. Schripsema, et al. (1995). "Terpenoid indole alkaloid biosynthesis and enzyme activities in two cell lines of Tabernaemontana divaricata." Phytochemistry 39(2): 341-349. The possible limitation of the rate of biosynthesis of terpenoid indole alkaloids was investigated in 2 cell lines of T. divaricata with different terpenoid indole alkaloid biosynthetic capacities. The activities of tryptophan decarboxylase (TDC), strictosidine synthase (SSS), strictosidine glucosidase (SG), isopentenyl pyrophosphate isomerase (IPP) and geraniol 10-hydroxylase (G10H) were compared. The activities of TDC, SSS and IPP did not show a direct relationship with biosynthetic capacity, but SG and G10H activities might be limiting. Loganin was fed to the cultures to determine whether the availability of terpenoid precursors limits the biosynthesis of terpenoid indole alkaloids. Loganin-feeding did not influence any of the measured enzyme activities, but increased the accumulation of terpenoid indole alkaloids to similar concentrations in both cell lines. A 5-fold increase was observed for the accumulating line; a >100-fold increase was observed for the low-accumulating one. Strictosidine accumulated mainly in the low-accumulating cell line which has high TDC activity and low SG activity; the amounts and types of the other terpenoid indole alkaloids which accumulated were similar in both lines. It is concluded that the biosynthesis of terpenoid indole alkaloids in both cell lines is limited by the availability of terpenoid precursors; this pathway is not saturated with substrates under normal culture conditions.

Dai JunGui, Zhu WeiHua, et al. (2000). "Effects of precursors and fungal elicitors on GKB production in suspension cultured cells of Ginkgo biloba L." Acta Pharmaceutica Sinica 35(2): 151-155. The effects of precursors and fungal elicitors on ginkgolide B

(GKB) production by suspension cultured cells of G. biloba were investigated. Precursors and fungal elicitors were added to the media. Their effects on the biomass and GKB yields of the suspension of cultured cells were studied. The total GKB yields were enhanced 69, 13.8 and 11.4% compared with the control by adding 100 mg/litre isoprene and low concentrations (10 and 50 mg/litre) of geraniol in media, respectively. Of the 10 investigated fungal elicitors, mycelium extract of Rhizopus japonicus was the best.

de Groot, A. C., A. M. van der Kley, et al. (1993). "Frequency of false-negative reactions to the fragrance mix." Contact Dermatitis 28(3): 139-40. To estimate the frequency of false-negative reactions to the fragrance mix, the 8 constituents of the mix in concentrations of 5% (2% for cinnamic aldehyde) were added to the European standard series for routine testing. Patients with positive reactions to individual ingredients in the absence of a reaction to the mix were retested with serial dilutions. In a 4-month period, 677 patients were tested. 61 (9%) reacted to the mix and to 1 or more of the ingredients. 4 patients (0.6% of all patients tested and 6.2% of the patients allergic to fragrances) had false-negative reactions to the mix. They were allergic to cinnamic alcohol, geraniol, isoeugenol and oak moss (1 reaction each), in the absence of a reaction to the fragrance mix. It is concluded that the currently used concentration of the mix (8 x 1%) not infrequently results in false-negative reactions, and that further research should be done to overcome this problem.

de Groot, A. C., D. H. Liem, et al. (1985). "Patch tests with fragrance materials and preservatives." Contact Dermatitis 12(2): 87-92. 179 patients suspected of cosmetic allergy were patch tested with a series of 16 fragrance materials and 9 preservatives. In 67 patients (37.4%), 1 or more of these substances gave positive reactions. In the group of fragrance materials, the largest numbers of positive patch test reactions were seen to isoeugenol, oak moss, geraniol, alpha-amylcinnamic alcohol, and a mixture of alpha-amylcinnamic aldehyde and alpha-hexylcinnamic aldehyde. The fragrance mix in the ICDRG standard series detected nearly 80% of cases of contact allergy to fragrance materials other than its constituents. In the group of preservatives, Kathon CG and quaternium-15 scored the highest number of positive reactions. It is argued that the commonly used patch test concentrations of 2% for oak moss and geraniol may be too low to detect all cases of sensitization.

de Montellano, P. R., J. S. Wei, et al. (1977). "Inhibition of squalene synthetase by farnesyl pyrophosphate analogues." J Med Chem 20(2): 243-9. The pyrophosphates of the following farnesol analogues have been synthesized: 2-methylfarnesol; 7,11-dimethyl-3-ethyl-2,6,10-dodecatrien-1-ol; 3-demethylfarnesol; 4-methylthiofarnesol; 7,11-dimethyl-3-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyl02-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyldodeca-6,10-dien-2-yn-1-ol; phytol; 3,7,11-trimethyl-2-dodecen-1-ol; 3,7,11-trimethyldodecan-1-ol; and geraniol. The double bonds in all the above compounds were in the E configuration, except phytol, which was a 7:3 mixture of 2E and 2Z isomers. Each of the pyrophosphates inhibits the incorporation of labeled farnesyl pyrophosphate into squalene by a yeast enzyme preparation. Free alcohols and monophosphates are inactive. The analogues, listed in order of decreasing inhibitory strength, are, by kinetic analysis, competitive or mixed inhibitors. Irreversible inhibition

is not observed. The results suggest that binding to the enzyme is primarily mediated by the pyrophosphate moiety assisted by relatively nonspecific lipophilic interactions. Decreasing the chain length and saturating double bonds severely reduces binding, while substitution at the 2,3, and 4 positions, and lengthening of the chain, is well tolerated.

de Ropp, J. S. and F. A. Troy (1984). "Chemical synthesis and 2H NMR investigations of polyisoprenols: dynamics in model membranes." Biochemistry 23(12): 2691-5. Polyisoprenols (PIs) such as dolichol and undecaprenol have been shown to play an important role as enzymatic cofactors in the synthesis of glycoconjugates of both prokaryotic and eukaryotic cells. Presented here is a synthetic route used for obtaining specifically labeled [omega,omega-(C2H3)2]PIs that initiates with the selective oxidation of the omega-terminal double bond of the PI with N-bromosuccinimide. Continuation of the reaction sequence produces an omega-terminal aldehyde three carbons shorter than the original PI. A Wittig reaction with an appropriate deuterium-labeled phosphonium salt is then used to form an omega-terminal-deuterated PI identical with the starting material except for replacement of 1H with 2H at the two omega-terminal methyls of the PI. Deuterium NMR spectra of [omega, omega-(C2H3)2]geraniol and -farnesol incorporated into phospholipid multilamellar vesicles show powder patterns. The quadrupole splitting of the 2H NMR signals was interpretable in terms of the degree of orderedness of the 2H-labeled site. The pure trans isomer geraniol gave rise to a single set of splittings for each C2H3 group while farnesol, a mixture of isomers, showed multiple quadrupole splittings. The quadrupole splittings of the PIs increased with increasing concentration of label and with lowering of temperature. Deuterium NMR T1 measurements, revealing rates of motion of the 2H-labeled site, showed fast motion for [omega,omega-(C2H3)3]geraniol relative to [omega,omega-(C2H3)2]cholesterol under similar conditions. A correlation time of 5 X 10(-10) s was estimated for [omega,omega-(C2H3)2]geraniol, which was 1 order of magnitude faster than for [26,27-(C2H3)2]cholesterol.

Dharmendra Saikia, S. P. S. Khanuja, et al. (2001). "Comparative antifungal activity of essential oils and constituents from three distinct genotypes of Cymbopogon spp." Current Science 80(10): 1264-1266. The antifungal activity of the essential oils of palmarosa (C. martini) cv. CIMAP/PRC-1, lemon grass (C. flexuosus) cv. Pragati and citronella (C. winterianus) cv. BIO-13, as well as some essential oil components, viz. citral, geraniol, citronellol and citronellal, were tested against 4 human pathogenic fungi (Microsporum gypseum, Candida albicans, Sporothrix schenckii, Aspergillus niger) to identify plant substances for future antifungal formulations. Among the essential oils and components tested, lemon grass oil and geraniol, respectively, recorded the highest antifungal activity. M. gypseum was highly sensitive to all the essential oils and tested components, with inhibition zones 1.5- to 2-fold larger than the other fungal pathogens and generally low minimum inhibitory dilutions (MID). Citral produced the smallest inhibition zones but demonstrated the highest activity in terms of MID and minimum fungicidal concentration (MFC) values, which were comparable to lemon grass oil. Lemon grass, palmarosa oil and geraniol recorded the highest inhibition of C. albicans, A. niger and S. schenckii, respectively. However, the highest antifungal activity in terms of MFC was recorded by lemon grass for all organisms tested.

Dorries, K. M., E. Adkins-Regan, et al. (1995). “Olfactory sensitivity to the pheromone, androstenone, is sexually dimorphic in the pig.” Physiol Behav 57(2): 255-9. Sexually dimorphic pheromone pathways have been used successfully to study insect olfactory coding. As one of the few mammalian species with an identified sex pheromone, the domestic pig (Sus scrofa) may be an ideal vertebrate species in which to examine sex differences in olfactory processing of a specific stimulus. In this experiment, androstenone and control odor detection thresholds were measured in adult male, female, and castrated male pigs. In an operant task, pigs were tested with descending concentration series of both androstenone and geraniol. All groups were equally sensitive to geraniol, but there was a sex difference in sensitivity to the odor of androstenone. Female pigs' detection threshold was a dilution fivefold lower than the threshold for intact males. Castrated males did not differ significantly from either males or females. This is the first example of a sexual dimorphism in sensitivity to a mammalian pheromone.

Elson, C. E. and S. G. Yu (1994). “The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables.” J Nutr 124(5): 607-14. Anutritive isoprenoid constituents of fruits, vegetables, cereal grains and essential oils exhibit a spectrum of anticarcinogenic activities. The induction of hepatic Phase II detoxifying activities by dietary isoprenoids appears to underlie their blocking action. The second anticarcinogenic action of the dietary isoprenoids, suppression of the growth of chemically initiated and transplanted tumors is, we suggest, secondary to the inhibition of mevalonate pathway activities. Mevinolin, a competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity, depletes cells of the intermediate products of the pathway that are required for the posttranslational modification of proteins, a process giving the proteins lipophilic anchors that bind to membranes. As a consequence, nuclear lamins and ras oncoproteins remain in nascent states, and cells do not proliferate. gamma-Tocotrienol, perillyl alcohol, geraniol and d-limonene suppress hepatic HMG-CoA reductase activity, a rate-limiting step in cholesterol synthesis, and modestly lower serum-cholesterol levels of animals. These isoprenoids also suppress tumor growth. The HMG-CoA reductase of neoplastic tissues differs from that of sterologenic tissues in being markedly resistant to sterol feedback inhibition. Our review suggests that the mevalonate pathway of tumor tissues is uniquely sensitive to the inhibitory actions of the dietary isoprenoids.

Elson, C. E., G. L. Underbakke, et al. (1989). “Impact of lemongrass oil, an essential oil, on serum cholesterol.” Lipids 24(8): 677-9. To test the hypothesis that non-sterol mevalonate pathway end products lower serum cholesterol levels, we asked 22 hypercholesterolemic subjects (315 +/- 9 mg cholesterol/dl) to take a daily capsule containing 140 mg of lemongrass oil, an essential oil rich in geraniol and citral. The paired difference in serum cholesterol levels of subjects completing the 90-day study approached significance (P less than 0.06, 2-tailed t-test). The subjects segregated into two groups, one consisting of 14 subjects resistant to the protocol and the other consisting of 8 subjects who responded. Paired differences in cholesterol level at 30, 60 and 90 d for resistant subjects were +2 +/- 6, +2 +/- 7 and -1 +/- 6 mg/dl; paired differences for the

responding subjects were -25 +/- 10 (p less than 0.05), -33 +/- 8 (p less than 0.01) and -38 +/- 10 (p less than 0.025), respectively. The paired difference (+8 +/- 4) in the cholesterol levels of six responders 90 days after the discontinuation of lemongrass oil was not significant.

Everitt, Z. M. and G. B. Lockwood (1992). “Biotransformation of geraniol by agitating and immobilised cultures of Anethum graveolens.” Fitoterapia 63(6): 534-536. Free and immobilized suspensions, and callus, derived from seeds of A. graveolens (dill) were grown in supplemented Gamborg's BS medium. Terpenoid (limonene and carvone) accumulation ceased after the 7th generation of callus. Feeding geraniol at 20 or 30 p.p.m. resulted in the production of nerol by suspensions over 24 h; thereafter no geraniol or nerol was detected in the cells or medium of suspension cultures. Higher concentrations (50 and 100 p.p.m.) of geraniol inhibited conversion to nerol.

Figueiredo, A. C., M. J. Almendra, et al. (1996). “Biotransformation of monoterpenes and sesquiterpenes by cell suspension cultures of Achillea millefolium L. ssp. millefolium.” Biotechnology Letters 18(8): 863-868. The transformation capacity of Achillea millefolium spp. millefolium (yarrow) cell suspension cultures was investigated using geraniol (50 mg/l) and borneol, menthol, thymol and farnesols (25 mg/l) as substrates. Apart from converting these substrates into several biotransformation products, the cell suspension cultures were also able to glycosylate both the substrates and the biotransformation products.

Frosch, P. J., B. Pilz, et al. (1995). “Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes.” Contact Dermatitis 33(5): 333-42. The objective of this study was to determine the frequency of reactivity to a series of commonly used fragrances in dermatological patients. A total of 48 fragrances (FF) were chosen, based on the publication of Fenn in 1989 in which the top 25 constituents of 3 types (1. perfumes, 2. household products, 3. soaps) of 400 commercial products on the US market had been determined. In a pilot study on a total of 1069 patients in 11 centres, the appropriate test concentration and vehicle were examined. For most fragrances, 1% and 5% were chosen, and petrolatum proved to be the best vehicle in comparison to isopropyl myristate and diethyl phthalate. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8% fragrance mix (FM) and its 8 constituents. In patients with a positive reaction to any of the 48 FF, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were tested in 11 centres. The 8% FM was positive in 89 patients (8.3% of 1072 patients). Allergic reactions to the constituents were most frequent to oak moss (24), isoeugenol (20), eugenol (13), cinnamic aldehyde (10) and geraniol (8). Reactions read as allergic on day 3/4 were observed only 10X to 7 materials of the new series (Iso E Super (2), Lyral (3), Cyclacet (1), DMBCA (1), Vertofix (1), citronellol (1) and amyl salicylate (1)). The remaining 41 fragrances were negative. 28 irritant or doubtful reactions on day 3/4 were observed to a total of 19 FF materials (more than 1 reaction: 5% citronellol (2), 1% amyl salicylate (2),

1% isononyl acetate (3), 0.1% musk xylol (2), 1% citral (2), and 1% ionone beta (2)). Clinical relevance of positive reactions to any of the FF series was not proved in a single case. This included the 4 reactions in patients who were negative to the 8% FM. In conclusion, the top 25 fragrances commonly found in various products caused few reactions in dermatological patients and these few appeared to be clinically irrelevant, with the possible exception of Lyral. However, this data should be interpreted in the light of the relatively small number of patients tested (only 100 in most centres).

Frosch, P. J., B. Pilz, et al. (1995). “Testing with fragrance mix. Is the addition of sorbitan sesquioleate to the constituents useful?” Contact Dermatitis 32(5): 266-72. In a multicentre study, the value of adding sorbitan sesquioleate (SSO) to the constituents of the 8% fragrance mix (FM) was investigated. In 7 centres, 709 consecutive patients were tested with 2 types of FM from different sources, its 8 constituents with 1% SSO, its 8 constituents without SSO, and 20% SSO. 5 patients (0.71%) reacted to the emulsifier SSO itself, read as definitely allergic on day 3/4. 53 patients reacted to either one of the mixes with an allergic type of reaction. When tested with the constituents without SSO, 41.5% showed an allergic reaction versus 54.7% with SSO. If both types of reactions were considered (allergic and irritant) 38.3% of 73 patients showed a positive "breakdown" result without SSO, versus 54.8% with SSO. The differences were statistically significant. Reactivity to FM constituents was changed in a specific pattern by addition of SSO--irritant reactions increased, particularly for cinnamic alcohol, eugenol, geraniol, oak moss and hydroxycitronellal, whereas others showed only a slight change. Allergic reactions were also increased by SSO, but the rank order of the top 3 sensitizers (isoeugenol, oak moss and eugenol) did not change. Cinnamic alcohol was the only constituent with decreased reactivity after addition of SSO. A positive history of fragrance sensitivity (HFS) was clearly associated with a positive allergic reaction to either the mix or 1 of its constituents (51% versus 28.6% with a negative HFS). Irritant reactions were linked to a negative HFS in a high proportion (64.3%).

Fujita, K., T. Yamaguchi, et al. (2000). “Biosynthetic pathway of beta-thujaplicin in the Cupressus lusitanica cell culture.” Journal of Plant Physiology 156(4): 462-467. The biosynthesis pathway of beta-thujaplicin, which has a unique conjugated 7-membered ring structure, was investigated using a C. lusitanica cell culture. We also examined a transformation of the carbon skeleton during its biosynthesis. Incorporation of [10-14C]-geraniol into beta-thujaplicin in the cell culture revealed that this compound was biosynthesized via geranylpyrophosphate (GPP) and hence is a monoterpenoid. The biosynthesis pathway from glucose to GPP and a skeletal rearrangement of GPP to beta-thujaplicin are proposed, based on the NMR analysis of beta-thujaplicin biosynthesized in C. lusitanica cells from [1-13C]-, [2-13C]- and [U-13C]-glucose. beta- Thujaplicin was produced in the C. lusitanica cell from GPP, which was almost solely produced through the GAP/pyruvate pathway; contribution of the classical mevalonate pathway must be negligible. In addition, it was assumed that GPP was transformed to beta- thujaplicin via the limonane type skeleton, which is a common intermediate of cyclic monoterpene.

Geldof, A. A., C. Engel, et al. (1992). “Estrogenic action of commonly used fragrant agent citral induces prostatic hyperplasia.” Urol Res 20(2): 139-44. A rat model for

benign prostatic hyperplasia in man (BPH) was investigated. Citral treatment of male Copenhagen rats for 4 months via the transdermal route resulted in a marked hyperplasia of glandular epithelium and interglandular stroma in the ventral prostate. Despite the cellular hyperplasia there was not a significant increase in prostate weight. Investigations of the mechanism of action of citral showed that application of citral directly to the vagina in female, ovariectomized rats resulted in an increased proliferation of vaginal epithelium and a significant increase in the BrdUrd incorporation in vaginal epithelial cells, in short a similar effect to that of estrogen application. In an in vitro assay citral proved to inhibit estrogen binding to estrogen receptors, while no such inhibition was observed with testosterone for androgen receptors. These observations together with the estrogen implication in the BPH and the reported incidence of gynecomastia following exposure to geraniol, a precursor of citral, strongly suggest that the prostatic hyperplasia-inducing capacity of citral may be due to its estrogenic action.

Godwin, D. A. and B. B. Michniak (1999). "Influence of drug lipophilicity on terpenes as transdermal penetration enhancers." Drug Dev Ind Pharm 25(8): 905-15. Percutaneous absorption-enhancing effects on the skin of hairless mice of 11 monoterpenes [1, (+)-limonene; 2, (-)-menthone; 3, (+)-terpinen-4-ol; 4, alpha-terpineol; 5, 1,8-cineole; 6, (+)-carvone; 7, (-)-verbenone; 8, (-)-fenchone; 9, p-cymene; 10, (+)-neomenthol; and 11, geraniol] were investigated using three different model drugs (caffeine, hydrocortisone, triamcinolone acetonide [TA]) with varying lipophilicities. Terpenes were applied at 0.4 M in propylene glycol (PG) to mouse skin. The model drugs were applied as suspensions in PG 1 hr following enhancer pretreatment. The combination of terpenes in PG provided significant enhancement of the permeation of caffeine through mouse skin. The most active compounds 10 and 11 increased permeation by between 13-fold and 16-fold. The terpenes also enhanced the delivery of hydrocortisone, but not to as great an extent. The most active compounds 3 and 4 increased permeation between 3.9-fold and 5-fold. The compounds examined did not significantly increase the delivery of TA. The most active compound 4 only increased delivery 2.5-fold, while the next most active compound 6 only increased delivery 1.7-fold. Overall, these results indicate that the combination of terpenes with PG can significantly increase the transdermal penetration of the hydrophilic drug caffeine and the polar steroid hydrocortisone.

Guerra, P., A. Aguilar, et al. (1987). "Contact dermatitis to geraniol in a leg ulcer." Contact Dermatitis 16(5): 298-9.

Gurdip Singh, I. P. S. Kapoor, et al. (2000). "Studies on essential oils, part 28: chemical composition, antifungal and insecticidal activities of rhizome volatile oil of Homalomena aromatica Schott." Flavour and Fragrance Journal 15(4): 278-280. HPLC and GC-MS analysis of rhizome oil of Homalomena aromatica showed the presence of 39 components accounting for 96.9% of the total oil. The major component was linalool (62.1%), followed by terpinen-4-ol (17.2%), alpha-terpineol (2.4%), gamma-terpinene (1.9%), alpha- cadinol (1.5%), geraniol (1.4%), nerol (1.4%), alpha-terpinene (1.0%), spatulenol (1.0%) and T-cadinol (1.0%). However, the higher percentage of linalool (87.5%) was obtained in HPLC studies. This oil showed good antifungal activity against Curvularia pallescens [Cochliobolus pallescens], Aspergillus niger and Fusarium graminearum

[Gibberella zeae] as well as also showing insecticidal behaviour against white termite (Odontotermes obesus).

Hamada, H., H. Yasumune, et al. (1997). “Biotransformation of geraniol, nerol and (+)- and (-)-carvone by suspension cultured cells of Catharanthus roseus.” Phytochemistry 44(4): 615-621. Suspension cultured cells of C. roseus hydroxylate the allylic positions of geraniol, nerol and (+)- and (-)-carvone and reduce double bonds and ketone groups. After incubation for 7 days, the main products of (-)- and (+)-carvone were 5beta-hydroxyneodihydrocarveol and 5alpha-hydroxycarvone, respectively.

Hartlieb, E., P. Anderson, et al. (1999). “Appetitive learning of odours with different behavioural meaning in moths.” Physiol Behav 67(5): 671-7. Moth behaviour is to a great extent guided by olfactory stimuli with different relevance. We investigated whether olfactory learning of odours is influenced by the behavioural significance of the odorant. In proboscis extension conditioning experiments species-specific sex pheromones, which normally elicit an innate behaviour in males, and a flower odour were used as olfactory stimuli. After 10 conditioning trials, both sexes showed similar response levels to individual pheromone components and to the flower odour geraniol. However, when the female gland extract was used as conditioning stimulus, the response level was significantly lower than that for geraniol in both sexes. Significant learning nevertheless occurred in females, but not in males. Experiments with different numbers of training trials revealed that, in females, fewer learning trials with individual pheromone components were necessary for significant memory formation than in males.

Hausen, B. M. and D. Kulenkamp (1990). “[Geraniol contact allergy].” Z Hautkr 65(5): 492-4. A 32-year-old female patient working in a company for baking ingredients, who had been handling grated lemon peel and lemon oil (oleum citri) for several years, developed allergic contact dermatitis of the fingers of both her hands. By means of thin-layer chromatography, we identified geraniol in both lemon peel and lemon oil and proved it to be the only source of the allergic reaction.

Havel, C., E. R. Rector, 2nd, et al. (1986). “Isopentenoid synthesis in isolated embryonic Drosophila cells. Possible regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by shunted mevalonate carbon.” J Biol Chem 261(22): 10150-6. Our previous studies (Watson, J. A., Havel, C. M., Lobos, D. V., Baker, F. C., and Morrow, C. J. (1985) J. Biol. Chem. 260, 14083-14091) suggested that a matabolite, distal to isopentenyl 1-pyrophospate (IPP), served as a regulatory signal for sterol-independent modulation of Kc cell 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. This report summarizes efforts to localize the potential source of the post-IPP regulatory signal molecule. We found no direct correlation between mevalonate-mediated suppression of Kc cell HMG-CoA reductase activity and the rates of [1-14C]-, [3-14C]-, [5-14C]-, or [5-3H]mevalonate incorporation into either carbon dioxide, neutral lipids, water, or water-soluble isopentenoid pyrophosphate esters. [1-14C]Mevalonate's rate of conversion to 14CO2 (a measure of total isopentenyl 1-pyrophosphate synthesis) was minimally 5-fold greater than that for neutral isopentenoid lipid synthesis (measured with either [5-3H]-, [3-14C]-, or [5-14C]mevalonate). However, [5-3H]mevalonate's rate of

conversion into [3H]H2O (measure of shunted mevalonate carbon) was equivalent or greater than that measured for neutral isopentenoid lipid synthesis. [5-14C]Mevalonate radioactivity was incorporated into macromolecules and n-fatty acids. Kc cell extracts (100,000 X g supernatant fluid) readily oxidized alcohols with the following activity sequence: geraniol = nerol greater than farnesol = dimethylallyl alcohol greater than geranylgeraniol, isopentenyl alcohol, and allyl alcohol. Oxidation required NAD, and ethanol was not a substrate. We conclude that (a) Kc cells shunted a significant fraction (greater than or equal to 40%) of their post-IPP carbon to prenols for oxidative catabolism and (b) that shunted mevalonate carbon may play a significant role in the mevalonate-mediated regulation of Kc cell HMG-CoA reductase activity.

He, L., H. Mo, et al. (1997). “Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo.” J Nutr 127(5): 668-74. Sundry mevalonate-derived constituents (isoprenoids) of fruits, vegetables and cereal grains suppress the growth of tumors. This study estimated the concentrations of structurally diverse isoprenoids required to inhibit the increase in a population of murine B16(F10) melanoma cells during a 48-h incubation by 50% (IC50 value). The IC50 values for d-limonene and perillyl alcohol, the monoterpenes in Phase I trials, were 450 and 250 micromol/L, respectively; related cyclic monoterpenes (perillaldehyde, carvacrol and thymol), an acyclic monoterpene (geraniol) and the end ring analog of beta-carotene (beta-ionone) had IC50 values in the range of 120-150 micromol/L. The IC50 value estimated for farnesol, the side-chain analog of the tocotrienols (50 micromol/L) fell midway between that of alpha-tocotrienol (110 micromol/L) and those estimated for gamma- (20 micromol/L) and delta- (10 micromol/L) tocotrienol. A novel tocotrienol lacking methyl groups on the tocol ring proved to be extremely potent (IC50, 0.9 micromol/L). In the first of two diet studies, experimental diets were fed to weanling C57BL female mice for 10 d prior to and 28 d following the implantation of the aggressively growing and highly metastatic B16(F10) melanoma. The isomolar (116 micromol/kg diet) and the Vitamin E-equivalent (928 micromol/kg diet) substitution of d-gamma-tocotrienol for dl-alpha-tocopherol in the AIN-76A diet produced 36 and 50% retardations, respectively, in tumor growth ($P < 0.05$). In the second study, melanomas were established before mice were fed experimental diets formulated with 2 mmol/kg d-gamma-tocotrienol, beta-ionone individually and in combination. Each treatment increased ($P < 0.03$) the duration of host survival. Our finding that the effects of individual isoprenoids were additive suggests the possibility that one component of the anticarcinogenic action of plant-based diets is the tumor growth-suppressive action of the diverse isoprenoid constituents of fruits, vegetables and cereal grains.

Hinson, D. D., K. L. Chambliss, et al. (1997). “Post-translational regulation of mevalonate kinase by intermediates of the cholesterol and nonsterol isoprene biosynthetic pathways.” J Lipid Res 38(11): 2216-23. To assess the potential for feedback inhibition by isoprene intermediates in the cholesterol and nonsterol isoprene biosynthetic pathway, we expressed human cDNAs encoding mevalonate kinase (MKase), phosphomevalonate kinase (PMKase), and mevalonate diphosphate decarboxylase (MDDase) as fusion proteins in Escherichia coli DH5alpha, and purified these proteins by affinity chromatography. Several phosphorylated and non-phosphorylated isoprenes were

analyzed as inhibitors of the enzymes using a standard spectrophotometric assay. Of the three proteins, only MKase was inhibited through competitive interaction at the ATP-binding site. The intermediates studied (and their relative inhibitory capacity) were: geranylgeranyl-diphosphate (GGPP, C20) > farnesyl-diphosphate (FPP, C15) > geranyl-diphosphate (GPP, C10) > isopentenyl-diphosphate (IPP, C5) > or = 3,3-dimethylallyl-diphosphate (DMAPP, C5) > farnesol (C15) > dolichol-phosphate (DP, C(80-100)). Mevalonate-diphosphate, geraniol, and dolichol were not inhibitors. Our data further define the spectrum of physiologic inhibitors of MKase, and provide the first evidence for feedback inhibition of MKase by a nonsterol isoprene produced by the branched pathway, dolichol-phosphate. These results provide additional evidence that MKase may occupy a central regulatory role in the control of cholesterol and nonsterol isoprene biosynthesis.

Hirasuna, T. J., L. J. Pestchanker, et al. (1996). “Taxol production in suspension cultures of Taxus baccata.” Plant Cell, Tissue and Organ Culture 44(2): 95-102. The response of Taxus baccata (PC2) to basic manipulations of culture conditions is described. Suspension cultures of PC2 were maintained at 25 deg C on a modified B5 medium with two-week transfers. Under these conditions, no taxol(R) was formed. However, if the cells were left in the same medium for 7 or more additional days, taxol was produced and released (ca. 90%) into the extracellular medium. Levels as high as 13 mg/litre extracellular taxol were achieved in shake flask cultures, and taxol was the primary taxane formed representing between 50 and 80% of total taxane in the medium. The cells were sensitive to changes in culture conditions and cultures cycled through periods of high (13 mg/litre) and low (<0.1 mg/litre) levels of taxol production during extended culture. Picloram was the most effective of the auxins tested with respect to cell growth but it suppressed taxol production. Addition of fructose to moderately- productive cultures (ca. 4 mg/litre) improved taxol production, but cultures in a high producing state did not respond. Glucose suppressed taxane production. Two isoprenoids (geraniol and pinene) had a modest effect on taxol production when added to cultures at 10 mg/litre.

Hornby, J. M., E. C. Jensen, et al. (2001). “Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol.” Appl Environ Microbiol 67(7): 2982-92. The inoculum size effect in the dimorphic fungus Candida albicans results from production of an extracellular quorum-sensing molecule (QSM). This molecule prevents mycelial development in both a growth morphology assay and a differentiation assay using three chemically distinct triggers for germ tube formation (GTF): L-proline, N-acetylglucosamine, and serum (either pig or fetal bovine). In all cases, the presence of QSM prevents the yeast-to-mycelium conversion, resulting in actively budding yeasts without influencing cellular growth rates. QSM exhibits general cross-reactivity within C. albicans in that supernatants from strain A72 are active on five other strains of C. albicans and vice versa. The QSM excreted by C. albicans is farnesol (C(15)H(26)O; molecular weight, 222.37). QSM is extracellular, and is produced continuously during growth and over a temperature range from 23 to 43 degrees C, in amounts roughly proportional to the CFU/milliliter. Production is not dependent on the type of carbon source nor nitrogen source or on the chemical nature of the growth medium. Both commercial mixed isomer and (E,E)-farnesol exhibited QSM activity (the ability to

prevent GTF) at a level sufficient to account for all the QSM activity present in C. albicans supernatants, i.e., 50% GTF at ca. 30 to 35 microM. Nerolidol was ca. two times less active than farnesol. Neither geraniol (C(10)), geranylgeraniol (C(20)), nor farnesyl pyrophosphate had any QSM activity.

Hornok, L., J. Domokos, et al. (1992). “Effect of harvesting time on the production of Nepeta cataria var. citriodora Balb.” Acta Horticulturae(306): 290-294. Harvesting of 1- and 2-year-old plantings was carried out over a period of 48-54 days, starting from the appearance of the buds and continuing to the green seed stage. Herb yield was, on average 79% greater for the 2-year-old plants than for the 1-year-old ones. Essential oil yield was greatest at the beginning of flowering (6.44 g/100 m2) for the 1-year-old planting, and during flowering (12.24 g/100 m2) for the 2-year-old planting. One of the main components of the oil, nerol, increased from 25% at the first harvest to 33.6% during flowering, after which it decreased. Geraniol content varied between 25.8 and 36.1%.

Hunter, M. I., T. L. Olawoye, et al. (1981). “The effect of temperature on the growth and lipid composition of the extremely halophilic coccus, Sarcina marina.” Antonie Van Leeuwenhoek 47(1): 25-40. Sarcina marina (NCMB 778) grew over the temperature range 20-45 degrees C but no growth was recorded at 15 degrees C or 50 degrees C. At the optimum growth temperature of 34 degrees C the doubling time was 14.5 h. The major polar lipid components, tentatively identified as the diether analogues of phosphatidyl glycerophosphate (PGP), phosphatidyl glycerol (PG), diglycosyl diglyceride (DGD) and triglycosyl diglyceride (TGD), and the major neutral lipid components, tentatively identified as squalene, dihydrosqualene, tetrahydrosqualene, vitamin MK8, geranyl geraniol and di-O-phytanyl glycerol, are identical to those found in other extremely halophilic rods and cocci. The total lipid content varied with growth conditions from 0.6-3.2% of the dry cell weight, polar lipids accounted for between 94.3 and 83.6% of the total lipid, the remainder being neutral lipid. In response to both the transition from exponential to stationary phase and a reduction of 14 degrees C in growth temperature, batch cultures showed: (i) an increase in total lipid content; (ii) a decrease in PG and (iii) an increase in PGP. Specific responses to the temperature decrease were (i) increased total lipid content; (ii) no decrease in neutral lipids in stationary phase; (iii) marked reduction in PG and (iv) raised DGD. (i) and (ii) could be mechanisms for increasing membrane fluidity. In common with all other extreme halophiles investigated the alkyl side chains of S. marina polar lipids were identified as the phytanyl (3R, 7R, 11R, 15-tetramethylhexadecyl) group. Its structure did not appear to vary with temperature so that the normal mechanisms for modifying the structure of lipid alkyl side chains to modulate membrane fluidity in response to temperature changes probably does not occur in this group of microorganisms.

Inouye, S., H. Yamaguchi, et al. (2001). “Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method.” J Infect Chemother 7(4): 251-4. The purpose of this study was to examine the antibacterial effects of a wide variety of essential oils on major respiratory tract pathogens. The antibacterial activity of 14 essential oils and their major components was

evaluated by agar-plate dilution assay under sealed conditions, with agar used as a stabilizer for homogeneous dispersion. Of the selected strains of four major bacteria causing respiratory tract infection, Haemophilus influenzae was most susceptible to the essential oils, followed by Streptococcus pneumoniae and Streptococcus pyogenes. Staphylococcus aureus was less susceptible. No cross-resistance was observed between penicillin-sensitive and penicillin-resistant S. pneumoniae. Escherichia coli, used as a control bacterium, showed the lowest susceptibility. Essential oils containing aldehyde or phenol as a major component showed the highest antibacterial activity, followed by the essential oils containing terpene alcohols. Other essential oils, containing terpene ketone, or ether, had much weaker activity, and an oil containing terpene hydrocarbon was inactive. Based on these findings, thyme (wild, red, and geraniol types), cinnamon bark, lemongrass, perilla, and peppermint oils were selected for further evaluation of their effects on respiratory tract infection.

Inouye, S., K. Uchida, et al. (2001). "Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact." Journal of Essential Oil Research 13(1): 68-72. The flowers of Perovskia abrotanoides and Nepeta juncea and the leaves and flowers of Thymus linearis were collected at full bloom from different areas of Karakoram district, Pakistan, then dried and examined for essential oil composition. Dried plant parts were placed in air-tight boxes with 3 species of fungi (Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes or Trichophyton rubrum) for one, 3 and 5 days, respectively. P. abrotanoides extracts contained high concentrations of 1,8-cineole (24-27%) and alpha-pinene (18-23%) and low concentrations of borneol and bornyl acetate. N. juncea essential oils contained nepetalactone (5.8 plus or minus 0.54 mg per g of dried flowers), and several minor constituents such as nerol, 1,8-cineole, neryl acetate and an unidentified component. Two Thymus linearis chemotypes were collected; that from Hunza and Rupal valley had thymol and carvacrol as major components, and the other chemotype from the Rakaposhi area had geranyl acetate and geraniol as major components. N. juncea and Thymus linearis essential oils inhibited fungal growth by vapour contact, while P. abrotanoides showed no activity. The 2 Trichophyton species were the most susceptible and C. albicans was the least susceptible to the toxic effects of plant aromatic compounds, while A. fumigatus showed intermediate susceptibility.

Ishihara, M., F. Takayama, et al. (2000). "Cytotoxic activity of polyprenylalcohols and vitamin K2 derivatives." Anticancer Res 20(6B): 4307-13. Cytotoxic activity of 9 polyprenylalcohols and 6 vitamin K2 derivatives (MK-1 to MK-6) with various lengths of prenyl units was investigated. Among these compounds, geranylgeraniol with 4 prenyl units, and MK-2 with 2 prenyl units, showed the highest cytotoxic activity against human oral tumor cell lines (HSC-2, HSG), without induction of internucleosomal DNA fragmentation. Higher molecular weight compounds showed selective cytotoxicity against tumor cell lines than normal human gingival fibroblasts HGF. ESR spectroscopy showed that all polyprenylalcohols did not produce radical, nor scavenged O2- generated by hypoxanthine and xanthine oxidase reaction, and only slightly enhanced the radical intensity of sodium ascorbate. Vitamin K2 derivatives scavenged O2- more efficiently, but did not produce radical (except MK-3) and only slightly modified the ascorbate

radical intensity. Cytotoxic activity of these compounds might be affected by the molecular weight, hydrophobicity, van der Waals area and stabilization of hydration of the molecule.

Izumi, S., O. Takashima, et al. (1999). "Geraniol is a potent inducer of apoptosis-like cell death in the cultured shoot primordia of Matricaria chamomilla." Biochemical and Biophysical Research Communications 259(3): 519-522.

Jagdev, S. P., R. E. Coleman, et al. (2001). "The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence for synergy with paclitaxel." Br J Cancer 84(8): 1126-34. Bisphosphonates are well established in the management of breast-cancer-induced bone disease. Recent studies have suggested that these compounds are effective in preventing the development of bone metastases. However, it is unclear whether this reflects an indirect effect via an inhibition of bone resorption or a direct anti-tumour effect. The breast cancer cell lines, MCF-7 and MDA-MB-231 cells were treated with increasing concentrations of the bisphosphonate, zoledronic acid, for varying time periods, in the presence or absence of paclitaxel. The effects of zoledronic acid were determined by assessing cell number and rate of apoptosis by evaluating changes in nuclear morphology and using a fluorescence nick translation assay. Zoledronic acid caused a dose- and time-dependent decrease in cell number ($P < 0.001$) and a concomitant increase in tumour cell apoptosis ($P < 0.005$). Short-term exposure to zoledronic acid was sufficient to cause a significant reduction in cell number and increase in apoptosis ($P < 0.05$). These effects could be prevented by incubation with geranyl geraniol, suggesting that zoledronic acid-induced apoptosis is mediated by inhibiting the mevalonate pathway. Treatment with zoledronic acid and clinically achievable concentrations of paclitaxel resulted in a 4-5-fold increase in tumour cell apoptosis ($P < 0.02$). Isobologram analysis revealed synergistic effects on tumour cell number and apoptosis when zoledronic acid and paclitaxel were combined. Short-term treatment with zoledronic acid, which closely resembles the clinical setting, has a clear anti-tumour effect on breast cancer cells. Importantly, the commonly used anti-neoplastic agent, paclitaxel, potentiates the anti-tumour effects of zoledronic acid. These data suggest that, in addition to inhibiting bone resorption, zoledronic acid has a direct anti-tumour activity on breast cancer cells in vitro.

Johansen, J. D., S. C. Rastogi, et al. (1997). "Content and reactivity to product perfumes in fragrance mix positive and negative eczema patients. A study of perfumes used in toiletries and skin-care products." Contact Dermatitis 36(6): 291-6. The aim of the study was to investigate the elicitation potential of perfumes from 17 commonly sold lower-price cosmetic products. 8 of the perfumes were from stay-on cosmetics and 9 were from wash-off cosmetics. Each perfume was tested in 500 consecutive eczema patients, who also were tested with the European standard patch test series. 4.2% reacted to 1 or more of the wash-off product perfumes and 3.2% to 1 or more of the stay-on product perfumes. Concordant positive reactions between the fragrance mix and the product perfumes were found in 81.3% of positive reactions to the stay-on product perfumes and in 52.4% of the reactions to the wash-off product perfumes. Compared to the fragrance mix alone, only 1 additional case of contact allergy to the product perfumes was detected by balsam of Peru. Chemical analysis revealed that between 1 and 5 of the chemically-defined constituents of the fragrance mix were present in all of the product perfumes. Geraniol

was found in 12 of the 17 perfumes and was most often detected. The concentration of the target fragrance materials ranged from 0.005%-1.35 w/v%. It is concluded that the allergenic constituents of the fragrance mix are impossible to avoid if perfumed cosmetics are used. Furthermore, patients suspected of perfume allergy need to be tested with their own perfumed products, as far from all cases of perfume allergy are detected by the fragrance mix and/or balsam of Peru in the European standard patch test series.

Jorge Neto, J. and B. Mancini (1992). "Dialium guianense (Aubl.) Sandw., Leguminosae: chromatographic analysis of the essential oil." Revista de Ciencias Farmaceuticas 14: 125-132. The essential oil composition of leaves of the medicinal plant D. guianense, collected in Irece, Bahia, Brazil, was determined. About 90% of the isolated compounds were identified by spectral analyses. The major components included alpha-pinene (16.74%), beta-pinene (25.64%), citronellol (19.98%), farnesol (9.03%) and geraniol (4.45%).

Kahlos, K., J. L. Kiviranta, et al. (1994). "Volatile constituents of wild and in vitro cultivated Gloeophyllum odoratum." Phytochemistry 36(4): 917-22. The brown-rot fungus Gloeophyllum odoratum was collected from spruce stumps in southern Finland. The volatiles in the fruiting body and fungal cultures grown in malt extract and liquid medium were investigated. Chitin, chitosan and D-(+)-glucosamine at a concentration of 450 mgl-1 medium were used as elicitors. Chitosan completely inhibited growth in the solid medium. The main volatile(s) according to GC and GC-MS analysis were either linalool, citronellol, geraniol and methyl p-methoxyphenylacetate or drimenol depending on the culture type and elicitor. The composition of volatiles in the natural fungus differed slightly from that of the cultivated fungus since the major compound was methyl p-methoxyphenylacetate. The volatile oils were toxic to larvae of the brine shrimp, Artemia salina, indicating that they may possess insecticidal and cytotoxic activity.

Kalinkina, G. I., V. N. Tikhonov, et al. (1994). "Chemical composition and pharmacological properties of essential oil of Thymus serpyllum L. s.l. grown in Central Siberia Botanical Garden." Rastitel'nye Resursy 30(3): 66-70. Of the 37 essential oil components detected in the aerial parts of T. serpyllum (f. citriodora), 16 were identified and the data are tabulated. Geraniol was the main component (60.32%). The essential oil has a characteristic lemon aroma and flavour, and could be of value for perfumery/cosmetic and culinary uses, and in making vodka- liqueurs. The essential oil also has an antiphlogistic and wound- healing effect.

Karp, F. and R. Croteau (1982). "Evidence that sabinene is an essential precursor of C(3)-oxygenated thujane monoterpenes." Archives of Biochemistry and Biophysics 216(2): 616-624. The volatile oil of immature Artemisia absinthium leaves contained sabinyl acetate (42%), 3-thujone (32%), sabinene (12%) and alpha - thujene (3%), and label from [1-3H]geraniol was incorporated, under aerobic conditions, into these monoterpenes in proportion to their natural abundance. Light had little effect on synthesis from exogenous geraniol, but, at reduced O2 levels, label accumulated in sabinene, whereas much less sabinyl acetate and 3-thujone were formed, suggesting a route to the ester and ketone by the allylic, nonphotochemical oxygenation of sabinene. Supporting evidence for the

intermediary role of sabinene was provided by isotopic dilution studies. [10-3H]Sabinene was incorporated directly in A. absinthium leaves into both [10-3H]sabinyl acetate and 3-[10-3H]thujone, and in Tanacetum vulgare and Salvia officinalis it was specifically incorporated into 3-thujone and 3-isothujone, respectively, confirming the role of this bicyclic olefin as the essential precursor of C(3)-oxygenated thujane monoterpenes.

Kedzia, B., M. Krzyzaniak, et al. (1994). "Composition and antimicrobial characteristics of Ol. Melissae and its components." Herba Polonica 40(1-2): 5-11. Ol. Melissae [the essential oil of Melissa officinalis] was analysed, and the main constituents were citronellal (25.2%), geraniol (16.4%) and citronellol (11.0%). The essential oil inhibited the growth of Staphylococcus aureus, Streptococcus faecalis and Candida albicans (MIC values of 100, 250 and 300 micro g/ml, respectively), and inhibited the growth of Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa to a lesser extent (MIC values of 500 micro g/ml). Citronellol, beta-caryophyllene, thymol, carvacrol and citronellal were the most active oil components against the microorganisms.

Krueger, R. J. and D. P. Carew (1975). "Catharanthus roseus tissue culture. The effects of biosynthetic precursors on secondary product formation." 38(6): 542. Various compounds including geraniol, mevalonic acid lactone, secologanin glucoside, L-tryptophan and tryptamine hydrochloride were added, separately or in combination, to suspension cultures of C. roseus and metabolites were isolated. In further experiments anthocyanins (glucosides of hirsutidin, malvidin and petunidin) were produced by callus grown under continuous fluorescent lighting and were shown to match those in the floral corollas of intact plants.

Krueger, R. J. and D. P. Carew (1978). "Catharanthus roseus tissue culture: the effects of precursors on growth and alkaloid production." Lloydia 41(4): 327-331. Mevalonic acid lactone, geraniol, secologanin, l-tryptophan and tryptamine HCl were added separately and in various combinations to suspension cultures of C. roseus. Tryptamine HCl fed alone had the greatest effect in stimulating alkaloid production. The combination of any of the non-indolic precursors with tryptamine HCl did not significantly alter alkaloid production from that seen with tryptamine HCl alone. The 2 most prominent metabolites isolated from cultures fed tryptamine were identified as N-acetyltryptamine and N,N-dimethyltryptamine.

Kulevanova, S., A. Kaftandzieva, et al. (2000). "Investigation of antimicrobial activity of essential oils of several Macedonian Thymus L. species (Lamiaceae)." Boll Chim Farm 139(6): 276-80. Antimicrobial activity of twenty specimens of essential oils of eleven Thymus species, naturally occurring in the Macedonian flora, was investigated by agar diffusion and broth dilution methods. Inhibition of growth and microbicidal action was examined on three Gram positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumoniae). In spite of wide variability in essential oil composition, ranging from traces of thymol to the amount of about 50% thymol in oils, all examined samples of Thymus essential oil possessed strong antibacterial activity. Zones of inhibition of growth (for 25% dilution of oils) was from 10-54 mm in diameters. MICs ranging from 0.012-0.1% while MMCs were from 0.025-0.4% for essential oils

that contained large amounts of phenols and 0.2-1.6% for those which contained traces of phenols and large amounts of geraniol, linalool and (Z + E)-citral.

Kulevanova, S., M. Ristic, et al. (1996). “Composition of the essential oil from Thymus moesiacus from Macedonia.” Planta Medica 62(1): 78-79. T. moesiacus is used in traditional medicine to treat coughs, flu, pulmonary infections and abdominal disorders. The essential oil, hydrodistilled from plant material of T. moesiacus (collected from Macedonia), was examined by GC and GC-MS. The main constituents were geraniol (14.87-33.27%), linalool (8.14-25.02%), geranyl acetate (4.11-16.75%) and carvacrol (12.31-13.31%).

Kulevanova, S., M. Ristic, et al. (1997). “Composition of essential oils of Thymus tosevii ssp. tosevii and Thymus tosevii ssp. substriatus from Macedonia.” Pharmazie 52(5): 382-386. T. tosevii subsp. tosevii and T. tosevii subsp. substriatus are used in traditional medicine in Macedonia against cold, flu, pulmonary infection and abdominal throes. The essential oils of T. tosevii subsp. tosevii and T. tosevii subsp. substriatus, growing wild in Macedonia, were investigated by means of GC and GC-MS. The main components of the oils were thymol, carvacrol, linalool, geraniol, terpenyl acetate, p-cymene and gamma-terpinene. The essential oil composition varied according to the origin and the year of plant collection.

Lal, R. N., T. K. Sen, et al. (1978). “Gas chromatography of the essential oil of Ocimum sanctum L.” Parfum. u Kosmetik 59(7): 230-231. Water distillation of O. sanctum cultivated in India yielded 0.7% oil. Eugenol content of the oil was 70% by weight. Other constituents identified in the oil were nerol, eugenol methylether, caryophyllene, terpinene-4-ol, decylaldehyde gamma -selinene, alpha -pinene, beta - pinene, camphor and carvacrol.

Larsen, W., H. Nakayama, et al. (2002). “Fragrance contact dermatitis - a worldwide multicenter investigation (Part III).” Contact Dermatitis 46(3): 141-4. The purpose of this study was to determine the frequency of responses to selected fragrance materials in patients who were fragrance sensitive. 218 fragrance sensitive subjects were evaluated in eight centres worldwide with a fragrance mixture (FM) and 17 less well-studied fragrance materials. Reaction to the fragrance mixture (FM) occurred in 76% of the subjects. The (FM) detected all reactions to nerol and hydroxycitronellol and 93% of the reactions to clove bud oil. Ten fragrance materials were not detected by the FM and deserve further study: benzenepropanol, beta, beta, 3-trimethyl, hexyl-salicylate, dl-citronellol, synthetic ylang ylang oil, benzyl mixture, cyclohexyl-acetate, eugenyl methyl ether, isoeugenyl methyl ether, 3-phenyl-1-propanol, and 3, 7-dimethyl-7-methoxyoctan-2-ol.

Leal, W. S., M. Ono, et al. (1994). “Kairomone from dandelion, Taraxacum officinale, attractant for scarab beetle Anomala octiescostata.” Journal of Chemical Ecology 20(7): 1697-1704. The attraction of Anomala octiescostata to Taraxacum officinale was chemically mediated by a mixture of cis-3-hexenyl acetate, benzaldehyde, phenylacetaldehyde, benzyl alcohol, phenethyl alcohol, phenylacetonitrile and benzyl benzoate in the ratio 4:8:14:3:5:19:11. Combinations of the synthetic kairomone and sex

pheromone (buibuilactone + japonilure (R,Z)-5-(-)-(dec-1-enyl)oxacyclopenta-2- onein the ratio 8:2), significantly increased the total catches of A. octiescostata in golf courses in Honshu, Japan. Catches of male (but not female) beetles were significantly higher with the kairomone- pheromone blend than with the kairomone alone. The synergistic effect of the kairomone from T. officinale on the attractiveness did not significantly differ from that of a food-type lure (anethol, geraniol, and phenethyl propionate in the ratio 9:0.5:0.5). The latter combined with the synthetic sex pheromone resulted in better attraction of female (but not male) A. octiescostata than sex pheromone alone.

Lis-Balchin, M. and S. G. Deans (1997). “Bioactivity of selected plant essential oils against Listeria monocytogenes.” J Appl Microbiol 82(6): 759-62. Ninety-three different commercial essential oils were screened for activity against 20 Listeria monocytogenes strains in vitro and the results correlated against the actual chemical composition of each oil. There was a substantial difference in the activity between different essential oils as expected, but there was also a difference in activity between different samples of the same essential oil. Strong anti-Listeria activity was often correlated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamaldehyde, thymol, and sometimes with citronellol, limonene and geraniol. However, as there was often no correlation between the anti-Listeria activity and the main chemical components, it is possible that either there is a more complex relationship with the chemical composition (which includes the minor components) or that substantial adulteration had occurred in some essential oil samples.

Lis-Balchin, M. T. and S. L. Hart (1994). “A pharmacological appraisal of the folk medicinal usage of Pelargonium grossularioides and Erodium cicutarium.” Journal of Herbs, Spices & Medicinal Plants 2(3): 41-48. In Africa, P. grossularioides and E. cicutarium are used in traditional medicine for their abortifacient properties, and to treat fevers, dysentery, wounds and worm infestations. The pharmacological effects of extracts (hexane, methanol and water) of leaves of P. grossularioides (obtained from South Africa) and E. cicutarium (obtained from Cambridge University, UK), were studied in vitro using guinea pig ileum, rat uterus, rat phrenic nerve preparations, and rabbit hearts. Extracts from both plants increased the tone, and reduced the strength or inhibited contraction, of guinea pig ileum. Extracts stimulated contractions of the rat uterus, increased the tension of the isolated diaphragm muscle in phrenic nerve preparations, and produced a negative ionotropic action in the rabbit heart. Hexane extracts were the most active, followed by methanol extracts. The compositions of the essential oils from both species were compared. Both species contained methyl eugenol, geraniol, citronellol, isomenthone and linalool. Sesquiterpenes, which accounted for 10% of the E. cicutarium essential oil, were absent from P. grossularioides.

Lis-Balchin, M., J. Patel, et al. (1998). “Studies on the mode of action of essential oils of scented-leaf Pelargonium (Geraniaceae).” Phytotherapy Research 12(3): 215-217. Essential oils, steam-distilled from species, hybrids and cultivars of scented-leaf Pelargonium, were assessed for their mode of spasmolytic activity in vitro using an isolated smooth muscle preparation. Their mechanism of action was postsynaptic and not atropine-like. Spasmolytic action was correlated with the chemical composition of the

essential oils assessed by GC-MS. The spasmolytic effect of Pelargonium essential oils with a rose-like odour was most likely mediated through cAMP, and not through cGMP; the action of all other essential oils with diverse odours was neither through cAMP, cGMP, nor via calcium channel blockade nor potassium channel activation. The mechanism of action of the main components of the rose-like pelargoniums, citronellol and geraniol, reflected that of the whole oils.

Lopes, D., M. Koketsu, et al. (1999). "Chemical composition of Pourouma guianensis Aublet essential oils." Flavour and Fragrance Journal 14(4): 233-236. The essential oils from leaves, stem bark and pistillate flowers of P. guianensis (collected in Rio de Janeiro, Brazil; species used medicinally and for edible fruits) were isolated by hydrodistillation and the volatile constituents were determined by HRGC and HRGC-MS. Methyl salicylate was the major compound identified in all oils studied and was present in yields of 20.8% (leaves), 31.2% (stem bark) and 62.2% (pistillate flowers). Altogether, 50 constituents were identified in the essential oil obtained from leaves, representing 76.6% of the total oil. Aliphatic C6 alcohols and esters were, in number and in quantity, the principal constituents (29.5%). Oxygenated monoterpenes were found to be an important group of compounds and the most representative compound was linalol [linalool] (2.4%). Thirty-eight components were identified in the essential oil from stem bark, representing 79.3% of the total oil. Among the monoterpenes identified, linalol was the principal compound (0.8%). The total content of fatty acids amounted to 40.0%. Analysis of the essential oil from pistillate flowers allowed the identification of 36 compounds, representing 88.5% of the oil. Ten oxygenated monoterpenes were identified, whereas linalol and its furan derivatives (9.7%), nerol (0.4%) and geraniol (1.3%) were the most abundant. Five aromatic derivatives were identified in the pistillate flower essential oil: methyl salicylate (62.2%), ethyl salicylate (0.1%), benzyl salicylate (0.2%), benzyl benzoate (0.3%) and benzaldehyde (0.1%).

Lozano, V. C., E. Bonnard, et al. (1996). "Mecamylamine-induced impairment of acquisition and retrieval of olfactory conditioning in the honeybee." Behav Brain Res 81(1-2): 215-22. Mecamylamine, a nicotinic receptor antagonist, was injected into the honeybee brain haemolymph. The effects of the drug were investigated on Pavlovian conditioning of the proboscis extension reflex. The conditioned response was acquired after a one-trial learning session, consisting of an olfactory-conditioned stimulus combined with a gustatory antennal unconditioned stimulus. The drug was injected at different times before or after the learning session in order to dissociate its effects on acquisition, consolidation and retrieval processes. The performance was evaluated in short-delayed recall tasks. To control potential effects on sensory-motor activity, the effects of the drug were also investigated on sensory processes (through olfactory and gustatory functions) and on motor processes of proboscis extension. The results of conditioning experiments showed that pretrial injection induced a decrease of retention performance 1 h after the learning trial. Mecamylamine injected 20 min after the learning session induced a time-dependent impairment of retention performance, as has been shown by the performance level registered from 10 to 80 min after injection. A 5-min post-trial injection had no effect on retention performance. Control experiments did not reveal any effect of mecamylamine on the response reflex of proboscis extension and on

responsiveness to olfactory stimuli (geraniol, lavender and vanillin). The absence of effects on sensory perception combined with the amnestic effect induced by pre- or late post-trial injections lead us to conclude that mecamylamine specially impaired acquisition and retrieval processes. The involvement of nicotinic-like receptors in these processes is discussed.

Machida, K., T. Tanaka, et al. (1998). "Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast Saccharomyces cerevisiae." J Bacteriol 180(17): 4460-5. The mechanism of farnesol (FOH)-induced growth inhibition of Saccharomyces cerevisiae was studied in terms of its promotive effect on generation of reactive oxygen species (ROS). The level of ROS generation in FOH-treated cells increased five- to eightfold upon the initial 30-min incubation, while cells treated with other isoprenoid compounds, like geraniol, geranylgeraniol, and squalene, showed no ROS-generating response. The dependence of FOH-induced growth inhibition on such an oxidative stress was confirmed by the protection against such growth inhibition in the presence of an antioxidant such as alpha-tocopherol, probucol, or N-acetylcysteine. FOH could accelerate ROS generation only in cells of the wild-type grande strain, not in those of the respiration-deficient petite mutant ([rho0]), which illustrates the role of the mitochondrial electron transport chain as its origin. Among the respiratory chain inhibitors, ROS generation could be effectively eliminated with myxothiazol, which inhibits oxidation of ubiquinol to the ubisemiquinone radical by the Rieske iron-sulfur center of complex III, but not with antimycin A, an inhibitor of electron transport that is functional in further oxidation of the ubisemiquinone radical to ubiquinone in the Q cycle of complex III. Cellular oxygen consumption was inhibited immediately upon extracellular addition of FOH, whereas FOH and its possible metabolites failed to directly inhibit any oxidase activities detected with the isolated mitochondrial preparation. A protein kinase C (PKC)-dependent mechanism was suggested to exist in the inhibition of mitochondrial electron transport since FOH-induced ROS generation could be effectively eliminated with a membrane-permeable diacylglycerol analog which can activate PKC. The present study supports the idea that FOH inhibits the ability of the electron transport chain to accelerate ROS production via interference with a phosphatidylinositol type of signal.

Madyastha, K. M., T. D. Meehan, et al. (1976). "Characterization of a cytochrome P-450 dependent monoterpene hydroxylase from the higher plant Vinca rosea." Biochemistry 15(5): 1097-102. A monooxygenase isolated from 5-day old etiolated Vinca rosea seedlings was shown to catalyze the hydroxylation of the monoterpene alcohols, geraniol and nerol, to their corresponding 10-hydroxy derivatives. Hydroxylase activity was inpendent upon NADPH (neither NADH nor combination of NADH, NADP+ and ATP served as substitutes) and O2. Geraniol hydroxylation was enhanced by dithiothreitol (monothiols were less effective) and inhibited by phospholipases, thiol reagents, metyrapone, and cytochrome c, as well as other inhibitors of cytochrome P-450 systems. Geraniol was hydroxylated at a faster rate than nerol, but the alcohols possessed similar apparent Km values. The membrane-bound hydroxylase was solubilized by treatment with sodium cholate, Renex-30, or Lubrol-WX. Cholate-treated enzyme was resolved by DEAE-cellulose chromatography and reconstitution of the hydroxylase was effected

utilizing different fractions containing cytochrome P-450, a NADPH-cytochrome c reductase, and lipid.

Mahmoud, A. L. (1994). “Antifungal action and antiaflatoxigenic properties of some essential oil constituents.” Lett Appl Microbiol 19(2): 110-3. The effect of 20 essential oil constituents on Aspergillus flavus growth and aflatoxin production was tested at the level of 1000 ppm. Some of the tested oils exhibited inhibitory effects on fungal growth and toxin formation. Five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth and aflatoxin synthesis. Trials for determining the minimum inhibitory concentration (MIC) of these oils revealed that geraniol, nerol and citronellol were effective at 500 ppm, while thymol and cinnamaldehyde were highly effective at doses as low as 250 and 200 ppm, respectively. It was observed that citral, citronellol and eugenol prevented fungal growth and toxin formation for up to 8 d. However, after 15 d of incubation, toxin production was greater than the controls.

Martinez-Gonzalez, J., B. Raposo, et al. (2001). “3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition prevents endothelial NO synthase downregulation by atherogenic levels of native LDLs: balance between transcriptional and posttranscriptional regulation.” Arterioscler Thromb Vasc Biol 21(5): 804-9. Atherogenic levels of native low density lipoproteins (nLDLs) decrease the bioavailability of endothelium-derived NO and downregulate endothelial NO synthase (eNOS) expression in cultured human endothelial cells. Here, we show that simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, within the therapeutic range (0.01 to 1 &mgr;mol/L) prevented the downregulation of eNOS mRNA and protein promoted by nLDL (180 mg cholesterol/dL, 48 hours) in human umbilical vein endothelial cells. This effect of simvastatin was completely reversed by mevalonate, the product of the reaction, and to a lesser extent by farnesol and geranyl geraniol. Simvastatin significantly stabilized eNOS mRNA in cells treated with nLDL during 48 hours (eNOS mRNA half-life approximately 11 hours in controls versus >24 hours in nLDL per 0.1 micromol/L simvastatin-treated cells). The downregulation of eNOS by nLDL was abrogated by cycloheximide, an inhibitor of protein synthesis, and by N-acetyl-leucyl-leucyl-norleucinal, a protease inhibitor that reduces the catabolism of sterol regulatory element binding proteins. Sterol deprivation increased the downregulation produced by nLDL on eNOS and sterol regulatory element binding protein-2 expression levels. However, no differential modulation of the retardation bands corresponding to the putative sterol-responsive element present in the eNOS promoter was detected by electrophoretic mobility shift assay. Our results suggest that nLDL promote eNOS downregulation operating at a transcriptional level, whereas simvastatin prevents such an effect through a posttranscriptional mechanism.

Mashanov, V. I. and I. E. Logvinenko (1979). “Artemisia balchanorum under cultivation.” Doklady Vsesoyuznoi Ordena Lenina Akademii Sel'skokhozyaistvennykh Nauk Imeni V.I. Lenina(1): 23-24. In 1972, the high-yielding varieties Krymchanka [Crimean], Balkhanka, Yuzhanka [Southerner], Evrika [Eurika] and Slavyanka were selected from a population in the Ukraine for high content of citral, linalool and geraniol.

During 1975-77, they were studied in various zones of the southern Ukraine. The best for yield of essential oil in all zones were Evrika and Balkhanka.

Masuda, Y., M. Nakaya, et al. (1997). “Geranylgeraniol potently induces caspase-3-like activity during apoptosis in human leukemia U937 cells.” Biochem Biophys Res Commun 234(3): 641-5. In a previous study, we showed that geranylgeraniol (GGO) is a potent inducer of apoptosis in human leukemia cells. The present study describes the effects of GGO on the activity of cysteine-dependent aspartate-directed proteases (caspases) in human leukemia U937 cells. The caspase-3 (CPP32) activity was increased in a time-dependent manner by treatment with 50 microM GGO, whereas no activation of caspase-1 (interleukin-1beta converting enzyme (ICE)) was observed in any time period under the same experimental conditions. Other isoprenyl compounds such as geraniol, geranylgerany-lacetone, and vitamin K2 had no measurable effects on the activities of either caspase-3 or caspase-1. A inhibitor that preferentially inhibits the caspase-3 related caspases, Z-DEVD-FMK, strongly blocked the GGO-induced DNA fragmentation. These results suggest the involvement of caspase-3 in GGO-induced apoptosis in U937 human leukemia cells.

Meehan, T. D. and C. J. Coscia (1973). “Hydroxylation of geraniol and nerol by a monooxygenase from Vinca rosea.” Biochem Biophys Res Commun 53(4): 1043-8.

Mehmood, Z., S. Ahmad, et al. (1997). “Antifungal activity of some essential oils and their major constituents.” Indian Journal of Natural Products 13(2): 10-13. Essential oils from lemongrass (Cymbopogon flexuosus), palmarosa (C. martini), cinnamon (Cinnamomum zeylanicum) and mint (Mentha arvensis) were tested for antifungal activity against Aspergillus, Fusarium and Cladosporium isolates from an ophthalmology specimen. Cinnamon oil was the most active against Aspergillus, and overall; palmarosa was the most active against Fusarium, and lemongrass against Cladosporium. The most active constituent found was eugenol, from citral and geraniol; citronellol and cinnamaldehyde were inactive.

Meijer, A. H., M. I. Lopes Cardoso, et al. (1993). “Isolation and characterization of a cDNA clone from Catharanthus roseus encoding NADPH:cytochrome P-450 reductase, an enzyme essential for reactions catalysed by cytochrome P-450 mono-oxygenases in plants.” Plant Journal 4(1): 47-60. The membrane-bound flavoprotein NADPH:cytochrome P-450 (cytochrome c) reductase that functions in electron transfer to cytochrome P-450 monoxygenases, was purified from a cell suspension culture of C. roseus. Antiserum raised against the purified protein was found to inhibit NADPH:cytochrome c reductase activity as well as the activities of the cytochrome P-450 enzymes geraniol 10-hydroxylase and trans-cinnamate 4-hydroxylase, which are involved in alkaloid and phenylpropanoid biosynthesis, respectively. Immunoscreening of a C. roseus cDNA expression library resulted in the isolation of a partial NADPH:cytochrome P-450 reductase cDNA clone, which was identified on the basis of sequence homology with NADPH:cytochrome P-450 reductase from yeast and animal species. The identity of the cDNA was confirmed by expression in Escherichia coli as a functional protein capable of NADPH-dependent reduction of cytochrome c and neotetrazolium, 2 in vitro substrates for the reductase. The N-terminal sequence of the

reductase, which was not present in the cDNA clone, was determined from a genomic NADPH:cytochrome P-450 reductase clone. It was demonstrated that the reductase probably is encoded by a single copy gene. A sequence comparison of this plant NADPH:cytochrome P-450 reductase (deposited under EMBL Data Library accession number X69791) with the corresponding enzymes from yeast and animal species showed that functional domains involved in binding of the cofactors FMN, FAD and NADPH are highly conserved between all kingdoms. In C. roseus cell cultures a rapid increase of the reductase steady state mRNA level was observed after addition of fungal elicitor preparations from Pythium aphanidermatum that are known to induce cytochrome P-450-dependent biosynthetic pathways.

Milone, C., M. L. Tropeano, et al. (2002). "Selective liquid phase hydrogenation of citral on Au/Fe2O3 catalysts." Chem Commun (Camb)(8): 868-9. Gold supported on iron oxide hydrogenates citral (an alpha,beta-unsaturated aldehyde) to the corresponding alpha,beta-unsaturated alcohols (geraniol and nerol) with a selectivity higher than 95%.

Miquel, K., A. Pradines, et al. (1996). "Farnesol and geranylgeraniol induce actin cytoskeleton disorganization and apoptosis in A549 lung adenocarcinoma cells." Biochem Biophys Res Commun 225(3): 869-76. The effects of exogenous isoprenoids were investigated on A549 human lung adenocarcinoma cells. Among the tested isoprenoids, only farnesol and geranylgeraniol induce actin cytoskeleton disorganization, growth inhibition, and apoptosis. In contrast, desmosterol leads only to growth inhibition. We show that all tested isoprenoids are potent inhibitors of HMG CoA reductase activity, the sterols being the most powerful while they induce neither F-actin disorganization nor apoptosis. Thus the molecular mechanisms induced by farnesol and geranylgeraniol appear independent of reductase regulation. Our results point out the specific role of farnesol and geranylgeraniol on actin cytoskeleton organization and apoptosis in adenocarcinoma cells.

Mo, H., D. Tatman, et al. (2000). "Farnesyl anthranilate suppresses the growth, in vitro and in vivo, of murine B16 melanomas." Cancer Lett 157(2): 145-53. The numbers of isoprene residues and unsaturated bonds, cis/trans configuration, and head group polarity influence the tumor-suppressive potency of acyclic isoprenoid hydrocarbons and alcohols; within the series tested, trans, trans farnesol had the greatest potency. Geraniol esters had increased potency relative to that of the free alcohol. Farnesyl anthranilate induced a concentration-dependent decrease in the B16 melanoma cell population, in part due to an increased proportion of cells in the G1 phase of the cell cycle and in part by the increased the proportion of apoptotic cells. Farnesyl anthranilate (1.5 mmol/kg diet) significantly suppressed the growth of implanted B16 melanomas and lowered the plasma cholesterol levels of tumor-free mice.

Moleyar, V. and P. Narasimham (1992). "Antibacterial activity of essential oil components." Int J Food Microbiol 16(4): 337-42. Antibacterial activity of fifteen essential oil components towards food borne Staphylococcus sp., Micrococcus sp., Bacillus sp. and Enterobacter sp. was studied by an agar plate technique. Cinnamic aldehyde was the most active compound followed by citral, geraniol, eugenol and

menthol. At 500 micrograms/ml, cinnamic aldehyde completely inhibited the bacterial growth for more than 30 days at 30 degrees C that was comparable to 200 micrograms/ml of butylated hydroxy anisole (BHA). At lower temperatures, 25 and 20 degrees C, antibacterial activity of the five essential oil components increased. Addition of sodium chloride at 4% level (w/v) in the medium had no effect on the inhibitory activity of cinnamic aldehyde. In mixtures of cinnamic aldehyde and eugenol or BHA an additive effect was observed.

Momin, R. A. and M. G. Nair (2002). "Pest-managing efficacy of trans-asarone isolated from Daucus carota L. seeds." J Agric Food Chem 50(16): 4475-8. The bioactive hexane extract of Daucus carota seed yielded 2,4,5-trimethoxybenzaldehyde (1), oleic acid (2), trans-asarone (3), and geraniol (4). Compounds 1-4 were evaluated for their mosquitocidal, nematicidal, antifeedant, and antimicrobial activities. Only trans-asarone was active in the assays performed, causing 100% mortality to fourth-instar mosquito larvae, Aedes aegyptii, at 200 microg mL(-1) and the nematodes Caenorhabditis elegans and Panagrellus redivivus at 100 microg mL(-1). In feeding trials, trans-asarone also caused significant weight reductions of the caterpillars Helicovarpa zea, Heliothis virescens, and Manduca sexta when incorporated into artificial diet at a concentration of 100 microg mL(-1). Also, it exhibited slight activity at 100 microg mL(-1) against the yeasts Candida albicans, Candida parapsilasis, and Candida kruseii.

Moody, J. O., S. A. Adeleye, et al. (1995). "Analysis of the essential oil of Cymbopogon nardus (L.) Rendle growing in Zimbabwe." Pharmazie 50(1): 74-75. A total of 36 compounds were identified in the steam-distilled essential oil of C. nardus, collected from Zimbabwe in 1989. The major compound was trans-geraniol (29.47%), followed by its ester form geraniol formate (8.79%).

Mumcuoglu, K. Y., R. Galun, et al. (1996). "Repellency of essential oils and their components to the human body louse, Pediculus humanus humanus." Entomologia Experimentalis et Applicata 78(3): 309-314. Five essential oils and 9 of their components were compared with diethyltoluamide (DEET) for their repellent activity against P. humanus humanus [P. humanus]. The absolute or intrinsic repellency of the compounds was tested by applying the repellent to corduroy patches and comparing them with untreated patches. It was found that the most effective repellents were DEET and citronella, whose activity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days. The repellent activity of the oil components such as citronellal and geraniol lasted more than 15 and 8 days, respectively. DEET remained effective at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1. The comparative or standard repellency of the candidate repellents was examined with the aid of a new screening technique using hairs treated with ammonium bicarbonate which is attractive to lice. Using this technique it could be shown that the repellent activity of citronella and geraniol lasted 2 days and that of rosemary and citronellal for only 1 day. DEET was active for <1 day. Serial dilutions of these substances also revealed that citronella was the most potent repellent for lice, followed by citronellal, rosemary, geraniol and DEET. The differences however, were not significant.

Nin, S., P. Arfaioli, et al. (1995). “Quantitative determination of some essential oil components of selected Artemisia absinthium plants.” Journal of Essential Oil Research 7(3): 271-277. In traditional medicine, A. absinthium is used as an anthelmintic, insecticide, stomachic, and tonic. The essential oils, steam- distilled from leaves and flowers of plants propagated from 49 mother plants obtained from Italy (21 plants), Austria (10), Germany (5), France (4) or USA (9), were analysed by GC. More than 90 compounds were detected, most of which occurred only in trace amounts. Quantitative and qualitative differences were observed in the contents of 8 antibacterial components (alpha- and beta-thujone, terpinen-4-ol, linalool, nerol, geraniol, alpha-pinene, and 1,8-cineole [eucalyptol]). These variations were observed between individual accessions, and between plants obtained from the same geographical location. The essential oils of some genotypes were characterized by particularly high percentages of active principles.

Nishikitani, M., K. Kubota, et al. (1996). “Geranyl 6-O-alpha-L-arabinopyranosyl-beta-D-glucopyranoside isolated as an aroma precursor from leaves of a green tea cultivar.” Biosci Biotechnol Biochem 60(5): 929-31. A new glycosidic aroma precursor was isolated from green tea leaves (Camellia sinensis var. sinensis cv. Yabukita) along with the known primeverosides of cis-linalool 3,6-oxide, linalool and geraniol. These glycosides were separated by chromatographic isolation on Amberlite XAD-2, ODS flash chromatography, and finally HPLC. The chemical structure of the new unknown glycoside was confirmed as geranyl 6-O-alpha-L-arabinopyranosyl-beta-D-glucopyranoside (geranyl beta-vicianoside) by spectrometric analyses and by an enzymatic hydrolysis with glycosidase followed by GC-MS and HPLC analyses. Moreover the vicianoside was hydrolyzed with acetone powder obtained from fresh tea leaves to generate the same compounds, suggesting this glycoside to be a tea aroma precursor.

Nishio, E., K. Tomiyama, et al. (1996). “3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor impairs cell p4fferentiation in cultured adipogenic cells (3T3-L1).” Eur J Pharmacol 301(1-3): 203-6. Lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, inhibits the synthesis of mevalonic acid. We examined the effect of lovastatin on the differentiation of the fibroblast/adipocyte cell line (3T3-L1). Lovastatin inhibits the differentiation of 3T3-L1 cells in a dose-dependent fashion. The inhibitory effect of lovastatin was partially reversed by adding exogenous mevalonic acid to the 3T3-L1 cells. Exogenous cholesterol (15 micrograms/ml) did not prevent lovastatin inhibition of adipocyte conversion. The isoprenoids, farnesol and geraniol, partially prevented lovastatin inhibition of adipocyte conversion but squalene did not prevent lovastatin inhibition of adipocyte conversion. We conclude that the inhibitory effect of lovastatin was partially due to the blockade of the pathway leading to synthesis of isoprenoids, which are downstream products of mevalonic acid.

Nishioka, K., I. Nakatsuka, et al. (1988). “An improved method for 14C-labelling of farnesylacetic acid and its geranyl ester.” Radioisotopes 37(3): 133-9. Farnesylacetic acid was efficiently labelled with 14C at the 5-position and gefarnate, a potent ulcer inhibitor, was prepared from it in radioactive form for use in metabolic studies. Condensation of

[carbonyl-14C]acetyl chloride (5) with t-butyl 2-ethoxymagnesiomalonate (6) followed by acid-catalyzed deprotection and decarboxylation gave ethyl 3-oxo[3-14C]butanoate (8). Alkylation of the keto ester (8) with geranyl bromide (9) afforded the unsaturated keto ester (10), which was hydrolyzed and decarboxylated to give geranyl[2-14C]acetone (11). Grignard reaction of 11 with cyclopropylmagnesium bromide followed by treatment with hydrobromic acid yielded [4-14C]homofarnesyl bromide (13). Cyanation of 13 with potassium cyanide and subsequent hydrolysis gave [5-14C]farnesylacetic acid (1) in 6.1% yield from barium [14C]carbonate (3). Chlorination of 1 followed by esterification with geraniol afforded [5-14C]gefarnate (2) in 88% yield.

Oh, K., H. Matsuoka, et al. (1993). “Automatic evaluation of antifungal volatile compounds on the basis of the dynamic growth process of a single hypha.” Appl Microbiol Biotechnol 38(6): 790-4. An automatic analysing system was developed and employed for the evaluation of antifungal activity of volatile compounds in the gas phase. Aspergillus niger was inoculated on agar medium in the reaction vessel. The reaction vessel was incubated at 28 degrees C for 24 h and then a volatile compound was introduced into the vessel either in a batch or flow manner. The antifungal activity of the respective compounds estimated in situ was expressed by the dynamic response parameters of a single hypha. All volatiles tested in the present system inhibited hyphal growth, except linalyl acetate: Limone and geraniol were the most inhibitory. In contrast, linalyl acetate promoted hyphal growth. By definition of the parameters, the fungicidal and fungistatic effects could be distinguished.

Ohnuma, S., M. Watanabe, et al. (1996). “Identification and characterization of geranylgeraniol kinase and geranylgeranyl phosphate kinase from the Archaebacterium Sulfolobus acidocaldarius.” J Biochem (Tokyo) 119(3): 541-7. Geranylgeranyl diphosphate is an important precursor of archaebacterial ether-linked lipids, and it has been thought that all of this compound is "de novo" synthesized by geranylgeranyl diphosphate synthase. We studied the phosphorylation of geranylgeraniol, which seems to be related to the salvage pathway of biosynthesis of archaebacterial ether-linked lipids, in the Archaebacterium Sulfolobus acidocaldarius. Activities of geranylgeraniol kinase and geranylgeranyl phosphate kinase were detected in a cell lysate of S. acidocaldarius. The two enzymes were easily separated by ultracentrifugation. The membrane fraction and the cytosolic fraction contained geranylgeraniol kinase activity and geranylgeranyl phosphate kinase activity, respectively. Geranylgeraniol kinase, which requires divalent cation such as Mg2+, Co2+, and Mn2+ and NTP (ATP, GTP, CTP, UTP), catalyzes monophosphorylation of (all-E)-geranylgeraniol to produce geranylgeranyl phosphate. (all-E)-Farnesol, (all-E)-hexaprenol, and (all-E)-octaprenol were also active substrates, though they were less effective than (all-E)-geranylgeraniol. However, neither geraniol nor (22E,6E,10Z,14Z,18Z,22Z,26Z,++ +30Z,34Z,38Z)-undecaprenol was active. This enzyme is extremely thermostable and its pH optimal is between 6.5 and 8.5. The Michaelis constants for (all-E)-geranylgeraniol and ATP are 27 nM and 650 microM, respectively.

Okutani, F., J. J. Zhang, et al. (2002). “Non-specific olfactory aversion induced by intrabulbar infusion of the GABA(A) receptor antagonist bicuculline in young rats.”

Neuroscience 112(4): 901-6. On postnatal day 12, young rats show an aversion to an odor to which they had been exposed along with presentations of foot shock on postnatal day 11. The acquisition of this aversive learning involves and requires disinhibition of the mitral/tufted cells induced by centrifugal noradrenergic activation during somatosensory stimulation. This olfactory learning is established only for the odor to which the rat has been exposed during conditioning. Infusion of the GABA(A) receptor antagonist bicuculline at a high dose (2.0 nmol/each olfactory bulb) into the olfactory bulb in the presence of an odor is capable of developing olfactory aversive responses without somatosensory stimulation in young rats. The purpose of this study is to characterize the properties of bicuculline-induced aversive responses. In contrast to the odor specificity of aversive learning produced by odor-shock conditioning, bicuculline-induced aversive responses lack odor specificity. Namely, bicuculline infusion in the presence of a citral odor results, in a dose-dependent manner, in subsequent aversive responses to strange odors (benzaldehyde and vanillin) that have never been presented. Moreover, bicuculline infusion alone is sufficient to produce dose-dependent aversive responses to strange odors (citral, benzaldehyde and geraniol).From these results we suggest that disinhibition of mitral/tufted cells from granule cells by bicuculline infusion makes young rats aversive to strange odors non-specifically, as if the rats had learned the odor aversion as a result of odor exposure paired with foot shock. Different mechanisms of disinhibition of the mitral/tufted cells may underlie both the pharmacological manipulation and noradrenergic activation by somatosensory stimulation.

Onisei, T., E. T. Toth, et al. (1995). “Growth and volatile oil production of two different vitroclones of Pelargonium roseum Ait.” Rivista Italiana EPPOS(16): 13-19. In vitro-cultured plants of P. roseum, derived by direct organogenesis from stem nodes taken from 2 plants of different origin (Fundulea and Chisinau), were transplanted in the field in June. At the end of July and in mid Sep., plants were assessed for growth parameters and essential oil yield (data presented). Differences in in vitro culture establishment, shoot regeneration, shoot multiplication, root induction, plant acclimatization and essential oil production are discussed in relation to the explant source. The plants retained the characteristics of the parental genotype. Fundulea plants were more productive with regard to all the parameters assessed (plant height, number of branches, number of axillary buds, and plant FW and DW). The major essential oil constituents were geraniol, linalool and citronellal; these were present in higher quantities in plants derived from Fundulea explants, compared with those of Chisinau origin. Citronellol was only detected in plants of Fundulea origin.

Overbosch, P., R. de Wijk, et al. (1989). “Temporal integration and reaction times in human smell.” Physiol Behav 45(3): 615-26. A model description of intensity perception in human taste and smell developed earlier has now been verified experimentally to determine parameter values for odorants. The final objective is to quantify and understand odour-odour interaction phenomena in e.g., masking, deo-perfumes and flavour enhancement. Five types of olfactometer experiments were carried out, viz. determination of thresholds, determination of reaction times, scaling of perceived intensity after 5 sec stimulation, scaling of perceived intensity of a fixed concentration at

variable duration, and measurement of intensity/time relationships. Four subjects were used and the odorants cineole, geraniol and hexane.

Padayatty, S. J., M. Marcelli, et al. (1997). “Lovastatin-induced apoptosis in prostate stromal cells.” J Clin Endocrinol Metab 82(5): 1434-9. Benign prostatic hyperplasia (BPH) is a common disease of aging men. Current medical treatment for this condition is only partially effective, therefore many patients must undergo surgery for symptomatic relief. BPH is caused by an increase in prostate epithelial and stromal cells, especially the latter. Since BPH stromal cells have a long life span and are not very responsive to androgen withdrawal, cultured BPH stromal cells were used to explore the feasibility of pharmacologically inducing apoptosis in these cells. We obtained BPH tissue during surgery, and stromal cells were isolated and maintained in culture. After cells achieved confluence, we induced apoptosis with the HMGCoA reductase inhibitor, lovastatin (30 micromol/L). The effects of testosterone (100 micromol/L), dihydrotestosterone (DHT; 100 micromol/L) and finasteride (100 micromol/L) on lovastatin-induced apoptosis were studied on cells grown in media containing charcoal stripped serum. Similarly, we examined the effect of the cholesterol pathway metabolites, mevalonic acid (30 micromol/L), geranyl geraniol (30 micromol/L), farnesol (10 micromol/L), squalene (30 micromol/L) and 7-ketocholesterol (3 micromol/L) on lovastatin-induced apoptosis. We demonstrated apoptosis by DNA laddering in agarose gels, by fluorescence microscopy following acridine orange staining, and by flow cytometry after end-labeling of DNA strand breaks with biotin-16-dUTP using deoxynucleotidyl exotransferase (TdT). Lovastatin at 30 micromol/L, but not at lower concentrations, induced apoptosis in BPH prostate stromal cells. This was seen (by flow cytometry) in 16.6 +/- 7.3% (mean +/- SD) of BPH cells treated with lovastatin at 72 h vs. 2.5 +/- 1.2% of cells treated with ethanol. Lovastatin-induced apoptosis was not increased in stripped serum or by the addition finasteride, and was not inhibited by testosterone or DHT. Only mevalonate and geranyl geraniol, prevented lovastatin-induced apoptosis whereas farnesol, squalene, or 7-ketocholesterol did not. We conclude that lovastatin can induce apoptosis in BPH stromal cells in vitro, and this is not affected by androgen withdrawal or stimulation. It is unlikely that lovastatin, per se, will be an effective treatment for BPH in vivo, but it does provide a means for inducing apoptosis in vitro. Understanding the apoptotic process in BPH stromal cells ultimately may lead to new therapeutic strategies for BPH.

Padula, L. Z., A. M. Collura, et al. (1977). “Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil.” Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Passet, J., J. P. Laget, et al. (1994). “[Systemic emulsions. 2. Use of different methods of formulation: the effect of essential oils of thyme on stability].” J Pharm Belg 49(6): 469-78. As part of an ongoing investigation on emulsifying techniques, we studied the

influence of different essential oils from Thymus vulgaris on emulsion stability. All four chimiotypes tested (geraniol, linalol, carvacrol, and thymol) caused a marked decrease in stability. This instability cannot be explained by a change in the hydrophilic lipophilic balance since the HLBc of the new oil phase (essential oil + paraffine oil) was not significantly different from that of paraffine alone.

Pattnaik, S., V. R. Subramanyam, et al. (1997). "Antibacterial and antifungal activity of aromatic constituents of essential oils." Microbios 89(358): 39-46. Five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) were tested for antimicrobial activity against 18 bacteria (including Gram positive cocci and rods, and Gram negative rods) and 12 fungi (Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, A. oryzae, Alternaria citrii, Fusarium oxysporum, F. solani, Helminthosporium compactum, Macrophoma phaseolina, Sclerotium rolfsii, Sporothrix schenckii and Trichophyton mentagrophytes). In terms of antibacterial activity linalool was the most effective and inhibited 17 bacteria, followed by cineole, geraniol (each of which inhibited 16 bacteria), menthol and citral aromatic compounds, which inhibited 15 and 14 bacteria, respectively. Citral and geraniol oils were the most effective against fungi (inhibiting all 12 fungi), followed by linalool (which inhibited 10 fungi), cineole and menthol (each of which inhibited 7 fungi) compounds.

Paubert-Braquet, M., H. Cousse, et al. (1998). "Effect of the lipidosterolic extract of Serenoa repens (Permixon) and its major components on basic fibroblast growth factor-induced proliferation of cultures of human prostate biopsies." Eur Urol 33(3): 340-7. OBJECTIVE: To assess the effect of the lipidosterolic extract of Serenoa repens (LSESr) on in vitro cell proliferation in biopsies of human prostate MATERIAL AND METHODS: Cell proliferation was assessed by incorporation of [3H]thymidine followed by historadiography. RESULTS: Basic fibroblast growth factor (b-FGF) induced a considerable increase in human prostate cell proliferation (from +100 to +250%); the glandular epithelium was mainly affected, minimal labeling being recorded in the other regions of the prostate. Similar results were observed with epidermal growth factor (EGF), although the increase in cell proliferation was not recorded in some cases. Lovastatin, an inhibitor of hydroxymethylglutaryl coenzyme A, antagonized both the basal proliferation and the growth factor-stimulated proliferation of human prostate epithelium (EGF, mean inhibition approximately 80-95%; b-FGF, mean inhibition approximately 40-90%). Geraniol, a precursor of both farnesyl pyrophosphate and geranylgeranyl pyrophosphate, and farnesol, the precursor of farnesyl pyrophosphate, increased cell proliferation only in some prostate specimens, this effect being antagonized by lovastatin. LSESr did not affect basal prostate cell proliferation, with the exception of two prostate specimens in which a significant inhibition of basal proliferation was observed with the highest concentration of LSESr (30 micrograms/ ml). In contrast, LSESr inhibited b-FGF-induced proliferation of human prostate cell cultures; this effect was significant for the highest concentration of LSESr (30 micrograms/ml). In some prostate samples, a similar inhibition was also noted with lower concentrations. Unsaturated fatty acids (UFA), in the range 1-30 ng/ml), did not affect the basal prostate cell proliferation, only a slight increase in cell proliferation was noted in 1 prostate specimen. UFA (1, 10 or 30 micrograms/ml) markedly inhibited the b-FGF-induced cell

proliferation down to the basal value. Lupenone, hexacosanol and the unsaponified fraction of LSESr markedly inhibited the b-FGF-induced cell proliferation, whereas a minimal effect on basal cell proliferation was noted. CONCLUSIONS: Despite the large variability in the response of the prostate samples to b-FGF, these results indicate that LSESr and its components affect the proliferative response of prostate cells to b-FGF more than their basal proliferation.

Pawar, P. V., R. N. Sharma, et al. (1991). "Action of some insect growth regulators on mosquito vectors: Part I--Citronellol based diethers." J Commun Dis 23(2): 118-22. New series of compounds starting from common terpenoids (Geraniol, citronellol) have been examined for biological activity on mosquito larvae. Many of these exhibited development disruption on eggs as well larvae. Some also affected adult oviposition behaviour. Developmental disturbances were classified as JH type by inducing typical metamorphosis inhibition in Dysdercus koenigii in the standard Hemipteran JH bioassay. Where indicated simulated field trials were also undertaken. This report describes results of bioevaluation of the citronellol based compounds. The results indicate that these show multifarious activity against mosquitoes but at relatively high doses, suggesting that exploration of further structural variety is needed before truly promising analogues can be obtained.

Pawar, P. V., S. P. Pisale, et al. (1995). "Effect of some new insect growth regulators on metamorphosis & reproduction of Aedes aegypti." Indian J Med Res 101: 13-8. Fourth instar larvae and pupae of Ae. aegypti were treated with four most active insect growth regulators from a new series of mixed alkyl and aryl diethers based on geraniol. Considerable reduction in fecundity and fertility of adults was obtained. Treatment of pupae or pharate adults did not affect adult emergence. Topical treatment of adult females caused great reduction in fertility and fecundity in older as compared to younger females. In addition to the effects on reproduction, adult survival was also reduced in the treated younger females.

Perry, N. S. L., P. J. Houghton, et al. (2000). "In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes." Journal of Pharmacy and Pharmacology 52(7): 895-902. The effects of S. lavandulaefolia [S. lavandulifolia] essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole [eucalyptol] (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma- terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha- pinene and were 0.03 micro g/ml, 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma- terpinene) were less potent (camphor IC50 of >10 mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro

cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant

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Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S., P. J. Houghton, et al. (2000). “In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes.” J Pharm Pharmacol 52(7): 895-902. Sage (Salvia spp) is reputed in European herbal encyclopaedias to enhance memory, and current memory-enhancing/anti-dementia drugs are based on enhancing cholinergic activity by inhibiting cholinesterase. In this study the effects of Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil batch used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma-terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha-pinene and were 0.03 microL [corrected] mL(-1), 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma-terpinene) were less potent (camphor IC50: >10mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. These findings suggest that if the inhibitory activity of the essential oil is primarily due to the main inhibitory terpenoid constituents identified, there is a major synergistic effect among the constituents. Since no single constituent tested was

particularly potent, it remains to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” J Pharm Pharmacol 53(10): 1347-56. Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in-vitro and in-vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, anti-inflammatory and estrogenic effects. Results of in-vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg mL(-1)) and the monoterpenoids (0.1 M) alpha- and beta-pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible anti-inflammatory activity (eicosanoid inhibition in rat leucocytes) was found in the EtOH extract (50 microg mL(-1)) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible estrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg mL(-1)) and the monoterpenoid geraniol (0.1-2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit estrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Rajab, M. S., C. L. Cantrell, et al. (1998). “Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study.” Planta Med 64(1): 2-4. The crude methanol extract of the Kenyan shrub Leucas volkensii Gurke (Labiatae) displayed in a radiorespirometric bioassay antimycobacterial activity against Mycobacterium tuberculosis. Bioassay-guided fractionation of the crude extract led to the identification of (E)-phytol as the principal active component with a minimum inhibitory concentration (MIC) of 2 micrograms/ml, a value also observed for (3R,S,7R,11R)-phytanol, (Z)-phytol, and a commercially available 2:1 mixture of (E)- and (Z)-phytol. The derivatives (E)-phytol acetate, a mixture of the (2S,3S)- and (2R,3R)-isomers of (E)-phytol epoxide and (3R,S,7R,11R)-phytanic acid displayed lower activities with MICs of 8, 16, and > 128 micrograms/ml, respectively. Geraniol and farnesol, displayed MICs of 64 and 8 micrograms/ml, respectively. The activities of (E)-phytol, (Z)-phytol and (3R,S,7R,11R)-phytanol were found to be in the same range as ethambutol, a clinically useful drug with an MIC in the range 0.95-3.8 micrograms/ml.

Rastogi, S. C., J. D. Johansen, et al. (1996). “Natural ingredients based cosmetics. Content of selected fragrance sensitizers.” Contact Dermatitis 34(6): 423-6. In the present study, we have investigated 42 cosmetic products based on natural ingredients for content of 11 fragrance substances: geraniol, hydroxycitronellal, eugenol, isoeugenol, cinnamic

aldehyde, cinnamic alcohol, alpha-amylcinnamic aldehyde, citral, coumarin, dihydrocoumarin and alpha-hexylcinnamic aldehyde. The study revealed that the 91% (20/22) of the natural ingredients based perfumes contained 0.027%-7.706% of 1 to 7 of the target fragrances. Between 1 and 5 of the chemically defined synthetic constituents of fragrance mix were found in 82% (18/22) of the perfumes. 35% (7/20) of the other cosmetic products (shampoos, creams, tonics, etc) were found to contain 0.0003-0.0820% of 1 to 3 of the target fragrances. Relatively high concentrations of hydroxycitronellal, coumarin, cinnamic alcohol and alpha-amyl cinnamic aldehyde were found in some of the investigated products. The detection of hydroxycitronellal and alpha-hexylcinnamic aldehyde in some of the products demonstrates that artificial fragrances, i.e., compounds not yet regarded as natural substances, may be present in products claimed to be based on natural ingredients.

Rastogi, S. C., J. D. Johansen, et al. (1998). “Deodorants on the European market: quantitative chemical analysis of 21 fragrances.” Contact Dermatitis 38(1): 29-35. Deodorants are one of the most frequently used types of cosmetics and side-effects from them are common. Recent studies relate perfume allergy to this type of product. 73 deodorants were analyzed by gas chromatography--mass spectrometry for the determination of the contents of 7 wellknown fragrance allergens from the fragrance mix and 14 other commonly used fragrance materials. The deodorants were purchased at retail outlets in 5 European countries. It was found that in general, fragrance mix ingredients were more frequently present in vapo- and aerosol sprays than in roll-on products. The levels of the fragrance mix substances ranged from 0.0001-0.2355%. The products investigated contained cinnamic aldehyde and isoeugenol less frequently (17% and 29% respectively), and eugenol and geraniol most frequently (57% and 76% respectively). The 14 other fragrance materials were found in 40-97% of the deodorants, with hedione and benzyl acetate the most frequently found substances. The concentration of these 14 substances ranged from 0.0001-2.7%. It is concluded that the levels of cinnamic aldehyde and isoeugenol found in the deodorants could prove to be relevant for elicitation of contact dermatitis. No conclusions could be drawn about the other fragrance mix constituents, as threshold levels in sensitized individuals have not been investigated. Furthermore, all of the fragrance materials investigated were frequently found in deodorants and, apart from the fragrance mix ingredients, the extent of problems with sensitization to these fragrance materials is largely unknown.

Rastogi, S. C., J. D. Johansen, et al. (1999). “Contents of fragrance allergens in children's cosmetics and cosmetic-toys.” Contact Dermatitis 41(2): 84-8. Fragrances are one of the major causes of allergic contact dermatitis from use of cosmetics. The aim of the current study was to assess the possible exposure of infants and children to fragrance allergens from cosmetic products and "toy-cosmetics". 25 children's cosmetics or toy-cosmetic products were analysed by gas chromatography - mass spectrometry. Target substances were the fragrance allergens from the fragrance mix and 14 other fragrance substances, most of which have been described as contact allergens. The fragrance mix ingredients were either not present in children's shampoos/shower gels and cream/lotions, or they were present in fairly low concentrations. In hydro-alcoholic products, such as eau de parfum, eau de toilette, several ingredients of the fragrance mix were found: geraniol was

present in 7/7 products, hydroxycitronellal in 6/7 and isoeugenol in 2/7 products. Isoeugenol was present in a maximum concentration of 0.07%. In one cosmetic-toy, cinnamic alcohol was present at 3.7% which exceeds the current industry guideline for safe products by a factor of 5. In all types of products other fragrance allergens were frequently found. In conclusion, children are already exposed at an early age to well-known allergens, sometimes at concentrations which are considered to be unsafe. As contact allergy usually persists for life, manufacturers of children's cosmetics should be aware of their special responsibility and apply the highest possible safety standards.

Rastogi, S. C., S. Heydorn, et al. (2001). “Fragrance chemicals in domestic and occupational products.” Contact Dermatitis 45(4): 221-5. Epidemiological studies have described an increasing prevalence of fragrance allergy and indicated an association with hand eczema. 59 domestic and occupational products intended for hand exposure were subjected to gas chromatography-mass spectrometric (GC-MS) analyses to test the hypothesis that fragrance chemicals known to have the potential to cause contact allergy but not included in fragrance mix (FM) may be common ingredients in these products. A quantitative analysis of 19 selected fragrances was performed by GC-MS. Further analysis of GC-MS data revealed the presence of 43 other fragrance chemicals/groups of fragrance chemicals in the products investigated. Among the 19 target substances the most commonly detected were limonene in 78%, linalool in 61% and citronellol in 47% of the products investigated. The FM ingredients were present in these products with the following frequencies: oak moss (evernic acid methylester) 2%, cinnamic alcohol 2%, cinnamic aldehyde (cinnamal) 3%, isoeugenol 5%, alpha-amylcinnamic aldehyde (amyl cinnamal) 8%, hydroxycitronellal 12%, eugenol 27%, and geraniol 41%. Thus, the chemical analyses of domestic and occupational products indicates that investigation of potential contact allergy related to these products types should consider fragrance allergens additional to those in the FM, since these may occur with high frequency.

Riou, C., J. M. Salmon, et al. (1998). “Purification, characterization, and substrate specificity of a novel highly glucose-tolerant beta-glucosidase from Aspergillus oryzae.” Appl Environ Microbiol 64(10): 3607-14. Aspergillus oryzae was found to secrete two distinct beta-glucosidases when it was grown in liquid culture on various substrates. The major form had a molecular mass of 130 kDa and was highly inhibited by glucose. The minor form, which was induced most effectively on quercetin (3,3',4',5,7-pentahydroxyflavone)-rich medium, represented no more than 18% of total beta-glucosidase activity but exhibited a high tolerance to glucose inhibition. This highly glucose-tolerant beta-glucosidase (designated HGT-BG) was purified to homogeneity by ammonium sulfate precipitation, gel filtration, and anion-exchange chromatography. HGT-BG is a monomeric protein with an apparent molecular mass of 43 kDa and a pI of 4.2 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing polyacrylamide gel electrophoresis, respectively. Using p-nitrophenyl-beta-D-glucoside as the substrate, we found that the enzyme was optimally active at 50 degreesC and pH 5.0 and had a specific activity of 1,066 &mgr;mol min-1 mg of protein-1 and a Km of 0.55 mM under these conditions. The enzyme is particularly resistant to inhibition by glucose (Ki, 1. 36 M) or glucono-delta-lactone (Ki, 12.5 mM), another powerful beta-glucosidase inhibitor present in wine. A comparison of the enzyme

activities on various glycosidic substrates indicated that HGT-BG is a broad-specificity type of fungal beta-glucosidase. It exhibits exoglucanase activity and hydrolyzes (1-->3)- and (1-->6)-beta-glucosidic linkages most effectively. This enzyme was able to release flavor compounds, such as geraniol, nerol, and linalol, from the corresponding monoterpenyl-beta-D-glucosides in a grape must (pH 2.9, 90 g of glucose liter-1). Other flavor precursors (benzyl- and 2-phenylethyl-beta-D-glucosides) and prunin (4',5,7-trihydroxyflavanone-7-glucoside), which contribute to the bitterness of citrus juices, are also substrates of the enzyme. Thus, this novel beta-glucosidase is of great potential interest in wine and fruit juice processing because it releases aromatic compounds from flavorless glucosidic precursors.

Roesyanto-Mahadi, I. D., A. M. Geursen-Reitsma, et al. (1990). “Sensitization to fragrance materials in Indonesian cosmetics.” Contact Dermatitis 22(4): 212-7. 2 different groups of patients were patch tested with 2 test series (A and B) containing extracts of fragrance raw materials, traditionally used in Indonesian cosmetics. Series A consisted of diluted extracts of commercially available Indonesian fragrances. Series B consisted of extracts prepared in our department from corresponding indigenous flowers and fruits. Group 1 consisted of 32 patients positive to fragrance-mix, of whom 8 (25%) had positive tests to 1 or more of the different extracts of fragrance raw materials. Reactions were observed to extracts of: Rosa hybrida Hort (7); Canangium odoratum Baill (5); Citrus aurantifolia Swingle (4); Jasminum sambac Ait (2). 6 of the 8 patients had reactions to 1 or more of the components of fragrance-mix: oakmoss (3); cinnamic alcohol (2), isoeugenol (1); cinnamic aldehyde (1) and geraniol (1). Group 2 consisted of 159 patients patch tested on suspicion of contact dermatitis, who were fragrance-mix negative. Only 2 (1.2%) had a positive patch test to the extracts of fragrance raw materials. Specimens taken (as is) from the flowers and citrus fruits (being the basis sources of the fragrance raw materials) were less antigenic. The use of additional test series in Indonesia to detect allergy to traditional cosmetics and perfumes merits further investigation.

Rojas, M. C., L. Chayet, et al. (1983). “Substrate and metal specificity in the enzymic synthesis of cyclic monoterpenes from geranyl and neryl pyrophosphate.” Arch Biochem Biophys 222(2): 389-96. A partially purified enzyme (carbocyclase) from the flavedo of Citrus limonum formed alpha-pinene, beta-pinene, limonene, and gamma-terpinene from geranyl pyrophosphate (GPP) and neryl pyrophosphate. The maximum specific activities obtained were 7.0 and 3.6 nmol/min/mg, respectively. Cross-inhibition by the two substrates were observed and the ability to utilize neryl pyrophosphate was almost completely lost with aging. Citronellyl pyrophosphate and dimethylallyl pyrophosphate were the most effective inhibitors of carbocyclase. Isopentenyl pyrophosphate, the monophosphate esters of nerol and geraniol, as well as inorganic pyrophosphate were much less effective inhibitors. The enzyme had an absolute requirement for Mn2+. It could be replaced with about 2% effectiveness by Mg2+ and Co2+. Kinetic studies showed that the observed reaction rate correlates with the calculated concentration of the GPP (Mn2+)2 species. Previous evidence with nonenzymatic reactions and the results presented support the view that the mechanism of carbocyclase may be the intramolecular analog of prenyltransferase.

Romaguera, C., F. Grimalt, et al. (1986). “Geraniol dermatitis.” Contact Dermatitis 14(3): 185-6.
Romaguera, C., F. Grimalt, et al. (1987). “[Contact dermatitis caused by perfumes and essences contained in various preparations for topical use].” Med Cutan Ibero Lat Am 15(5): 367-70. Regarding two cases of allergic contact dermatitis caused by geraniol, containing in two different pharmaceutical products, a revision has been made of the preparations of topical application which contain this and other fragrances. At the same time the clinical picture and examination of these cause is reported.

Roullet, J. B., U. C. Luft, et al. (1997). “Farnesol inhibits L-type Ca2+ channels in vascular smooth muscle cells.” J Biol Chem 272(51): 32240-6. Earlier experiments with animal and human arteries have shown that farnesol, a natural 15-carbon (C15) isoprenoid, is an inhibitor of vasoconstriction (Roullet, J.-B., Xue, H., Chapman, J., McDougal, P., Roullet, C. M., and McCarron, D. A. (1996) J. Clin. Invest. 97, 2384-2390). We report here that farnesol reduced KCl- and norepinephrine-dependent cytosolic Ca2+ transients in fura-2-loaded intact arteries. An effect on Ca2+ signaling was also observed in cultured aortic smooth muscle cells (A10 cells). In these cells, farnesol reduced KCl-induced [Ca2+]i transients and mimicked the inhibitory effect of Ca2+-free medium on the [Ca2+]i response to both 12,13-phorbol myristate acetate, a protein kinase C activator, and thapsigargin, a specific endoplasmic reticulum ATPase inhibitor. Perforated patch-clamp experiments further showed in two vascular smooth muscle cell lines (A10 and A7r5), a reversible, dose-dependent inhibitory effect of farnesol on L-type Ca2+ currents (IC50 = 2.2 microM). Shorter (C10, geraniol) and longer (C20, geranylgeraniol) isoprenols were inactive. L-type Ca2+ channel blockade also occurred under tight (gigaohm) seal configuration using cell-attached, single-channel analysis, thus suggesting a possible action of farnesol from within the intracellular space. We finally demonstrated that farnesol did not affect Ca2+-sensitive pathways implicated in smooth muscle contraction, as tested with alpha-toxin permeabilized arteries. Altogether, our results indicate that farnesol is an inhibitor of vascular smooth muscle Ca2+ signaling with plasma membrane Ca2+ channel blocker properties. The data have implications for the endogenous and pharmacological regulation of vascular tone by farnesol or farnesol analogues.

Sargenti, S. R. and F. M. Lancas (1997). “Supercritical fluid extraction of Cymbopogon citratus (DC.) Stapf.” Chromatographia 46(5/6): 285-290. Steam extraction of the leaves of C. citratus (known as lemon grass) produces a volatile oil used for medicinal purposes. Supercritical fluid extraction of C. citratus in sequential and dynamic extraction modes is described. Different modifiers for supercritical carbon dioxide were used for the extractions. Principal compounds in the essential oils were separated by GC and GC-MS and identified as neral, geraniol, geranial, nerolic acid and geranic acid. Different chromatographic profiles were obtained when the type and proportion of modifier were changed. When the modifier was 10% hexane, the extract yield was similar to that for steam extraction but with additional compounds; with 30% hexane the extract was similar to that from Soxhlet extraction with hexane; 10 or 20% acetone liberated compounds not extracted by other agents; 10% methanol was not selective.

Saxena, V. K. and R. N. Sharma (1998). “Constituents of the essential oil from Commiphora mukul gum resin.” Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Schaneberg, B. T. and I. A. Khan (2002). “Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC.” J Agric Food Chem 50(6): 1345-9. A gas chromatography flame ionization detection method for the quantification of bioactive marker compounds (neral, geranial, geraniol, limonene, citronellal, and beta-myrcene) in the essential oil of Cymbopogon citratus (lemon grass) was developed. Four procedures for the extraction of essential oils from C. citratus were compared including solvent extraction, steam distillation extraction, accelerated solvent extraction, and supercritical fluid extraction. Solvent extraction by sonication with nonpolar solvents showed comparable results to the steam distillation method. Several commercial products prepared from C. citratus and Cymbopogon flexuosus were analyzed and compared.

Schmidt, C., U. Schmidt, et al. (1984). “The effect of N-methyl-formimino-methylester on the neural olfactory threshold in albino mice.” Arch Otorhinolaryngol 239(1): 25-9. The effects of N-methyl-formimino-methylester were studied in albino mice. Very short exposure (0.5 and 1 s) to the concentrated vapour led to an increase of the neural olfactory threshold to geraniol by a factor of 10(5)-10(7). There was a slow recovery of the olfactory sensitivity and after about 40 days the threshold values returned to normal.

Schmidt, U. and M. Eckert (1988). “The influence of early odour experience on the neural response of the olfactory bulb in laboratory mice.” J Comp Physiol [A] 163(6): 771-6. In mice (strain NMRI) the influence of olfactory rearing conditions on the ontogenetic development of the bulbar electroencephalogram (EEG) was investigated. The cages of control animals were perfused continually with filtered air, whereas in the three experimental groups geraniol was added to the atmosphere at different times (group G0-13, from birth till day 13; group G0-6, from birth till day 6; group G6-12, from day 6 till day 12). At various ages the EEG of the bulbus olfactorius was studied by means of permanently implanted tungsten electrodes, and the neural response to nest odour and geraniol (10(-2) vol. %) was recorded. No differences were found between the groups regarding the overall development of the bulbar EEG, nor did the raising conditions affect the neural response to nest odour. However, in groups G0-13 and G6-12 a marked response to the odour of geraniol was recorded, while in the controls and the individuals that had experienced geraniol only during their first week of life, the bulbar response to this odourant did not differ from that obtained following stimulation with clean air. In the animals of group G0-13, which were investigated as adults (day 70), the prominent geraniol response was still recordable 2 months after the last contact with the odour. These results indicate that odours experienced during a sensitive period in the nest evoke

neuronal alterations in the olfactory system of the mouse that facilitate processing of a known odourant.

Serrano, G., C. Pujol, et al. (1989). “Riehl's melanosis: pigmented contact dermatitis caused by fragrances.” J Am Acad Dermatol 21(5 Pt 2): 1057-60. We report a case of a 27-year-old woman with a patchy, dark brown hyperpigmentation on the face. Patch tests were positive to lemon oil, geraniol, and hydroxycitronellal. A compact face powder that the patient used contained two of these chemicals. Hyperpigmentation disappeared within 6 months after the patient avoided contact with cosmetics containing these fragrances.

Sharma, R. N., P. V. Pawar, et al. (1993). “Action of some new insect growth regulators on mosquito vectors. Part II: Geraniol based diethers.” J Commun Dis 25(1): 30-5. Biological activity of saturated diethers viz. 1-benzyloxy/phenoxy-8-alkoxy and 1-alkoxy-8-benzyloxy-3,7-dimethyl-1, 8-octanes (IIa-IIq) prepared from Geraniol, were studied on three mosquito species and the bug Dysdercus koenigii. These diethers exhibited oviposition deterrent and developmental inhibition activities of greater magnitudes than the compounds based on citronellol reported in Part I of this paper. Some of these new compounds inhibit development of mosquitoes at 0.05 ppm and deter oviposition at 0.05 per cent doses. Tests were extended to field simulated conditions in selected cases.

Shibuya, H., K. Ohashi, et al. (1994). “Chemical transformation of terpenoids. X. Ionophoretic activities of macrocyclic lactone epoxides synthesized from geraniol.” Chem Pharm Bull (Tokyo) 42(2): 293-9. Two coronand-type 18-membered lactone epoxides, i.e., geranyl dimeric lactone diepoxide (GL2E2, 10) and tetraepoxide (GL2E4, 11), were synthesized from geraniol as diastereomeric mixtures. Among them, GL2E4 (11) was shown to exhibit ion-transport activity for Ca2+ ion in the test using a W-07 (liquid-membrane type) apparatus and ion-permeation activities for Ca2+ and K+ ions across the human erythrocyte membrane. Isolation of six component diastereomers of GL2E4 (11) [GL2E4-1 (11c), -2 (11d), -3 (11e), -4 (11f), -5 (11g), -6 (11h)], was effected by HPLC separation of two diastereomeric tetraepoxides (11a, 11b) which were prepared from two diepoxides (GL2E2-1, 10a and GL2E2-2, 10b). The relative stereostructures of these diastereomers were determined by a combination of X-ray diffraction and 1H-NMR analyses. Among the six diastereomers, S2-symmetrical GL2E4-4 (11f) exhibited the strongest ion-transport activity for Ca2+ ion while C2-symmetrical GL2E4-6 (11h) exhibited the strongest ion-permeation activity for Ca2+ ion across the human erythrocyte membrane.

Shoff, S. M., M. Grummer, et al. (1991). “Concentration-dependent increase of murine P388 and B16 population doubling time by the acyclic monoterpene geraniol.” Cancer Res 51(1): 37-42. Geraniol, an acyclic end product of a plant isoprene pathway and a pyrophosphorylated intermediate in plant and animal pathways, caused a concentration-dependent increase in the population doubling time of murine P388 leukemia cells in suspension culture and of B16 melanoma cells in monolayer culture. The suppression of the growth of P388 cells by geraniol (0-0.9 mM) and by mevinolin (0-0.25 microM), a competitive inhibitor of mevalonate biosynthesis, was reversed by the addition of 0.5 mM

mevalonolactone to the growth medium. Flow cytometry of asynchronous B16 cells grown with geraniol (0-0.15 mM) revealed a population characterized by larger cells with altered nuclear characteristics. Over the course of four studies, dietary geraniol increased the 50% survival time of mice by 10, 29, 33, and 50% following the i.p. transfer of P388 cells. The results of the latter study showed that, following the i.p. transfer of 1 x 10(5) P388 cells, the control group of female C57BL x DBA/2 F1 mice had a 50% survival time of 24 days and a maximum survival of 27 days. Mice fed a diet containing 0.1% geraniol for 14 days prior to and following the P388 cell transfer had a 50% survival time of 36 days, and 20% of the mice remained free of tumors during the 50-day trial. These studies support the possibility that monoterpenes and other isoprenoid products of plant metabolism are in part responsible for the anticarcinogenic actions of diverse fruits, vegetables, and cereal products.

Shoji, Y., H. Ishige, et al. (1998). “Enhancement of anti-herpetic activity of antisense phosphorothioate oligonucleotides 5' end modified with geraniol.” J Drug Target 5(4): 261-73. We have previously shown that antisense phosphorothioate oligonucleotide (SON) targeted against immediate early (IE) pre-mRNA5 of the herpes simplex virus type I (HSV-I) possessed potent anti-herpetic activities in vitro system. However, anti-herpetic activities of SON were not still efficient enough. Lipophilic compounds have been often conjugated with antisense oligonucleotide to enhance the biological activity. In this study, we selected geraniol as a lipophilic compound and newly synthesized SON bearing 5' terminal geraniol (geranyl-SON) toward IE pre-mRNA 5 of the HSV-1 to enhance the anti-herpetic activity. Geraniol is a olefinic terpene alcohol which is found in many essential oils. It possesses lipophilic characteristic. It is thought to be absorbed in tissue. Geraniol enhanced the anti-herpetic activity of SON with less cytotoxicity in a sequence specific manner. Terminal modification with geraniol did not affect binding affinity with complimentary DNA. Cytoplasm distribution of geranyl-SON was confirmed by confocal microscope. While some of the geranyl-SON was seen in the nucleus, unmodified SON had a punctate distribution in the cytoplasm with little in the nucleus. These results suggested that geranyl modification enhances anti-herpetic activity by changing the subcellular distribution of the oligonucleotides. Consequently geraniol-modifica-tion could provide new means for the efficient delivery of oligo-nucleotides.

Sidibe, L., J. C. Chalchat, et al. (2001). “Aromatic plants of Mali (IV): chemical composition of essential oils of Cymbopogon citratus (DC) Stapf and C. giganteus (Hochst.) Chiov.” Journal of Essential Oil Research 13(2): 110-112. The composition of the essential oils of C. citratus and C. giganteus from Mali and Cote d'Ivoire, collected in 1993 and 1994, was determined by GC and GC/MS, and they were found to contain 19 and 27 constituents, respectively. C. citratus oil from Mali contained a high proportion of citral (approximately 75%) (geranial/neral ca 2/1), some myrcene (6.2-9.1%) and geraniol (3.0-5.6%). It differed from the oil of the Ivory Coast in which the contents of geranial, neral and myrcene each ranged between 18-35%. C. giganteus oil was characterized by high proportions of cis- and trans-p-mentha-1(7), 8- dien-2-ols (approx. 50%) and p-mentha-2,8-dien-1-ols (approx.25%) together with isopiperitenol-carveol (approx. 10%) and traces of carvone (<5%).

Szabo, K., E. Nemeth, et al. (1996). “Morphological and chemical variability of basil genotypes.” Beitrage zur Zuchtungsforschung - Bundesanstalt fur Zuchtungsforschung an Kulturpflanzen 2(1): 76-79. The morphological and chemotaxonomic variability of 13 basil (Ocimum basilicum) genotypes was investigated. The genotypes were classified into 3 main groups on the basis of essential oil composition: (1) linalool; (2) linalool-estragole; and (3) linalool-geraniol-eugenol- gamma-murolene chemotypes. Data on plant height, secondary branches, leaves : stem : flower ratio and essential oil contents in different plant organs are also presented.

Tachibana, A., T. Tanaka, et al. (1996). “Evidence for farnesol-mediated isoprenoid synthesis regulation in a halophilic archaeon, Haloferax volcanii.” FEBS Lett 379(1): 43-6. Farnesol strongly inhibited growth of a halophilic archaeon, Haloferax volcanii, with an IC50 value of only 2 microM (0.4 microgram/ml) in rich medium and 50 nM (0.01 microgram/ml) in minimal medium without lysis. Other isoprenoid alcohols such as isopentenol, dimethylallyl alcohol, geraniol, and geranylgeraniol at 500 microM did not affect its growth. Mevalonate, which is the precursor of all isoprenoid membrane lipids in archaea, led to recovery of the growth inhibition of H. volcanii, but acetate had no such effect. Farnesol inhibited incorporation of acetate, but not mevalonate, into the lipid fraction. These results suggest that farnesol inhibited the biosynthetic pathway from acetate (acetyl-CoA) to mevalonate. Farnesol is known to be derived from the important intermediate of isoprenoids, farnesyl diphosphate (FPP), and found in neutral lipid fraction from this archaeon. Moreover, the cell-free extracts from H. volcanii could phosphorylate farnesol with ATP to generate farnesyl monophosphate and FPP. We conclude that farnesol-mediated isoprenoid synthesis regulation system by controlling farnesol concentration is present in H. volcanii.

Tse, G., D. Blankschtein, et al. (1999). “Thermodynamic prediction of active ingredient loading in polymeric microparticles.” J Control Release 60(1): 77-100. The growing use of microparticles as a controlled-delivery system for pharmaceutical and non-pharmaceutical active ingredients (AIs) has prompted a costly trial-and-error development of new and effective microparticle systems. In order to facilitate a more rational design and optimization of AI loadings in microparticles, we have developed a molecular-thermodynamic theory to predict the loading of liquid AIs in polymeric microparticles that are manufactured by a solvent evaporation process. This process involves the emulsification of a liquid polymer solution (consisting of polymer and AI dissolved in a volatile solvent) in an aqueous surfactant solution. The theory describes the equilibrium distribution of the AI between the aqueous phase and the dispersed polymeric droplets. The universal functional activity coefficient (UNIFAC) and UNIFAC-Free Volume (FV) group-contribution methods are utilized to model the nonidealities in the water and polymeric droplet phases, respectively. The inputs to the theory are: (i) the chemical structures, densities and total masses of the manufacturing ingredients, (ii) the manufacturing temperature and (iii) the glass transition temperature of the polymer. Since surfactant concentrations exceeding the critical micellar concentration (CMC) are often required in order to stabilize the dispersed polymeric droplets during the emulsion manufacturing process, the theory also accounts for AI solubilization in surfactant micelles present in the manufacturing solution. To test the AI loading predictions, we

compare theoretical predictions of AI loadings in poly(lactic acid), poly(methyl methacrylate) and polystyrene microparticles to experimentally measured ones for five model AIs with varying degrees of hydrophobicity (benzyl alcohol, n-octanol, geraniol, farnesol and galaxolide). We also demonstrate how the developed theory can be utilized to screen polymers with respect to their abilities to load a given AI, as well as to provide guidelines for manufacturing microparticles having the desired AI loading.

Tsuchiya, H. (2001). "Biphasic membrane effects of capsaicin, an active component in Capsicum species." J Ethnopharmacol 75(2-3): 295-9. Capsaicin, an active component in Capsicum species, not only stimulates sensory afferent neurons but also inhibits bacterial growth and platelet aggregation. To address the pharmacological mechanism of non-neuronal actions, the effects of capsaicin and its structural analog (N-vanillylnonanamide) on membrane fluidity were studied by measuring fluorescence polarization of liposomes prepared with different phospholipids and cholesterol. Capsaicin and the analog changed membrane fluidity over the concentration range of 50-500 microM differentially with varying concentrations and membrane lipid composition. They showed biphasic effects on 100 mol% 1-palmitoyl-2-oleoylphosphatidylcholine liposomes and 40 mol% cholesterol-containing 1-palmitoyl-2-oleoylphosphatidylcholine liposomes to fluidize and rigidify both liposomal membranes at low and high concentrations, respectively. Changes in membrane fluidity occurred at concentrations corresponding to their reported antibacterial and antiplatelet concentrations. Antibacterial (geraniol and lidocaine) and antiplatelet reference compounds (4-ethylphenol and benzyl alcohol) concentration-dependently fluidized membranes, while not showing biphasic effects. Comparing the potency to fluidize membranes, capsaicin was almost comparable to geraniol and 4-ethylphenol, and more active than lidocaine and benzyl alcohol. The membrane effects of capsaicinoids are responsible for their non-neuronal antibacterial and antiplatelet actions, although they are not the simple membrane fluidizers.

Van Dessel, G., H. Van Meirvenne, et al. (1992). "Uptake of dolichol by Vero cells." Biochem Cell Biol 70(6): 475-80. Characterization and kinetics of dolichol uptake by a Vero cell line are reported. Vero cells incorporate dolichol in a time- and dose-dependent manner. Optimal uptake is found at 37 degrees C and at a pH of 7.4. In contrast to cholesterol, an inhibitory effect on the dolichol incorporation is found for farnesol, geraniol, and retinol. Long chain polyprenols were slightly stimulatory. The translocation seems not to be highly energy dependent. The lack of substantial inhibition by chloroquine does not plead for a receptor-mediated endocytosis. Incorporated dolichol was distributed over both membranes and supernatant fractions, paralleling the distribution of the lysosomal marker beta-N-acetylhexosaminidase. The incorporated dolichol is subject to a fast efflux process, which is potentiated by the presence of lipid acceptors in the extracellular medium.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). "Constituents of essential oil of Myrtus communis." Planta Medica 39(2): 164-167. beta-Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported

constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Vieira, R. F., R. J. Grayer, et al. (2001). “Genetic diversity of Ocimum gratissimum L. based on volatile oil constituents, flavonoids and RAPD markers.” Biochemical Systematics and Ecology 29(3): 287-304. Morphological, chemical and genetic differences of 12 tree basil (O. gratissimum) accessions were studied to determine whether volatile oils and flavonoids can be used as taxonomic markers and to examine the relationship between random amplified polymorphic DNA (RAPD)s and these chemical markers. Eugenol, thymol, and geraniol were the major volatile oil constituents found in O. gratissimum. Xanthomicrol and cirsimaritin were the major external flavones. The accessions morphologically described as O. gratissimum var. gratissimum contained eugenol as the major volatile oil constituent, and cirsimaritin as the major flavone. O. gratissimum var. macrophyllum accessions contained thymol as the major volatile oil constituent, and xanthomicrol as the major flavone. A distinct essential oil and flavone chemotype (producing geraniol and a mixture of the flavones cirsimaritin, isothymusin, xanthomicrol, and luteolin) were found in an accession genetically more distant from the other two groups when analysed by molecular markers. The accessions could be divided based on volatile oil constituents into six groups: (1) thymol: alpha- copaene (ot24, ot25, ot26, and ot28); (2) eugenol:spathulenol (ot17, ot63, and ot52); (3) thymol:p-cymene (ot65); (4) eugenol:gamma- muurolene (ot27 and ot29); (5) eugenol:thymol:spathulenol (ot85); and (6) geraniol (ot84). Cluster analysis of RAPD markers showed that there were three groups genetically distinct and highly correlated (r = 0.814) to volatile oil constituents.

Vilaplana, J., C. Romaguera, et al. (1991). “Contact dermatitis from geraniol in Bulgarian rose oil.” Contact Dermatitis 24(4): 301.

Viollon, C. and J. P. Chaumont (1994). “Antifungal properties of essential oils and their main components upon Cryptococcus neoformans.” Mycopathologia 128(3): 151-3. Cryptococcus neoformans opportunistic fungus met in the last phasis of AIDS is inhibited in vitro by several essential oils on natural volatile compounds. The minimal inhibitory concentration may reach 100 microliters/l and minimal fungicidal concentration 200 microliters/l with Palmarosa or Cinnamon oils. Among phenolic compounds, thymol and carvacrol are most fungitoxic. Terpenoids, citral, geraniol, and citronellol show best activities.

Wainwright, G., S. G. Webster, et al. (1998). “Neuropeptide regulation of biosynthesis of the juvenoid, methyl farnesoate, in the edible crab, Cancer pagurus.” Biochem J 334(Pt 3)): 651-7. The neuropeptide mandibular organ (MO)-inhibiting hormone (MO-IH), synthesized and secreted from the X-organ-sinus-gland complex of the eyestalk, regulates the biosynthesis of the putative crustacean juvenile hormone, methyl farnesoate (MF). Using radiolabelled acetate as a precursor for isoprenoid biosynthesis, farnesoic acid (FA), farnesol, farnesal, MF and geranyl geraniol were detected in MOs cultured for 24 h. Treatment of MOs with extract of sinus gland inhibited the final step of biosynthesis of MF, catalysed by FA O-methyltransferase. Additionally, treatment of MOs with purified MO-IH exhibited a dose-dependent inhibition of this final step of MF synthesis. The

extent of this inhibition was dependent on the ovary stage of the MO-donor animal, being maximal in MOs from animals in the early stages of ovarian development. Assay of FA O-methyltransferase activity, using [3H]FA in the presence of S-adenosyl-l-methionine, demonstrated that the enzyme was located in the cytosolic fraction of MOs and was inhibited by incubation of MOs with MO-IH prior to preparation of subcellular fractions. For cytosolic preparations taken from vitellogenic animals, both Vmax and Km were appreciably lower than for those taken from non-vitellogenic animals. Conversely, eyestalk ablation of early-vitellogenic animals, which removes the source of MO-IH in vivo, resulted in enhancement of the cytosolic FA O-methyltransferase activity. Although both Vmax and Km show an appreciable increase upon eyestalk ablation, the increased enzyme activity is probably reflected by the fact that Vmax/Km (an approximate indication of kcat) has increased 5-fold. The combined evidence demonstrates that MO-IH inhibits FA O-methyltransferase, the enzyme which catalyses the final step of MF biosynthesis in MOs.

Wang, G., X. Zhu, et al. (1992). "[Analysis of chemical constituent of essential oil in Lonicera japonnica Thunb. cultivated on the northern plain of Henan Province]." Zhongguo Zhong Yao Za Zhi 17(5): 268-70, 319. The chemical constituents of the essential oil in the dry flower and fresh flower of Lonicera japonica were analyzed by the GC-MS-DS technique and the superimposition of authentic samples. 27 and 30 monoterpenoids and sesquiterpenoids were identified from the essential oil of the dry flower and fresh flower respectively. The major constituents have been found to be linalool, geraniol, aromadendrene and eugenol etc.

Wheeler, C. J., C. A. Mihaliak, et al. (1990). "Uncompetitive inhibition of monoterpene cyclases by an analog of the substrate geranyl pyrophosphate and inhibition of monoterpene biosynthesis in vivo by an analog of geraniol." Arch Biochem Biophys 279(2): 203-10. Monoterpene cyclases catalyze the divalent metal ion-dependent conversion of the acyclic precursor geranyl pyrophosphate to a variety of monocyclic and bicyclic monoterpene skeletons. Examination of the kinetics of inhibition of cyclization by the pyrophosphate ester of (E)-4-[2-diazo-3-trifluoropropionyloxy]-3-methyl-2-buten-1-o l, a photolabile structural analog of the substrate, using a partially purified preparation of geranyl pyrophosphate:(+)-pinene cyclase and geranyl pyrophosphate:(+)-bornyl pyrophosphate cyclase from common sage (Salvia officinalis) evidenced (under dark conditions) strictly uncompetitive inhibition with K'i values of 3.2 and 4.7 microM, respectively. These values are close to the corresponding Km values for the substrate with these two enzymes. This novel property of the substrate analog was also examined in the presence of two other inhibitors which bind to different domains of the cyclase active site (inorganic pyrophosphate and a sulfonium ion analog of a cyclic carbocationic intermediate of the reaction sequence (dimethyl-(4-methylcyclohex-3-en-1-yl)sulfonium iodide)) in order to address the mechanistic origins of the uncompetitive inhibition of cyclization. It was not possible, however, to rule out either an induced-fit mechanism or a sequential binding mechanism since the substrate is recognized by at least two binding domains and because direct examination of the effects of binding on cyclase conformation is currently not feasible. The substrate analog, although photoactive, did not give rise to light-dependent enzyme inactivation of greater magnitude than that

obtained from ultraviolet light alone. The unusual behavior of the analog was attributed to intramolecular interaction of the electron-rich carbonyl group of the diazoester with the required divalent metal ion that is chelated by the pyrophosphate group. A photostable analog of geraniol that resembled the photoactive substrate analog in bearing a carbonyl function at C6 (6-oxo-3,7-dimethyloct-2(trans)en-1-ol) was prepared. Following foliar application to rapidly growing sage plants, this analog was seemingly activated to the corresponding pyrophosphate ester in vivo and selectively inhibited the activity of several cyclases in this tissue as evidenced by diminished production of the corresponding monoterpene end products.

Williams, S. N., M. L. Anthony, et al. (1998). "Induction of apoptosis in two mammalian cell lines results in increased levels of fructose-1,6-bisphosphate and CDP-choline as determined by 31P MRS." Magn Reson Med 40(3): 411-20. Programmed cell death or apoptosis was induced in human promyelocytic leukemia (HL-60) and Chinese hamster ovary (CHO-K1) cells using several cytotoxic drugs that have different modes of action, including camptothecin, ceramide, chelerythrine, etoposide, farnesol, geranyl geraniol, and hexadecylphosphocholine. The consequent changes in cellular metabolism were monitored using 31P MRS measurements on intact cells and cell extracts. Cells undergoing programmed cell death exhibited characteristic changes in the levels of glycolytic and phospholipid metabolites. The most significant changes were increases in the concentration of the glycolytic intermediate, fructose-1,6-bisphosphate and in the concentration of CDP-choline, which is an intermediate in phosphatidylcholine biosynthesis. In HL-60 cells, the increase in fructose-1,6-bisphosphate levels could be explained by depletion of cellular NAD(H) levels. All of the agents used to induce apoptosis caused the accumulation of CDP-choline. Since the resonances of this compound occur in a relatively well resolved region of tissue spectra, it could provide a marker for apoptosis that would allow the noninvasive detection of the process in vivo using 31P MRS measurements.

Yamamoto, A., A. Morita, et al. (2002). "Contact urticaria from geraniol." Contact Dermatitis 46(1): 52.

Yamamoto, H., N. Katano, et al. (1999). "Transformation of loganin and 7-deoxyloganin into secologanin by Lonicera japonica cell suspension cultures." Phytochemistry 50(3): 417-422. Cell suspension cultures of L. japonica used in this study did not produce any iridoid and secoiridoid glucosides. Cells had the ability to convert loganin into secologanin. Cells also converted 7- deoxyloganin into both loganin and secologanin, but not geraniol into either iridoid glucoside or secologanin. The lack of enzymes converting geraniol into iridoids might result in the non-production of secologanin.

Yan, D. W., Z. J. Zhang, et al. (1994). "Analysis of the chemical compositions of essential oils from scented leaves of Pelargonium hybrids acclimated in Yunnan Province." Fruits (Paris) 49(1): 22. The leaves of Pelargonium hybrids, collected from plants grown at Kunming, in May, June, Aug., Sept. and Oct. [year unspecified], were analysed for their essential oil compositions, using GC. Whereas geraniol contents continously increased between May and Oct., citronellol contents slightly decreased. The

geraniol concentration of plants grown in Kunming was 4-13% higher than that of plants grown in Bin Chuang (Yunnan Province, China), Reunion or Morocco.

Yanai, T. and M. Sato (2000). "Purification and characterization of an alpha-L-rhamnosidase from Pichia angusta X349." Biosci Biotechnol Biochem 64(10): 2179-85. An intracellular alpha-L-rhamnosidase from Pichia angusta X349 was purified to homogeneity through four chromatographic steps. The alpha-L-rhamnosidase appeared to be a monomeric protein with a molecular mass of 90 kDa. The enzyme had an isoelectric point at 4.9, and was optimally active at pH 6.0 and at around 40 degrees C. The Ki for L-rhamnose inhibition was 25 mM. The enzyme was inhibited by Cu2+, Hg2+, and p-chloromercuribenzoate. The alpha-L-rhamnosidase was highly specific for alpha-L-rhamnopyranoside and liberated rhamnose from naringin, rutin, hesperidin, and 3-quercitrin. The alpha-L-rhamnosidase was active at the ethanol concentrations of wine. It efficiently released monoterpenols, such as linalool and geraniol, from an aroma precursor extracted from Muscat grape juice.

Yu, S. G., L. A. Hildebrandt, et al. (1995). "Geraniol, an inhibitor of mevalonate biosynthesis, suppresses the growth of hepatomas and melanomas transplanted to rats and mice." Journal of Nutrition 125(11): 2763-2767. The impact of dietary geraniol (an acyclic monoterpenoid alcohol) on the growth of hepatomas and melanomas was examined in rats and mice. First, male buffalo rats were fed on geraniol (23 mmol/kg diet, 350 micro mol/day) for 14 days before and for 42 days after the transplant of Morris 7777 hepatomas. Neoplasm growth was suppressed ($P<0.001$). Secondly, the dose-dependent impact of geraniol on the growth of B16 melanomas was investigated. Female C57BL mice were fed on geraniol (0.65, 6.5 and 65 mmol/kg diet) for 14 days before and for 21 days after transplant of B16 melanomas. Neoplasm growth was suppressed ($P<0.02$) by geraniol 6.5 and 65 mmol/kg diet.

Zhao, J., Q. Hu, et al. (2001). "Effects of stress factors, bioregulators, and synthetic precursors on indole alkaloid production in compact callus clusters cultures of Catharanthus roseus." Appl Microbiol Biotechnol 55(6): 693-8. Compact callus cluster (CCC) cultures established from Catharanthus roseus consist of cohesive callus aggregates displaying certain levels of cellular or tissue differentiation. CCC cultures synthesize about two-fold more indole alkaloids than normal dispersed-cell cultures. Our studies here show that additions of KCl, mannitol, and a variety of synthetic precursors and bioregulators to the CCC cultures markedly improved indole alkaloid production and release of these alkaloids into the medium. Treatment with 250 mM mannitol and 4 g/l KCl yielded 42.3 mg l(-1) and 33.6 mg l(-1)of ajmalicine, respectively; these amounts were about four-fold higher than the control. Succinic acid, tryptamine, and tryptophan feedings also significantly increased ajmalicine (41.5 mg l(-1), 36.9 mg l(-1), and 31.8 mg l(-1), respectively) and catharanthine (21.1 mg l(-1), 17.2 mg l(-1), and 18 mg l(-1), respectively) production by the CCC cultures, while geraniol feeding inhibited biomass and alkaloid accumulation. We also found that tetramethyl ammonium bromide could significantly improve ajmalicine production (49.3 mg l(-1)) and catharanthine production (18.3 mg l(-1)) in C. roseus CCC cultures. The mechanisms responsible for these treatment effects are discussed herein.

Zivanovic, L. J., M. Jovanovic, et al. (1990). “Densitometric determination of monoterpenoids in Melissa extracts.” Fitoterapia 61(1): 82-83. Melissa fluid extract is used as a diaphoretic and a stimulant in pharmaceutical preparations, as a skin antiirritant in emulsions and lotions, and because of its antiviral effect in some pharmaceutical ointments. A densitometric method for the determination of geraniol, linalool, citral and citronellal in Melissa officinalis leaf extracts is presented.

Thujene CAS# 546-80-5 (Isothujone, 3-thujanone, thujon, thujone, Bicyclo hexan-3-one 4-methyl methylethyl)

Banthorpe, D. V., B. M. Modawi, et al. (1978). “Redox interconversions of geraniol and nerol in higher plants.” Phytochemistry 17(7): 1115-1118. The use of 14C, 3H-labelled precursors showed that for plant feedings carried out in winter, isothujone (trans-thujan-3-one) was formed in Tanacetum vulgare from nerol (3,7-dimethyl-octa-cis-2,6-dien-1-ol) without loss of hydrogen from C-1 of the precursor. In contrast, formation from geraniol (the corresponding trans-isomer) involved stereospecific loss of the pro-(1S) hydrogen. This suggests that geraniol and nerol were interconverted by a redox system. However, anomalous results were obtained from similar studies at other seasons with T. vulgare, and on the biosynthesis of alpha - and beta -pinenes (pin-3-ene and pin-2-(10)-ene) in Pinus pinaster, 1,8-cineole (1,8- oxidomethane) in Mentha piperita and Eucalyptus globulus, and of carvone (menth-6,8(9)-dien-2-one) in M. spicata.

Banthorpe, D. V., O. Ekundayo, et al. (1978). “Evidence for geraniol as an obligatory precursor of isothujone.” Phytochemistry 17(7): 1111-1114. The results of in vivo (leaves) and in vitro studies on Tanacetum vulgare are reported. They indicated that the precursor mevalonic acid-[14C, 3H] was converted in the sequence geraniol 'right arrow' nerol 'right arrow' isothujone.

Karp, F. and R. Croteau (1982). “Evidence that sabinene is an essential precursor of C(3)-oxygenated thujane monoterpenes.” Archives of Biochemistry and Biophysics 216(2): 616-624. The volatile oil of immature Artemisia absinthium leaves contained sabinyl acetate (42%), 3-thujone (32%), sabinene (12%) and alpha - thujene (3%), and label from [1-3H]geraniol was incorporated, under aerobic conditions, into these monoterpenes in proportion to their natural abundance. Light had little effect on synthesis from exogenous geraniol, but, at reduced O2 levels, label accumulated in sabinene, whereas much less sabinyl acetate and 3-thujone were formed, suggesting a route to the ester and ketone by the allylic, nonphotochemical oxygenation of sabinene. Supporting evidence for the intermediary role of sabinene was provided by isotopic dilution studies. [10-3H]Sabinene was incorporated directly in A. absinthium leaves into both [10-3H]sabinyl acetate and 3-[10-3H]thujone, and in Tanacetum vulgare and Salvia officinalis it was specifically incorporated into 3-thujone and 3-isothujone, respectively, confirming the role of this bicyclic olefin as the essential precursor of C(3)-oxygenated thujane monoterpenes.

Mara Donelles Viera, C., F. De Paris, et al. (1998). “The utilization of Tanacetum parthenium in the migraine and arthritis.” Revista Brasileira de Farmacia 79(1-2): 42-44. In this work we discuss the antimigraine and antiinflammatory activities attributed to Tanacetum parthenium. The drug (leaves and inflorescences) contains a series of compounds, specially sesquiterpene lactones, being parthenolide regarded as the main responsible for the therapeutic properties of the plant. Parthenolide inhibits serotonin release and this effect could explain the antimigraine action. Likewise, this lactone acts inhibiting prostaglandins and leucotrienes, mediators in inflammatory processes such as arthritis. Along lactones, the plant contains flavonoids such as tanacetin which presents antiinflammatory activity. This product could present a synergy with parthenolide.

Nin, S., P. Arfaioli, et al. (1995). “Quantitative determination of some essential oil components of selected Artemisia absinthium plants.” Journal of Essential Oil Research 7(3): 271-277. In traditional medicine, A. absinthium is used as an anthelmintic, insecticide, stomachic, and tonic. The essential oils, steam- distilled from leaves and flowers of plants propagated from 49 mother plants obtained from Italy (21 plants), Austria (10), Germany (5), France (4) or USA (9), were analysed by GC. More than 90 compounds were detected, most of which occurred only in trace amounts. Quantitative and qualitative differences were observed in the contents of 8 antibacterial components (alpha- and beta-thujone, terpinen-4-ol, linalool, nerol, geraniol, alpha-pinene, and 1,8-cineole [eucalyptol]). These variations were observed between individual accessions, and between plants obtained from the same geographical location. The essential oils of some genotypes were characterized by particularly high percentages of active principles.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major

constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” J Pharm Pharmacol 53(10): 1347-56. Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in-vitro and in-vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, anti-inflammatory and estrogenic effects. Results of in-vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents.

Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg mL(-1)) and the monoterpenoids (0.1 M) alpha- and beta-pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible anti-inflammatory activity (eicosanoid inhibition in rat leucocytes) was found in the EtOH extract (50 microg mL(-1)) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible estrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg mL(-1)) and the monoterpenoid geraniol (0.1-2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit estrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Seidakhmetova, R. B., A. A. Beisenbaeva, et al. (2002). “Chemical composition and biological activity of the essential oil from.” Pharmaceutical Chemistry Journal 36(3): 135-138.

Strang, J., W. N. Arnold, et al. (1999). “Absinthe: What's your poison?” British Medical Journal 319(7225): 1590-1592.

Tomassoni, A. J. and K. Simone (2001). “Herbal medicines for children: An illusion of safety?” Current Opinion in Pediatrics 13(2): 162-169. Herbal medicaments are in common use. In general, the judicious use of carefully selected and prepared herbal medications seems to cause few adverse effects and may be beneficial. However, toxic effects of these products have been reported with increasing frequency. Infants and children may be even more susceptible to some of the adverse effects and toxicity of these products because of differences in physiology, immature metabolic enzyme systems, and dose per body weight. Although information promoting the use of herbal medicine is widespread, true evidence-based information about the efficacy and safety of herbal medications is limited. Although the most conservative approach is to recommend against use of herbal medicine until such evidence is available, some patients are not receptive to this approach. A reasonable approach for health care providers may be to follow such use closely, assist in herbal therapeutic decisions, and monitor for adverse effects and interactions. This manuscript discusses general concepts about herbal medicines, public health implications, and a framework for mechanisms of adverse effects from the use of botanicals. Adverse effects and toxicity of selected herbal products, including Chinese herbal medicines, are presented. The authors propose a risk reduction approach in which physicians actively seek information about the use of complementary or alternative medicine while taking medical histories. (c) 200 Lippincott Williams & Wilkins, Inc.

Woolf, A. (1999). “Essential oil poisoning.” Journal of Toxicology - Clinical Toxicology 37(6): 721-727.

Yarnell, E. and K. Ballen (1999). “Misunderstood 'toxic' herbs.” Alternative and Complementary Therapies 5(1): 6-11.

Curcumene CAS# 458-37-7 (Curcumin, Curouma, Diferuloylmethane, 4-Hydroxy methoxyphenyl 1,6-Heptadiene 3,5-Dione)

(1993). “Curcumin.” Drugs of the Future 18(4): 367.

(1995). “Curcumin.” Drugs of the Future 20(4): 411.

(1996). “Curcumin.” Drugs of the Future 21(4): 413.

(2001). “Monograph: Curcuma longa (Turmeric).” Alternative Medicine Review 6(SUPPL.): S62-S66.

Abe, Y., S. Hashimoto, et al. (1999). “Curcumin inhibition of inflammatory cytokine production by human.” Pharmacological Research 39(1): 41-47. Curcumin, a dietary pigment responsible for the yellow colour of curry, has been used for the treatment of inflammatory diseases and exhibits a variety of pharmacological effects such as anti-inflammatory activity. The mechanism in anti-inflammatory activity of curcumin has been investigated; however, little is known about the effect of curcumin on cytokine production by human peripheral blood monocytes and alveolar macrophages. In the present study, we shed light on the effect of curcumin on inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. To this end, we determined the concentrations of interleukin-8 (IL-8), monocyte inflammatory protein-1 (MIP-1alpha), monocyte chemotactic protein-1 (MCP-1), interleukin-1beta (IL-1beta), and tumour necrosis factor-alpha (TNF-alpha) in the culture supernatants from phorbor ester, 4beta phorbor 12beta-myristate-13alpha acetate (PMA)- or lipo-polysaccharide (LPS)-stimulated monocytes and alveolar macrophages in the presence or absence of curcumin. Curcumin inhibited the production of IL-8, MIP-1alpha, MCP-1, IL-1beta, and TNF-alpha by PMA- or LPS-stimulated monocytes and alveolar macrophages in a concentration- and a time-dependent manner. These results show that curcumin exhibits an inhibitory effect on the production of IL-8, MIP-1alpha, MCP-1, IL-1beta, and TNF-alpha by PMA- or LPS-stimulated monocytes and alveolar macrophages.

Afaq, F. and H. Mukhtar (2002). “Photochemoprevention by botanical antioxidants.” Skin Pharmacology and Applied Skin Physiology 15(5): 297-306. The trend towards an increase in incidence and higher prevalence of skin cancer makes identification of effective chemopreventive agents an urgent priority. Excessive exposure to solar ultraviolet (UV) B radiation has been implicated as its main cause. Since these trends are likely to continue in the foreseeable future, the adverse effect of UVB has become a major human health concern. Therefore, the development of novel strategies to reduce the occurrence of skin cancer has become a highly desirable goal. Because UV radiation is known to cause excessive generations of reactive oxygen species (ROS) which in turn results in a situation known as oxidative stress, the approaches aimed at counteracting ROS production may be useful for the prevention of skin cancer. One approach to reduce its occurrence is through 'photochemoprotection', which we define as 'the use of agents capable of ameliorating the adverse effects of UVB on the skin'. Among many

photochemoprotective agents, botanical antioxidants are showing promise. This review focuses on photochemopreventive effects of selected botanical antioxidants. We suggest that the use of botanical antioxidants in combination with the use of sunscreens and educational efforts to avoid excessive sun exposure may be an effective strategy for reducing incidence of skin cancer and other UV-mediated damages in humans. Copyright (c) 2002 S. Karger AG, Basel.

Afzal, M., D. Al-Hadidi, et al. (2001). "Ginger: An ethnomedical, chemical and pharmacological review." Drug Metabolism and Drug Interactions 18(3-4): 159-190. Powerful medicinal properties have been recorded for Zingiber officinale, commonly known as ginger. All of these medicinal activities have been compiled with 99 references to the present status of the plant in the literature. Volatile components and the presence of trace metals are included. In addition, details of individual medicinal activities are given and the molecular structures of identified organic metabolites and their synthesis are described.

Aisen, P. S. (2002). "Development of antiinflammatory therapy for Alzheimer's disease." Drug Development Research 56(3): 421-427. Inflammatory mechanisms are active in the Alzheimer's disease (AD) brain. Studies that range from epidemiological surveys to therapeutic trials in transgenic mice provide growing support for the theory that antiinflammatory drugs may be useful in the prevention and/or treatment of the disease. Randomized controlled trials in humans have not yet confirmed this theory. However, prevention and treatment trials continue to test specific antiinflammatory strategies for AD. (c) 2002 Wiley-Liss, Inc.

Ali, M., A. Bagati, et al. (1995). "Comparison of anti-inflammatory activity of curcumin analogues." Indian Drugs 32(10): 502-505. Various semi-synthetic analogues of curcumin, isolated from Curcuma longa (turmeric), were screened for anti-inflammatory activity using carrageenin-induced rat paw oedema and compared with phenylbutazone. Diacetylcurcumin, diacetoxy-curcumin and tetrabromocurcumin were the most potent analogues among the seven analogues studied. The presence of keto groups at C-3 and C-5 positions is important for the anti-inflammatory activity. Substitution of alkyl groups at o-positions of the aromatic rings decreased the anti-inflammatory activity.

Ammon, H. P. T. and M. A. Wahl (1991). "Pharmacology of Curcuma longa." Planta Medica 57(1): 1-7. The data reviewed indicate that extracts of Curcuma longa exhibit anti-inflammatory activity after parenteral application in standard animal models used for testing anti-inflammatory activity. It turned out that curcumin and the volatile oil are at least in part responsible for this action. It appears that when given orally, curcumin is far less active than after i.p. administration. This may be due to poor absorption, as discussed. Data on histamine-induced ulcers are controversial, and studies on the secretory activity (HCl, pepsinogen) are still lacking. In vitro, curcumin exhibited antispasmodic activity. Since there was a protective effect of extracts of Curcuma longa on the liver and a stimulation of bile secretion in animals, Curcuma longa has been advocated for use in liver disorders. Evidence for an effect on liver disease in humans is not yet available. From the facts that after oral application only tracers of curcumin were found in the blood and that, on the other hand, most of the curcumin is excreted via the

faeces it may be concluded that curcumin is absorbed poorly by the gastrointestinal tract and/or underlies pre-systemic transformation. Systemic effects therefore seem to be questionable after oral application except that they occur at very low concentrations of curcumin. This does not exclude a local action in the gastrointestinal tract.

Ammon, H. P. T., M. I. Anazodo, et al. (1992). “Curcumin: A potent inhibitor of leukotriene Binf 4 formation in rat.” Planta Medica 58(2): 226.

Ammon, H. P. T., N. Safayhi, et al. (1993). “Mechanism of antiinflammatory actions of curcumine and boswellic acids.” Journal of Ethnopharmacology 38(2-3): 113-119. Curcumine from Curcuma longa and the gum resin of Boswellia serrata, which were demonstrated to act as antiinflammatories in in vivo animal models, were studied in a set of in vitro experiments in order to elucidate the mechanism of their beneficial effects. Curcumine inhibited the 5-lipoxygenase activity in rat peritoneal neutrophils as well as the 12-lipoxygenase and the cyclooxygenase activities in human platelets. In a cell free peroxidation system curcumine exerted strong antioxidative activity. Thus, its effects on the dioxygenases are probably due to its reducing capacity. Boswellic acids were isolated from the gum resin of Boswellia serrata and identified as the active principles. Boswellic acids inhibited the leukotriene synthesis via 5-lipoxygenase, but did not affect the 12-lipoxygenase and the cyclooxygenase activities. Additionally, boswellic acids did not impair the peroxidation of arachidonic acid by iron and ascorbate. The data suggest that boswellic acids are specific, non-redox inhibitors of leukotriene synthesis either interacting directly with 5-lipoxygenase or blocking its translocation.

Anto, R. J., G. Kuttan, et al. (1998). “Anti-inflammatory activity of natural and synthetic curcuminoids.” Pharmacy and Pharmacology Communications 4(2): 103-106. The anti-inflammatory activity of three natural curcuminoids and eight synthetic curcuminoids was studied using the mouse paw oedema method. Among the natural curcuminoids, curcumin III was the most effective anti-inflammatory agent. Curcumin III produced an inhibition of 86.8% of the mice paw oedema at a concentration of 50 mg kgsup -sup 1 body weight whereas curcumin I and curcumin II produced an inhibition of 72.4% and 65.1%, respectively at the same concentration. Among the synthetic curcuminoids, salicyl (63.0% inhibition), veratryl (62.6%) and piperonal (61.1%) curcuminoids were found to be potent anti-inflammatory agents, followed by p-anisyl curcuminoid (58.6%). There was a correlation between the anti-inflammatory activities of the curcuminoids and their observed antioxidant activities, reported earlier.

Anto, R. J., G. Kuttan, et al. (1998). “Anti-inflammatory activity of natural and synthetic curcuminoids.” Pharmacy and Pharmacology Communications 4(2): 103-106. The antiinflammatory activity of 3 natural curcuminoids and 8 synthetic curcuminoids was studied using the mouse paw oedema method. Among the natural curcuminoids (isolated from an alcoholic extract of Curcuma longa), curcumin III was the most effective antiinflammatory agent: at a concentration of 50 mg/kg body weight it gave 86.8% inhibition of mouse paw oedema, compared with 72.4% for curcumin I and 65.1% for curcumin II. Among the synthetic curcuminoids, salicyl (63.0% inhibition), veratryl (62.6%) and piperonal (61.1%) curcuminoids were found to be potent antiinflammatory

agents, followed by p-anisyl curcuminoid (58.6%). There was a correlation between the antiinflammatory activities of the curcuminoids and their previously reported antioxidant activities. Antioxidant activity was associated with phenolic structures (in either raw or metabolized compounds), and antiinflammatory activity with olefinic double bonds and hydroxy groups.

Anto, R. J., G. Kuttan, et al. (1998). “Anti-inflammatory activity of natural and synthetic curcuminoids.” Pharmacy and Pharmacology Communications 4(2): 103-106. The antiinflammatory activity of 3 natural curcuminoids and 8 synthetic curcuminoids was studied using the mouse paw oedema method. Among the natural curcuminoids (isolated from an alcoholic extract of Curcuma longa), curcumin III was the most effective antiinflammatory agent: at a concentration of 50 mg/kg body weight it gave 86.8% inhibition of mouse paw oedema, compared with 72.4% for curcumin I and 65.1% for curcumin II. Among the synthetic curcuminoids, salicyl (63.0% inhibition), veratryl (62.6%) and piperonal (61.1%) curcuminoids were found to be potent antiinflammatory agents, followed by p-anisyl curcuminoid (58.6%). There was a correlation between the antiinflammatory activities of the curcuminoids and their previously reported antioxidant activities. Antioxidant activity was associated with phenolic structures (in either raw or metabolized compounds), and antiinflammatory activity with olefinic double bonds and hydroxy groups.

Ara, G. and B. A. Teicher (1996). “Cyclooxygenase and lipoxygenase inhibitors in cancer therapy.” Prostaglandins Leukotrienes and Essential Fatty Acids 54(1): 3-16.

Araujo, C. A. C. and L. L. Leon (2001). “Biological activities of Curcuma longa L.” Memorias do Instituto Oswaldo Cruz 96(5): 723-728. A review is presented discussing the chemical structure and pharmacologic activities of curcumin, an extract from C. longa, to invite researchers to investigate new drugs for the treatment of numerous diseases. Curcumin has anti-inflammatory, anti-oxidant, anti-protozoal, nematicidal, anti-bacterial, anti-venom, anti-human immunodeficiency virus, and anti-neoplastic activities.

Araujo, C. A. C. and L. L. Leon (2001). “Biological activities of Curcuma longa L.” Memorias do Instituto Oswaldo Cruz 96(5): 723-728. A review is presented discussing the chemical structure and pharmacologic activities of curcumin, an extract from C. longa, to invite researchers to investigate new drugs for the treatment of numerous diseases. Curcumin has anti-inflammatory, anti-oxidant, anti-protozoal, nematicidal, anti-bacterial, anti-venom, anti-human immunodeficiency virus, and anti-neoplastic activities.

Awasthi, S., U. Pandya, et al. (2000). “Curcumin-glutathione interactions and the role of human glutathione S.” Chemico-Biological Interactions 128(1): 19-38. Curcumin (diferuloylmethane), a yellow pigment of turmeric with antioxidant properties has been shown to be a cancer preventative in animal studies. It contains two electrophilic alpha, beta-unsaturated carbonyl groups, which can react with nucleophilic compounds such as glutathione (GSH), but formation of the GSH-curcumin conjugates has not previously been demonstrated. In the present studies, we investigated the reactions of curcumin with GSH and the effect of recombinant human glutathione S- transferase(GST)P1-1 on

reaction kinetics. Glutathionylated products of curcumin identified by FAB-MS and MALDI-MS included mono- and di- glutathionyl-adducts of curcumin as well as cyclic rearrangement products of GSH adducts of feruloylmethylketone (FMK) and feruloylaldehyde (FAL). The presence of GSTP1-1 significantly accelerated the initial rate of GSH- mediated consumption of curcumin in 10 mM potassium phosphate, pH 7.0, and 1 mM GSH. GSTP1-1 kinetics determined using HPLC indicated substrate inhibition (apparent K(m) for curcumin of 25 +/- 11 muM, and apparent K(i) for curcumin of 8 +/- 3 muM). GSTP1-1 was also shown to catalyze the reverse reaction leading to the formation of curcumin from GSH adducts of FMK and FAL. (C) 2000 Elsevier Science Ireland Ltd.

Barnes, J. (2002). "(4) hyperlipidaemia." Pharmaceutical Journal 269(7210): 193-195.

Barr, R. K. and M. A. Bogoyevitch (2001). "The c-Jun N-terminal protein kinase family of mitogen-activated protein." International Journal of Biochemistry and Cell Biology 33(11): 1047-1063. The c-Jun N-terminal protein kinase mitogen-activated protein kinases (JNK MAPKs) are an evolutionarily-conserved family of serine/threonine protein kinases. First identified in 1990 when intraperitoneal injection of the protein synthesis inhibitor cycloheximide activated a 54 kDa protein kinase, the JNK MAPKs have now taken on a prominent role in signal transduction. This research has revealed a number of levels of complexity. Alternative gene splicing is now recognised to result in ten different JNK MAPK isoforms of 46-55 kDa, and these isoforms differ in their substrate affinities. Furthermore, although originally classified as stress-activated protein kinases (SAPKs), or SAPKs, the JNK MAPKs are also critical mediators of signal transduction in response to stimulation by cytokines and some growth factors. JNK MAPKs have been shown to be critical mediators in dorsal closure in developing Drosophila embryos, and targeted knockout of murine JNK MAPKs has suggested a critical involvement of these kinases in mammalian embryonic development. Recent work has also highlighted their importance in programmed cell death. Thus, the JNK MAPKs may provide a critical target for regulation in both normal and diseased states. (c) 2001 Elsevier Science Ltd. All rights reserved.

Barthelemy, S., L. Vergnes, et al. (1998). "Curcumin and curcumin derivatives inhibit Tat-mediated transactivation." Research in Virology 149(1): 43-52. The transcription of HIV1 provirus is regulated by both cellular and viral factors. Various evidence suggests that Tat protein secreted by HIV1- infected cells may have additional action in the pathogenesis of AIDS because of its ability to also be taken up by non-infected cells. Curcumin (diferuloylmethane or 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the yellow pigment in turmeric Curcuma longa (Linn). It exhibits a variety of pharmacological effects including antiinflammatory and antiretroviral activities. Here, we demonstrated that curcumin used at 10 to 100 nM inhibited Tat transactivation of HIV1-LTR lacZ by 70 to 80% in HeLa calls. In order to develop more efficient curcumin derivatives, we synthesized and tested in the same experimental system the inhibitory activity of reduced curcumin (Cinf 1), which lacks the spatial structure of curcumin; allyl-curcumin (Cinf 2), which possesses a condensed allyl derivative on curcumin that plays the role of metal chelator; and tocopheryl-curcumin (Cinf 3), which enhances the

antioxidant activity of the molecule. Results obtained with Cinf 1, Cinf 2 and Cinf 3 curcumin derivatives showed a significant inhibition (70 to 85%) of Tat transactivation. Despite the fact that tocopheryl-curcumin (Cinf 3) failed to scavenge Oinf 2/-sup -, this curcumin derivative exhibited the most activity; 70% inhibition was obtained at 1 nM, while only 35% inhibition was obtained with the curcumin.

Bertagnolli, M. M. (1999). “APC and intestinal carcinogenesis. Insights from animal models.” Annals of the New York Academy of Sciences 889(-): 32-44. The APC protein is a crucial regulator of intestinal cell growth, and mutations in the APC gene are a common initial event in the process of human colorectal carcinogenesis. Animals bearing germline mutations in Apc are therefore important models for human colorectal cancer. These animals have been used both to understand the biology of human colorectal cancer and to screen for agents able to prevent malignant transformation of susceptible intestinal cells.

Bina, J., U. J. S. P. Rao, et al. (1997). “Presence of an acidic glycoprotein in the serum of arthritic rats: modulation by capsaicin and curcumin.” Molecular and Cellular Biochemistry 169(1/2): 125-134. Levels of various serum proteins were found to change in adjuvant- induced arthritis. Increased levels of a glycoprotein with an apparent MW of 72 kDa (Gp A72) were observed in the sera of arthritic rats. Gp A72 is an acidic glycoprotein with a pI of 5.1. Gp A72 also showed antitryptic activity. The appearance of Gp A72 in the serum preceded the onset of paw inflammation in arthritic rats and persisted in the chronic phase. Oral administration of the antiinflammatory spice principles, capsaicin (from red pepper; daily dose 5 mg/kg) and curcumin (from turmeric; 30 mg/kg), for 15 days lowered the levels of Gp A72 by 88 and 73%, respectively, with concomitant lowering of paw inflammation in arthritic rats.

Bina, J., U. J. S. P. Rao, et al. (1997). “Presence of an acidic glycoprotein in the serum of arthritic rats: modulation by capsaicin and curcumin.” Molecular and Cellular Biochemistry 169(1/2): 125-134. Levels of various serum proteins were found to change in adjuvant- induced arthritis. Increased levels of a glycoprotein with an apparent MW of 72 kDa (Gp A72) were observed in the sera of arthritic rats. Gp A72 is an acidic glycoprotein with a pI of 5.1. Gp A72 also showed antitryptic activity. The appearance of Gp A72 in the serum preceded the onset of paw inflammation in arthritic rats and persisted in the chronic phase. Oral administration of the antiinflammatory spice principles, capsaicin (from red pepper; daily dose 5 mg/kg) and curcumin (from turmeric; 30 mg/kg), for 15 days lowered the levels of Gp A72 by 88 and 73%, respectively, with concomitant lowering of paw inflammation in arthritic rats.

Boland, C. R., F. A. Sinicrope, et al. (2000). “Colorectal cancer prevention and treatment.” Gastroenterology 118(2 SUPPL.): S115-S128.

Boone, C. W., J. W. Bacus, et al. (1997). “Properties of intraepithelial neoplasia relevant to the development of.” Journal of Cellular Biochemistry 67(SUPPL. 28/29): 1-20. Cancer chemoprevention is concerned with the development of drugs or diet supplements that will avert the onset or stop the progression of the intraepithelial neoplasia which

precedes invasive cancer. Two basic processes underlie the onset and development of intraepithelial neoplasia. First is genomic instability (often associated with chronic diffuse epithelial hyperplasia), which is the increased production of genomic structural variants due to unrepaired DNA breaks with secondary formation of abnormal structures, including 'mutator' mutations in genes responsible for genomic stability, gene copy amplification or loss from DNA breakage-fusion-anaphase bridge cycles, unequal sister chromatid exchange, and accumulation of double minutes. Second is the development within an epithelium having genomic instability of multicentric neoplastic lesions that independently progress through each of the following processes at a continuously accelerating rate: clonal evolution, hyperproliferation, production of genomic structural variants, and apoptosis. Recommended chemoprevention strategies based on these mechanisms are (1) early diagnosis and treatment of genomic instability before the appearance of intraepithelial neoplasia, i.e., during the 'predysplastic' or 'premorphologic' phase, (2) development of multiple agents that block intralesional proliferation at steps along the 'command' pathways of mitotic signal transduction and along the 'execute' pathways of synthesis of daughter cell components, (3) development of nontoxic antiinflammatory agents, antioxidants, antimutagens, and proapoptotics, (4) avoidance of 'clonal escape' through use of drug combinations, and (5) use of computer- assisted quantitative image analysis to assay modulation of surrogate endpoints in chemoprevention clinical trials.

Bremner, P. and M. Heinrich (2002). “Natural products as targeted modulators of the nuclear factor-kappaB.” Journal of Pharmacy and Pharmacology 54(4): 453-472. The use of plant extracts to alleviate inflammatory diseases is centuries old and continues to this day. This review assesses the current understanding of the use of such plants and natural products isolated from them in terms of their action against the ubiquitous transcription factor, nuclear factor kappa B (NF-kappaB). As an activator of many pro-inflammatory cytokines and inflammatory processes the modulation of the NF-kappaB transduction pathway is a principal target to alleviate the symptoms of such diseases as arthritis, inflammatory bowel disease and asthma. Two pathways of NF-kappaB activation will first be summarised, leading to the IKK (IkappaB kinase) complex, that subsequently initiates phosphorylation of the NF-kappaB inhibitory protein (IkappaB). Natural products and some extracts are reviewed and assessed for their activity and potency as NF-kappaB inhibitors. A large number of compounds are currently known as NF-kappaB modulators and include the isoprenoids, most notably kaurene diterpenoids and members of the sesquiterpene lactones class, several phenolics including curcumin and flavonoids such as silybin. Additional data on cellular toxicity are also highlighted as an exclusion principle for pursuing such compounds in clinical development. In addition, where enough data exists some conclusions on structure-activity relationship are provided.

Brouet, I. and H. Ohshima (1995). “Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits.” Biochemical and Biophysical Research Communications 206(2): 533-540. L-Arginine-derived nitric oxide (NO) and its derivatives, such as peroxynitrite and nitrogen dioxide, play a role in inflammation and also possibly in the multistage process of carcinogenesis. We investigated the effect of various non-steroidal anti-inflammatory agents and related compounds on the induction of NO synthase (NOS)

in RAW 264.7 macrophages activated with lipopolysaccharide (LPS) and interferon-gamma (IFN-gamma). Low concentrations of curcumin, a potent anti-tumour agent having anti-inflammatory and anti-oxidant properties, inhibited NO production, as measured by the amount of nitrite released into the culture medium in 24 h (IC$D5inf 0 = 6 muM). NOS activity in soluble extracts of macrophages activated for 6 - 24 h in the presence of curcumin (10 muM) was significantly lower than that of macrophages activated without curcumin. Northern-blot and immunoblotting analyses demonstrated that significantly reduced levels of the mRNA and 130-kDa protein of inducible NOS were expressed in macrophages activated with curcumin, compared to those without curcumin. Inhibition of NOS induction was maximal when curcumin was added together with LPS and IFN-gamma and decreased progressively as the interval between curcumin and LPS/IFN-gamma was increased to 18 h.

Chan, M. M. Y. (1995). “Inhibition of tumor necrosis factor by curcumin, a phytochemical.” Biochemical Pharmacology 49(11): 1551-1556. Curcumin, contained in the rhizome of the plant Curcuma longa Linn, is a naturally occurring phytochemical that has been used widely in India and Indonesia for the treatment of inflammation. The pleiotropic cytokine tumor necrosis factor-alpha (TNF) induces the production of interleukin-1beta (IL-1), and, together, they play significant roles in many acute and chronic inflammatory diseases. They have been implicated in the pathogenesis of intracellular parasitic infections, atherosclerosis, AIDS and autoimmune disorders. This report shows that, in vitro, curcumin, at 5 muM, inhibited lipopolysaccharide (LPS)-induced production of TNF and IL-1 by a human monocytic macrophage cell line, Mono Mac 6. In addition, it demonstrates that curcumin, at the corresponding concentration, inhibited LPS-induced activation of nuclear factor kappa B and reduced the biological activity of TNF in L929 fibroblast lytic assay.

Chan, M. M. Y., C. T. Ho, et al. (1995). “Effects of three dietary phytochemicals from tea, rosemary and turmeric.” Cancer Letters 96(1): 23-29. In chronic inflammation, cytokines induce the production of nitric oxide (NO(.)) that is converted to DNA damaging and carcinogenic peroxynitrite and nitrite. The compounds epigallocatechin gallate (EGCG), carnosol, and curcumin are non-vitamin phytochemicals contained in commonly consumed dietary plants. They are known to be anti-inflammatory and cancer preventive. Therefore, we studied their effect on the generation of peroxynitrite radicals and nitrite. They inhibited lipopolysaccharide (LPS) and interferon-gamma (IFNgamma) induced nitrite production by mouse peritoneal cells by more than 50% at 2.5-10 muM. Cell viability assays verified that the inhibition was not due to general cellular toxicity.

Chan, M. M. Y., H. I. Huang, et al. (1998). “In vivo inhibition of nitric oxide synthase gene expression by.” Biochemical Pharmacology 55(12): 1955-1962. Curcumin is a naturally occurring, dietary polyphenolic phytochemical that is under preclinical trial evaluation for cancer preventive drug development and whose working pharmacological actions include anti-inflammation. With respect to inflammation, in vitro, it inhibits the activation of free radical-activated transcription factors, such as nuclear factor kappaB (NFkappaB) and AP-1, and reduces the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFalpha), interleukin-1beta (IL-1beta), and interleukin-

8. Inducible nitric oxide synthase (iNOS) is an inflammation-induced enzyme that catalyzes the production of nitric oxide (NO), a molecule that may lead to carcinogenesis. Here, we report that in ex vivo cultured BALB/c mouse peritoneal macrophages, 1-20 muM of curcumin reduced the production of iNOS mRNA in a concentration-dependent manner. Furthermore, we demonstrated that, in vivo, two oral treatments of 0.5 mL of a 10-muM solution of curcumin (92 ng/g of body weight) reduced iNOS mRNA expression in the livers of lipopolysaccharide(LPS)-injected mice by 50-70%. Although many hold that curcumin needs to be given at dosages that are unattainable through diet to produce an in vivo effect, we were able to obtain potency at nanomoles per gram of body weight. This efficacy is associated with two modifications in our preparation and feeding regimen: 1) an aqueous solution of curcumin was prepared by initially dissolving the compound in 0.5 N NaOH and then immediately diluting it in PBS; and 2) mice were fed curcumin at dusk after fasting. Inhibition was not observed in mice that were fed ad lib., suggesting that food intake may interfere with the absorption of curcumin. Copyright (C) 1998 Elsevier Science, Inc.

Chan, M., I. Huang Hsing, et al. (1998). “In vivo inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties.” Biochemical Pharmacology 55(12): 1955-1962. Curcumin is a naturally occurring, dietary polyphenolic phytochemical, present in Curcuma longa rhizomes, that is under preclinical trial evaluation for cancer preventive drug development and whose pharmacological actions include antiinflammatory properties. With respect to inflammation, in vitro, it inhibits the activation of free radical-activated transcription factors, such as nuclear factor kappaB (NFkappaB) and AP-1, and reduces the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFalpha), interleukin-1beta (IL-1beta), and IL-8. Inducible nitric oxide synthase (iNOS) is an inflammation-induced enzyme that catalyses the production of nitric oxide (NO), a molecule that may lead to carcinogenesis. In this study, with ex vivo cultured BALB/c mouse peritoneal macrophages, 1-20 micro M curcumin reduced the production of iNOS mRNA in a concentration-dependent manner. In vivo, 2 oral treatments of 0.5 ml of a 10-micro M solution of curcumin (92 ng/g) reduced iNOS mRNA expression in the livers of lipopolysaccharide (LPS)-injected mice by 50-70%. Although many hold the view that curcumin needs to be given at dosages that are unattainable through diet to produce an in vivo effect, potency was obtained at nanomoles per gram of body weight. This efficacy was associated with 2 modifications in the preparation and feeding regimen: (1) an aqueous solution of curcumin was prepared by initially dissolving the compound in 0.5 N NaOH and then immediately diluting it in PBS; and (2) mice were fed curcumin at dusk after fasting. Inhibition was not observed in mice that were fed ad lib, suggesting that food intake may interfere with the absorption of curcumin.

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Chang, D. M. (2001). “Curcumin: A heat shock response inducer and potential cytoprotector.” Critical Care Medicine 29(11): 2231-2232.

Chang, J. H., E. E. Gabison, et al. (2001). “Corneal neovascularization.” Current Opinion in Ophthalmology 12(4): 242-249. Corneal neovascularization (NV) is a sight-threatening condition usually associated with inflammatory or infectious disorders of the ocular surface. It has been shown in the field of cancer angiogenesis research that a balance exists between angiogenic factors (such as fibroblast growth factor and vascular endothelial growth factor) and anti-angiogenic molecules (such as angiostatin, endostatin, or pigment epithelium derived factor) in the cornea. Several inflammatory, infectious, degenerative, and traumatic disorders are associated with corneal NV, in which the balance is tilted towards angiogenesis. The pathogenesis of corneal NV may be influenced by matrix metalloproteinases and other proteolytic enzymes. New medical and surgical treatments, including angiostatic steroids, nonsteroidal inflammatory agents, argon laser photocoagulation, and photodynamic therapy have been effective in animal models to inhibit corneal NV and transiently restore corneal "angiogenic privilege." (c) 2001 Lippincott Williams & Wilkins, Inc.

Chauhan, D. P. (2002). “Chemotherapeutic potential of curcumin for colorectal cancer.” Current Pharmaceutical Design 8(19): 1695-1706. Colorectal cancer is one of the leading causes of cancer deaths in the Western world. More than 56,000 newly diagnosed colorectal cancer patients die each year in the United States. Available therapies are either not effective or have unwanted side effects. Epidemiological data suggest that dietary manipulations play an important role in the prevention of many human cancers. Curcumin the yellow pigment in turmeric has been widely used for centuries in the Asian countries without any toxic effects. Epidemiological data also suggest that curcumin may be responsible for the lower rate of colorectal cancer in these countries. Curcumin is a naturally occurring powerful anti-inflammatory medicine. The anticancer properties of

curcumin have been shown in cultured cells and animal studies. Curcumin inhibits lipooxygenase activity and is a specific inhibitor of cyclooxygenase-2 expression. Curcumin inhibits the initiation of carcinogenesis by inhibiting the cytochrome P-450 enzyme activity and increasing the levels of glutathione-S-transferase. Curcumin inhibits the promotion/progression stages of carcinogenesis. The anti-tumor effect of curcumin has been attributed in part to the arrest of cancer cells in S, G2/M cell cycle phase and induction of apoptosis. Curcumin inhibits the growth of DNA mismatch repair defective colon cancer cells. Therefore, curcumin may have value as a safe chemotherapeutic agent for the treatment of tumors exhibiting DNA mismatch repair deficient and microsatellite instable phenotype. Curcumin should be considered as a safe, non-toxic and easy to use chemotherapeutic agent for colorectal cancers arise in the setting of chromosomal instability as well as microsatellite instability.

Chen, H. Y. and J. Y. Fang (2000). “Therapeutic patents for topical and transdermal drug delivery systems.” Expert Opinion on Therapeutic Patents 10(7): 1035-1043. For more than 30 years techniques of delivering drugs through skin have been elaborated as clinically relevant methods to administer therapeutic agents. The great contributions of transdermal and topical administrations on clinical therapeutics are firmly based on their distinctive properties. Progress in new technologies and compound is continuously stimulating the growth and revolution of topical and transdermal administration. This review summarises recent developments in the design of formulations and the patent literature in the area of topical and transdermal drug delivery systems.

Chuang, S. E., A. L. Cheng, et al. (2000). “Inhibition by curcumin of diethylnitrosamine-induced hepatic.” Food and Chemical Toxicology 38(11): 991-995. Curcumin (CCM), a major yellow pigment of turmeric obtained from powdered rhizomes of the plant Curcuma longa Linn, is commonly used as coloring agent in foods, drugs and cosmetics. In this study we report that gavage administration of 200 mg/kg or 600 mg/kg CCM effectively suppressed diethylnitrosamine (DEN)-induced liver inflammation and hyperplasia in rats, as evidenced by histopathological examination. Immunoblotting analysis showed that CCM strongly inhibited DEN-mediated the increased expression of oncogenic p21(ras) and p53 proteins in liver tissues of rats. In cell-cycle-related proteins, CCM selectively reduced the expression of proliferating cell nuclear antigen (PCNA), cyclin E and p34(cdc2), but not Cdk2 or cyclin D1. Moreover, CCM also inhibited the DEN-induced increase of transcriptional factor NF-kappa B. However, CCM failed to affect DEN-induced c-Jun and c-Fos expression. It has become widely recognized that the development of human hepatocellular carcinoma (HCC) is predominantly due to the chronic inflammation by virus, bacteria or chemical. Our results suggest a potential role for CCM in the prevention of HCC. Copyright (C) 2000 Elsevier Science Ltd.

Cipriani, B., G. Borsellino, et al. (2001). “Curcumin inhibits activation of Vgamma9Vdelta2 T cells by.” Journal of Immunology 167(6): 3454-3462. Curcumin, in addition to its role as a spice, has been used for centuries to treat inflammatory disorders. Although the mechanism of action remains unclear, it has been shown to inhibit the activation of NF-kappaB and AP-1, transcription factors required for induction of many proinflammatory mediators. Due to its low toxicity it is currently under consideration as a

broad anti-inflammatory, anti-tumor cell agent. In this study we investigated whether curcumin inhibited the response of gammadelta T cells to protease-resistant phosphorylated derivatives found in the cell wall of many pathogens. The results showed that curcumin levels >=30 muM profoundly inhibited isopentenyl pyrophosphate-induced release of the chemokines macrophage inflammatory protein-1alpha and -1beta and RANTES. Curcumin also blocked isopentenyl pyrophosphate-induced activation of NF-kappaB and AP-1. Commencing around 16 h, treatment with curcumin lead to the induction of cell death that could not be reversed by APC, IL-15, or IL-2. This cytotoxicity was associated with increased annexin V reactivity, nuclear expression of active caspase-3, cleavage of poly(ADP-ribose) polymerase, translocation of apoptosis-inducing factor to the nucleus, and morphological evidence of nuclear disintegration. However, curcumin led to only large scale DNA chromatolysis, as determined by a combination of TUNEL staining and pulse-field and agarose gel electrophoresis, suggesting a predominantly apoptosis-inducing factor-mediated cell death process. We conclude that gammadelta T cells activated by these ubiquitous Ags are highly sensitive to curcumin, and that this effect may contribute to the anti-inflammatory properties of this compound.

Cipriani, B., G. Borsellino, et al. (2001). “Curcumin inhibits activation of Vgamma9Vdelta2 T cells by phosphoantigens and induces apoptosis involving apoptosis-inducing factor and large scale DNA fragmentation.” Journal of Immunology 167(6): 3454-3462. Curcumin, in addition to its role as a spice, has been used for centuries to treat inflammatory disorders. Although the mechanism of action remains unclear, it has been shown to inhibit the activation of NF-kappaB and AP-1, transcription factors required for induction of many proinflammatory mediators. Due to its low toxicity it is currently under consideration as a broad anti-inflammatory, anti- tumour cell agent. In this study we investigated whether curcumin inhibited the response of gammadelta T cells to protease-resistant phosphorylated derivatives found in the cell wall of many pathogens. The results showed that curcumin levels more than or equal to 30 micro M profoundly inhibited isopentenyl pyrophosphate-induced release of the chemokines macrophage inflammatory protein-1alpha and -1beta and RANTES (regulated on activation normal t lymphocyte expressed and secreted). Curcumin also blocked isopentenyl pyrophosphate-induced activation of NF-kappaB and AP-1. Commencing around 16 h, treatment with curcumin lead to the induction of cell death that could not be reversed by APC, IL-15, or IL-2. This cytotoxicity was associated with increased annexin V reactivity, nuclear expression of active caspase-3, cleavage of poly(ADP-ribose) polymerase, translocation of apoptosis-inducing factor to the nucleus, and morphological evidence of nuclear disintegration. However, curcumin led to only large scale DNA chromatolysis, as determined by a combination of TUNEL staining and pulse-field and agarose gel electrophoresis, suggesting a predominantly apoptosis- inducing factor-mediated cell death process. We conclude that gammadelta T cells activated by these ubiquitous Ags are highly sensitive to curcumin, and that this effect may contribute to the anti-inflammatory properties of this compound.

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Cuendet, M. and J. M. Pezzuto (2001). "The role of cyclooxygenase and lipoxygenase in cancer chemoprevention." Drug Metabolism and Drug Interactions 17(1): 109-157. The involvement of prostaglandins (PGs) and other eicosanoids in the development of human cancer has been known for over two decades. Importantly, an increase in PG synthesis may influence tumor growth in human beings and experimental animals, and numerous studies have illustrated the effect of PG synthesis on carcinogen metabolism, tumor cell proliferation and metastatic potential. PGs produced by cyclooxygenases (COXs) are represented by a large series of compounds that mainly enhance cancer development and progression, acting as carcinogens or tumor promoters, with profound effects on carcinogenesis. Further investigations suggest that arachidonic acid (AA) metabolites derived from lipoxygenase (LOX) pathways play an important role in growth-related signal transduction, implying that intervention through these pathways should be useful for arresting cancer progression. We discuss here the implications of COX and LOX in colon, pancreatic, breast, prostate, lung, skin, urinary bladder and liver cancers. Select inhibitors of COX and LOX are described, including nonsteroidal antiinflammatory drugs (NSAIDs), selective COX-2 inhibitors, curcumin, tea, silymarin and resveratrol, as well as a method useful for evaluating inhibitors of COX. Although a substantial amount of additional work is required to yield a better understanding of the role of COX and LOX in cancer chemoprevention, it is clear that beneficial therapeutic effects can be realized through drug-mediated modulation of these metabolic pathways.

Dick, R. A. and T. W. Kensler (2002). “Chemoprotective potential of phase 2 enzyme inducers.” Expert Review of Anticancer Therapy 2(5): 581-592. In this review we summarize recent data on the use of phase 2 enzyme inducers as cancer chemopreventive agents in preclinical and clinical studies. These agents elevate the expression of genes involved in the detoxication of electrophiles and free radicals that contribute to carcinogenesis. Their mechanisms of action, efficacy and limitations are discussed. Particular attention is paid to isothiocyante and dithiolothione classes of agents, as these are the most developed.

Dikshit, M., L. Rastogi, et al. (1995). “Prevention of ischaemia-induced biochemical changes by curcumin and.” Indian Journal of Medical Research 101(JAN.): 31-35. Effect of myocardial ischaemia on the bioantioxidants levels in the cat heart was evaluated. In addition, effect of curcumin, an anti-inflammatory and anti-thrombotic drug, and quinidine, a standard antiarrhythmic drug, was also studied in the cat. Myocardial ischaemia was induced by the ligation of left descending coronary artery. Quinidine (1 mg/kg, iv) was administered 15 min prior to while curcumin (100 mg/kg, ip) was given 30 min before ligation. Hearts were removed 4 h post coronary artery ligation. Levels of glutathione (GSH), malonaldehyde (MDA), myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) were estimated in the ischaemic and non-ischaemic zones. Both the drugs protected the animals against decrease in the heart rate and blood pressure following ischaemia. In the ischaemic zone, after 4 h of ligation, an increase in the level of MDA and activities of MPO and SOD (cytosolic fraction) were observed. Quinidine and curcumin pretreatment prevented the ischaemia-induced elevation in MDA contents and LDH release. Curcumin pretreatment did not prevent the increase in MPO activity while quinidine did. Results obtained indicate alterations in the bioantioxidants following ischaemia and both curcumin and quinidine prevented ischaemia induced changes in the cat heart.

Dirsch, V. M., H. Stuppner, et al. (1998). “The griess assay: Suitable for a bio-guided fractionation of anti.” Planta Medica 64(5): 423-426. In the field of inflammation research the inducible nitric oxide synthase (iNOS) became an important pharmacological target, since overproduction of nitric oxide (NO) after induction of this enzyme seems to be associated with numerous pathological conditions. NO released from cells can be detected and quantified photometrically as its stable product nitrite by a simple colorimetric reaction (Griess reaction). The aim of our study was to investigate whether this method might be suitable for the bio-guided fractionation of anti-inflammatory plant extracts. For this purpose we assayed extracts as well as fractions of the roots of Curcuma zanthorrhiza Roxb. which contain the known iNOS inhibitor curcumin, and compared the obtained activity with their curcumin content. Furthermore, leaf extracts of Betula pendula Roth, to which defined amounts of curcumin were added, were examined to clarify the question whether chlorophyll might interfere with the test system. The presented results suggest that the Griess assay is indeed suitable to guide fractionation of plant extracts in order to isolate highly active compounds. Factors, however, which might restrict the broad application of this assay are the limited selection of solvents which do not interfere with the system and high contents of chlorophyll in plant extracts.

Durairaj, P., V. Narayanan, et al. (2000). “Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats.” British Journal of Pharmacology 131(2): 169-172. Curcumin, an anti-inflammatory, antioxidant, was evaluated for its ability to suppress bleomycin (BLM)-induced pulmonary fibrosis in rats. A single intratracheal instillation of BLM (0.75 U/100 g, killed 3, 5, 7, 14 and 28 days post-BLM) resulted in significant increases in total cell numbers, total protein, and angiotensin- converting enzyme (ACE), and alkaline phosphatase (AKP) activities in bronchoalveolar lavage fluid. Rats with fibrosis had a significant increase in lung hydroxyproline content. Alveolar macrophages from BLM-administered rats elaborated significant increases in tumour necrosis factor (TNF)-alpha release, and superoxide and nitric oxide production in culture medium. Interestingly, oral administration of curcumin (300 mg/kg 10 days before and daily thereafter throughout the experimental time period) inhibited BLM-induced increases in total cell counts and biomarkers of inflammatory responses in BALF. In addition, curcumin significantly reduced the total lung hydroxyproline in BLM rats. Furthermore, curcumin remarkably suppressed the BLM-induced alveolar macrophage production of TNF- alpha, superoxide and nitric oxide. These findings suggest curcumin as a potent anti-inflammatory and anti-fibrotic agent against BLM- induced pulmonary fibrosis in rats.

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Dwivedi, A. K., M. Raman, et al. (1992). “Combined thin-layer chromatography-densitometry for the quantitation of.” Indian Journal of Pharmaceutical Sciences 54(5): 174-177.

El-Ashmawy, M. B. (1999). “The role of natural antioxidants and synthetic nonsteroidal.” Saudi Pharmaceutical Journal 7(1-2): 1-13. Chemoprevention of cancer has become a promising approach, because chemotherapy and surgery have not been fully effective

against the high incidence or low survival rate of many tumors. This review focuses on the effect of some natural antioxidants and synthetic nonsteroidal anti- inflammatory drugs in cancer chemoprevention, particularly, their role on lipid peroxidation. Because lipid peroxidation and oxygen free radicals are interdependable, antioxidants are gaining popularity in interfering with the free radical chain-reactions, and thus, arresting tumor promotion. Different enzymes are also involved in either promoting the formation, or catalyzing inactivation of free radicals, other lipid oxygenated derivatives and mutagenic products. Peroxidases play complex roles in the process. Glutathione peroxidase, for example, represents an endogenous defense mechanism, while the peroxidase-activity of prostaglandin-endoperoxide synthases catalyzes the formation of some inflammatory and mutagenic compounds. Potentiators of the first and inhibitors of the second were proved to possess chemopreventive activity. Natural antioxidants of chemopreventive activities include the tea polyphenols, curcumin and resveratrol. Several mechanisms have been suggested to explain their anticarcinogenic effects, and interference with lipid peroxidation is one of these. Synthetic medicinals, such as aspirin, sulindac and piroxicam exhibited good results in colon cancer, perhaps due to their inhibitory effect on prostaglandin synthesis.

El-Ashmawy, M. B. (1999). “The role of natural antioxidants and synthetic nonsteroidal antiinflammatory drugs in the chemoprevention of cancer.” Saudi Pharmaceutical Journal 7(1/2): 1-13. This review focuses on the effect of natural antioxidants (retinoids, carotenoids, vitamin E, glutathione and selenium compounds, green tea polyphenols, curcumin and resveratrol) and synthetic nonsteroidal anti-inflammatory drugs in cancer chemoprevention, particularly their role in lipid peroxidation.

El-Ashmawy, M. B. (1999). “The role of natural antioxidants and synthetic nonsteroidal antiinflammatory drugs in the chemoprevention of cancer.” Saudi Pharmaceutical Journal 7(1/2): 1-13. This review focuses on the effect of natural antioxidants (retinoids, carotenoids, vitamin E, glutathione and selenium compounds, green tea polyphenols, curcumin and resveratrol) and synthetic nonsteroidal anti-inflammatory drugs in cancer chemoprevention, particularly their role in lipid peroxidation.

Epinat, J. C. and T. D. Gilmore (1999). “Diverse agents act at multiple levels to inhibit the Rel/NF-kappaB.” Oncogene 18(49): 6896-6909. Rel/NF-kappaB transcription factors regulate several important physiological processes, including developmental processes, inflammation and immune responses, cell growth, cancer, apoptosis, and the expression of certain viral genes. Therefore, they have also been sought-after molecular targets for pharmacological intervention. As details of the Rel/NF-kappaB signal transduction pathway are revealed, it is clear that modulators of this pathway can act at several levels. Inhibitors of the Rel/NF-kappaB pathway include a variety of natural and designed molecules, including anti-oxidants, proteasome inhibitors, peptides, small molecules, and dominant-negative or constitutively active polypeptides in the pathway. Several of these molecules act as general inhibitors of Rel/NF-kappaB induction, whereas others inhibit specific pathways of induction, Inhibitors of Rel/NF-kappaB are likely to gain stature as treatments for certain cancers and neurodegenerative and inflammatory diseases.

Fintelmann, V. and T. Wegener (2001). “Curcuma longa - An underestimated medicinal plant.” Deutsche Apotheker Zeitung 141(32): 53-63.

Flynn, D. L., M. F. Rafferty, et al. (1986). “Inhibition of 5-hydroxy-eicosatetraenoic acid (5-HETE) formation in.” Prostaglandins Leukotrienes and Medicine 22(3): 357-360. Various diarylheptanoids including curcumin, bis (3,4-dihydroxycinnamoyl)methane, and yakuchinones A & B were screened and found to potent inhibitors of 5-HETE production by intact human neutrophils, with respective IC50 values of 8.0, 4.4, 5.4, and 4.0 muM. These diarylheptanoids were found to be more potent than BW-755C, Phenidone, and AA-861. We have confirmed a previous report that several of these diarylheptanoids inhibit cyclooxygenase. Thus, curcuminoids and yakuchinones are more accurately characterized as dual inhibitors of arachidonic acid metabolism.

Frautschy, S. A., W. Hu, et al. (2001). “Phenolic anti-inflammatory antioxidant reversal of Abeta-induced.” Neurobiology of Aging 22(6): 993-1005. Both oxidative damage and inflammation have been implicated in age-related neurodegenerative diseases including Alzheimer's Disease (AD). The yellow curry spice, curcumin, has both antioxidant and anti-inflammatory activities which confer significant protection against neurotoxic and genotoxic agents. We used 22 month Sprague-Dawley (SD) rats to compare the effects of the conventional NSAID, ibuprofen, and curcumin for their ability to protect against amyloid beta-protein (Abeta)-induced damage. Lipoprotein carrier-mediated, intracerebroventricular infusion of Abeta peptides induced oxidative damage, synaptophysin loss, a microglial response and widespread Abeta deposits. Dietary curcumin (2000 ppm), but not ibuprofen, suppressed oxidative damage (isoprostane levels) and synaptophysin loss. Both ibuprofen and curcumin reduced microgliosis in cortical layers, but curcumin increased microglial labeling within and adjacent to Abeta-ir deposits. In a second group of middle-aged female SD rats, 500 ppm dietary curcumin prevented Abeta-infusion induced spatial memory deficits in the Morris Water Maze and post-synaptic density (PSD)-95 loss and reduced Abeta deposits. Because of its low side-effect profile and long history of safe use, curcumin may find clinical application for AD prevention. (c) 2001 Elsevier Science Inc. All rights reserved.

Fujiki, H. (1999). “Two stages of cancer prevention with green tea.” Journal of Cancer Research and Clinical Oncology 125(11): 589-597. Cancer chemoprevention is a new and important medical science in its own right. On the occasion of my presentation entitled 'Natural agents and cancer chemoprevention' at the 90th AACR Meeting in 1999, I summarized our recent results on cancer prevention with green tea. In this article, the present status of clinical trials supported by the Chemoprevention Branch of the National Cancer Institute in the United States is first described by way of introduction. Although various natural products are now under investigation in phase I clinical trials, green tea has, perhaps, the greatest potential for further development. In order to expand our understanding of the effects of tea polyphenols and green tea, I review their ability to inhibit growth and cause apoptosis of cancer cells, their distribution into target organs and their other cancer-preventing properties. In addition, the paper focuses on the significance of reducing tumor necrosis factor alpha (TNFalpha) gene expression in cells and TNFalpha release from cells as essential activities for cancer prevention. As for the

amounts of green tea effective in cancer prevention, I present two results from our Research Institute: a prospective cohort study with over 8000 individuals in Saitama Prefecture revealed that the daily consumption of at least ten Japanese-size cups of green tea resulted in delayed cancer onset, and a follow-up study of breast cancer patients conducted at our Hospital found that stages I and II breast cancer patients consuming over five cups per day experienced a lower recurrence rate and longer disease- free period than those consuming fewer than four cups per day. Thus, I propose here, for the first time, the two-stage approach to analyzing cancer prevention with green tea: cancer prevention before cancer onset and cancer prevention following cancer treatment. As an additional example of cancer prevention with natural agents, kava, a daily beverage in Fiji, is mentioned. All the evidence reminds us of the significance of alternative medicine in practical cancer prevention.

Garg, A. and B. B. Aggarwal (2002). “Nuclear transcription factor-kappaB as a target for cancer drug.” Leukemia 16(6): 1053-1068. Nuclear factor kappa B (NF-kappaB) is a family of inducible transcription factors found virtually ubiquitously in all cells. Since its discovery by Sen and Baltimore in 1986, much has been discovered about its mechanisms of activation, its target genes, and its function in a variety of human diseases including those related to inflammation, asthma, atherosclerosis, AIDS, septic shock, arthritis, and cancer. Due to its role in a wide variety of diseases, NF-kappaB has become one of the major targets for drug development. Here, we review our current knowledge of NF-kappaB the possible mechanisms of its activation, its potential role in cancer, and various strategies being employed to target the NF-kappaB signaling pathway for cancer drug development.

Gatof, D. and D. Ahnen (2002). “Primary prevention of colorectal cancer: Diet and drugs.” Gastroenterology Clinics of North America 31(2): 587-623. Primary prevention of colonic adenomas and cancer through dietary interventions or chemoprevention has great appeal. This article discusses primary prevention goals and promising nutritional or chemopreventive strategies. There is substantial observational evidence that diets high in total calories and fat and or low in fruits and vegetables or total fiber as well as low levels of physical activity are related to the risk of colonic neoplasia. Similar observational data indicate that diets high in specific nutrients such as antioxidant vitamins or calcium may be protective. The article describes some of the newer chemopreventive agents and reviews the data linking diet and lifestyle to colorectal cancer risk, focusing on interventions that have also been studied in prospective clinical trials. Finally the evidence supporting the role of non-steroidal antiinflammatory drugs for the chemoprevention of CRC is reviewed and the status of several other promising newer agents that are entering human trials is summarized.

Gautam, S. C., Y. X. Xu, et al. (1998). “Nonselective inhibition of proliferation of transformed and.” Biochemical Pharmacology 55(8): 1333-1337. We have investigated the antiproliferative effect of curcumin, an antitumor agent with antioxidant and anti-inflammatory properties, against a variety of transformed and nontransformed cell types. At equimolar concentrations ranging from 6.25 to 50 muM, curcumin inhibited DNA synthesis, as revealed by sup 3H-incorporation, in five leukemia lines, three

nontransformed hematopoietic progenitor cell populations, and four nontransformed fibroblastic cell lines in a concentration-dependent manner. Curcumin also inhibited the cellular growth of both transformed and nontransformed cells in clonogenic assays. Without discriminating between transformed or nontransformed cells, the inhibition of cell proliferation by curcumin was not always associated with programmed cell death. These findings have implications for developing curcumin-based anticancer and anti-inflammation therapies.

Gee, J. M. and I. T. Johnson (2001). "Polyphenolic compounds: Interactions with the gut and implications for." Current Medicinal Chemistry 8(11): 1245-1255. Polyphenolic compounds are abundant throughout the plant kingdom and are found in a wide variety of human foods. The flavonoids, which are the best defined group of polyphenols in the human diet, themselves comprise a large and complex group, all of which contain a three-ring structure with two aromatic centres and a central oxygenated heterocycle. Recent evidence suggests that significant quantities of quercetin and possibly myricetin and kaempferol are absorbed in the gut. A larger fraction probably remains in the lumen, and thus a substantial proportion of the gastrointestinal mucosa is exposed to biologically significant concentrations of these compounds. A substantial body of experimental work has established that flavonoids can suppress carcinogenesis in animal models and there is considerable interest in the biological effects of these compounds at the cellular level. Flavonoids interact with cellular signal pathways controlling the cell cycle, differentiation and apoptosis. Their potentially antineoplastic effects include antioxidant activity, induction of Phase II enzyme activity, inhibition of protein kinases and interactions with Type II estrogen binding sites. Naturally occuring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential for pharmaceutical uses as chemopreventive agents against neoplastic changes in the alimentary tract. Future research should therefore focus on the biological effects of flavonoids in the human body, using biomarkers to define their effects at each stage in the onset of neoplasia.

Gentile, J. M., G. Gentile, et al. (2001). "Antimutagenesis/anticarcinogenesis 2001: Mechanistic studies." Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 480-481(-): 1-7.

Gescher, A., U. Pastorino, et al. (1998). "Suppression of tumour development by substances derived from the diet." British Journal of Clinical Pharmacology 45(1): 1-12. The concept that cancer can be prevented, or its onset postponed, by certain diet-derived substances is currently eliciting considerable interest. Agents which interfere with tumour development at the stage of promotion and progression in particular are of potential clinical value. As chemopreventive agents have to be administered over a long period of time in order to establish whether they possess efficacy in humans, it is of paramount importance to establish their lack of toxicity. The desire to select the best chemopreventive drug candidates for clinical trial, and the necessity to monitor efficacy in the short and intermediate term, render the identification of specific mechanism-based in vivo markers of biological activity a high priority. Antioxidation, inhibition of arachidonic acid metabolism, modulation of cellular signal transduction pathways,

inhibition of hormone and growth factor activity and inhibition of oncogene activity are discussed as mechanisms by which the soya constituent genistein, the curry ingredient curcumin and the vitamin A analogue 13-cis retinoic acid exert tumour suppression. A better understanding of these mechanisms will help the establishment of screens for the discovery of new and better chemopreventive agents and the identification of surrogate markers to assess the outcome of clinical chemoprevention trials.

Gilmore, T., M. E. Gapuzan, et al. (2002). "Rel/NF-kappaB/IkappaB signal transduction in the generation and." Cancer Letters 181(1): 1-9. The Rel/NF-kappaB family is a group of structurally-related, tightly-regulated transcription factors that control the expression of a multitude of genes involved in key cellular and organismal processes. The Rel/NF-kappaB signal transduction pathway is misregulated in a variety of human cancers, especially ones of lymphoid cell origin, due either to genetic changes (such as chromosomal rearrangements, amplifications, and mutations) or to chronic activation of the pathway by epigenetic mechanisms. Constitutive activation of the Rel/NF-kappaB pathway can contribute to the oncogenic state in several ways, for example, by driving proliferation, by enhancing cell survival, or by promoting angiogenesis or metastasis. In many cases, inhibition of Rel/NF-kappaB activity reverses all or part of the malignant state. Thus, the Rel/NF-kappaB pathway has received much attention as a focal point for clinical intervention.

Greenwald, P., G. J. Kelloff, et al. (1995). "Genetic and cellular changes in colorectal cancer: Proposed targets of." Cancer Epidemiology Biomarkers and Prevention 4(7): 691-702. Progress in development of a genetic model for colorectal tumorigenesis and human chemoprevention research may allow the mechanism-based identification of targets and chemopreventive agents that will protect against colorectal cancer. For example, numerous mutagenic events can occur throughout colorectal carcinogenesis, including loss of heterozygosity in tumor suppressor genes such as APC, MCC, DCC, and p53, as well as in oncogenes such as K-ras. Chemopreventive agents that inhibit mutagenic activity such as N-acetyl-l-cysteine, oltipraz, and nonsteroidal anti-inflammatory drugs may protect against these mutations. Also, agents such as perillyl alcohol and lovastatin that interfere with protein isoprenylation and, hence, inhibit oncogene activation may protect against aberrant K-ras expression. Hyperproliferation in normal mucosa, leading to early adenomas, and cellular proliferation, leading to growth and progression of neoplasia, are also aspects of colorectal carcinogenesis that can be controlled by chemopreventive agents. Calcium is a chemopreventive agent for which there is both clinical and experimental evidence of inhibition of cell proliferation in colon mucosa. Other examples of antiproliferative agents with potential chemopreventive efficacy in colon are 2-difluoromethylornithine, dehydroepiandrosterone, and selenium. Differentiating agents such as retinoids and deltanoids also may slow proliferation and progression. Antioxidants have potential for interfering with both mutagenicity and proliferation (e.g., by preventing oxidative activation of carcinogens and scavenging activated oxygen species generated during inflammation). The same mechanistic principles apply to identification of dietary chemopreventive intervention for colorectal carcinogenesis. For example, lowering dietary fat and increasing dietary fiber lead to

lower colorectal mucosal proliferation, and cruciferous vegetables contain agents such as indoles and dithiolthiones that have shown antimutagenic activity.

Grilli, M. and M. Memo (1999). “Nuclear factor-kappaB/Rel proteins: A point of convergence of.” Biochemical Pharmacology 57(1): 1-7. Nuclear factor-kappaB (NF-kappaB)/Rel designates a family of transcription factors participating in the activation of a wide range of genes crucially involved in immune and inflammatory function. NF-kappaB/Rel proteins have been demonstrated recently in primary neurons and in several brain areas. Functional significance of these proteins is still not understood completely, but since certain subsets of neurons appear to contain constitutively active DNA-binding activity, it seems likely that they may participate in normal brain function. A growing body of evidence is accumulating for a specific activation of NF-kappaB/Rel proteins in the CNS, and in particular in neuronal cells, during neurodegenerative processes associated to etiologically unrelated conditions. Whether NF-kappaB activation is part of the neurodegenerative process or of protective mechanisms is a matter of debate. This issue will be reviewed here with particular attention to the available reports on the activity of NF-kappaB/Rel proteins in both experimental paradigms of neurodegeneration and post-mortem brain tissue of patients affected by various neurological diseases. We hypothesize that NF-kappaB/Rel proteins may represent the point of convergence of several signalling pathways relevant for initiating or accelerating the process of neuronal dysfunction and degeneration in many neurological diseases, including Parkinson's disease, Alzheimer's disease, CNS viral infections, and possibly others. If NF-kappaB/Rel proteins represent an integrating point of several pathways potentially contributing to neuronal degeneration, molecules that finely modulate their activity could represent a novel pharmacological approach to several neurological diseases.

Groten, J. P., W. Butler, et al. (2000). “An analysis of the possibility for health implications of joint actions.” Regulatory Toxicology and Pharmacology 31(1): 77-91. The possibility that structurally unrelated food additives could show either joint actions or interactions has been assessed based on their potential to share common sites and mechanisms of action or common pathways of elimination. All food additives approved in the European Union and allocated numerical acceptable daily intake values were studied, initially based on the reports by the FAO-WHO Joint Expert Committee for Food Additives. Target organs were identified based on the effects reported at doses above the no-observed-adverse-effect level (NOAEL) in animal and human studies. The descriptions of the pathological and other changes reported were used to assess whether different additives, sharing the same target organ, would produce a common toxic effect. In all but a very few cases, the possibility of joint actions or interactions could be excluded on scientific grounds. The exceptions were on the liver (curcumin, thiabendazole, propyl gallate, and BHT), the kidney (diphenyl, o-phenylphenol, and ferrocyanide salts), the blood (azorubine and propyl gallate), and the thyroid (erythosine, thiabendazole, and nitrate). Toxicokinetic interactions were considered unlikely because of the low dosages involved, the diverse nature of the routes of metabolism and elimination, and the fact that enzyme induction or inhibition would have influenced selection of the NOAEL. Many of those additives which could not be excluded from showing joint actions or interactions would have low intakes; in some cases they were

alternatives for the same application, thereby further lowering the combined intake. In consequence, joint actions or interactions between additives do not represent a significant health concern. (C) 2000 International Life Sciences Institute.

Grundman, M. and P. Delaney (2002). “Antioxidant strategies for Alzheimer's disease.” Proceedings of the Nutrition Society 61(2): 191-202. Oxidative damage is present within the brains of patients with Alzheimer's disease (AD), and is observed within every class of biomolecule, including nucleic acids, proteins, lipids and carbohydrates. Oxidative injury may develop secondary to excessive oxidative stress resulting from beta-amyloid-induced free radicals, mitochondrial abnormalities, inadequate energy supply, inflammation or altered antioxidant defences. Treatment with antioxidants is a promising approach for slowing disease progression to the extent that oxidative damage may be responsible for the cognitive and functional decline observed in AD. Although not a uniformly consistent observation, a number of epidemiological studies have found a link between antioxidant intake and a reduced incidence of dementia, AD and cognitive decline in elderly populations. In AD clinical trials molecules with antioxidant properties such as vitamin E and Ginkgo biloba extract have shown modest benefit. A clinical trial with vitamin E is currently ongoing to determine if it can delay progression to AD in individuals with mild cognitive impairment. Combinations of antioxidants might be of even greater potential benefit for AD, especially if the agents worked in different cellular compartments or had complementary activity (e.g. vitamins E, C and ubiquinone). Naturally-occurring compounds with antioxidant capacity are available and widely marketed (e.g. vitamin C, ubiquinone, lipoic acid, beta-carotene, creatine, melatonin, curcumin) and synthetic compounds are under development by industry. Nevertheless, the clinical value of these agents for AD prevention and treatment is ambiguous, and will remain so until properly designed human trials have been performed.

Gupta, B., V. K. Kulshrestha, et al. (1980). “Mechanism of curcumin induced gastric ulcer in rats.” Indian Journal of Medical Research 71(5): 806-814. Curcumin - an active principle isolated from Curcuma longa Linn. possesses significant anti-inflammatory activity. In a dose of 100 mg/kg (2Xanti-inflammatory ED$D5inf 0) administered orally for six consecutive days curcumin produced gastric ulceration in albino rats. Marked reduction in the mucin content of gastric juice seems to be responsible for this ulcerogenic response. Pretreatment with adrenergic, cholinergic, tryptaminergic and histaminergic (Hinf 2)receptor antagonists provided partial protection against curcumin induced gastric ulcers, while metiamide pretreatment almost completely prevented the development of gastric lesions and prevented a decrease in the mucin secretion. It would appear that Hinf 2receptors are predominantly involved in the ulcerogenicity of curcumin. Further, the change in the mucin content appears to be the determinant factor in the ulcerogenicity of curcumin.

Hamby, J. M. and H. D. H. Showalter (1999). “Small molecule inhibitors of tumor-promoted angiogenesis, including.” Pharmacology and Therapeutics 82(2): 169-193. Angiogenesis is an exciting and promising new area of research. The concept that tumor cells are absolutely dependent upon neovascularization to grow and metastasize has opened the door to a multitude of new approaches and targets for developing anticancer

therapies. These potential new antiangiogenic therapies offer the possibility for improved efficacy and reduced toxicity relative to conventional cancer treatments without the possibility of drug resistance. In particular, reports of small molecule inhibitors of tumor-promoted angiogenesis are appearing ever more frequently in the scientific literature. For this reason, the major focus of this review will be to cover small molecule inhibitors of tumor-promoted angiogenesis. The present review concentrates on selected literature, principally from mid-1996 to mid-1998, where there are sufficient biological data to support claims of antiangiogenic activities by small molecules. In addition, a historical background is presented and some of the important issues related to this field are discussed within. Copyright (C) 1999 Elsevier Science Inc.

Han, R. (1994). “Highlight on the studies of anticancer drugs derived from plants in.” Stem Cells 12(1): 53-63. Recent progress on the study of anticancer drugs originating from plants in China is reviewed in this paper. Guided by the experience of traditional Chinese medicine, several new drugs have been found. Indirubin from Indigofera tinctoria is useful for the treatment of chronic myelocytic leukemia. Irisquinone from Iris latea pallasii and 10-hydroxy camptothecin from Camptotheca accuminata have exhibited definite activity on rodent tumors. Recent studies indicate that ginsenoside Rhinf 2 is an inducer of cell differentiation in melanoma B-16 cells in vitro. Pharmacological studies have demonstrated that curcumin from Curcuma longa is an antimutagen as well as an antipromotor for cancer. Daidzein and acetyl boswellic acid have been shown to be effective inducers of cell differentiation in HL-60 cells. Guided by the chemotaxonomic principle of plants, harringtonine and homoharringtonine isolated from Cephalotaxus hainanensis have exhibited significant antileukemia activity and are widely used in clinics in China. Taxol from Taxus chinensis has been shown to be an important new anticancer drug with unique chemical structure and mechanism of action. The continuous search for new anticancer drugs from plants will be a fruitful frontier in cancer treatment and chemoprevention.

Heng, M. C. Y., M. K. Song, et al. (2000). “Drug-induced suppression of phosphorylase kinase activity correlates.” British Journal of Dermatology 143(5): 937-949. Background: Phosphorylase kinase (PhK), also known as adenosine triphosphate (ATP)-phosphorylase b phosphotransferase, integrates multiple calcium/calmodulin-dependent signalling pathways, including those involved in cell migration and cell proliferation, while coupling these pathways to glycogenolysis and ATP-dependent phosphorylation, thus ensuring continuing energy supply for these activities. Objectives: Our laboratory recently reported correlation of elevated PhK activity with psoriatic activity. This study further evaluates the significance of drug-induced suppression of PhK activity on psoriatic activity. Patients and methods: PhK activity was assayed in four groups, each with 10 patients: (i) Active untreated psoriasis; (ii) resolving psoriasis treated by calcipotriol (Dovonex(R), Bristol Myers Squibb, Princeton, NJ, U.S.A.), a vitamin Dinf 3 analogue and an indirect inhibitor of PhK; (iii) curcumin (diferuloylmethane), a selective PhK inhibitor; and (iv) 10 normal non-psoriatic subjects. Results: PhK activity in units mgsup -sup 1 protein was highest in active untreated psoriasis (1204 +/- 804.3; mean +/- SD), lower in the calcipotriol-treated group (550.7 +/- 192.9), lower in curcumin-treated group (207.2 +/- 97.6), and lowest in normal skin (105.4 +/- 44.6). One-way analysis of

variance performed on log-transformed PhK activity measure showed significant differences among the four groups, Finf 3inf .inf 3inf 6 = 48.79, P < 0.0001. Decreased PhK activity in curcumin-and calcipotriol-treated psoriasis was associated with corresponding decreases in keratinocyte transferrin receptor (TRR) expression, severity of parakeratosis and density of epidermal CD8 + T cells. Conclusions: Our results demonstrate that drug-induced suppression of PhK activity is associated with resolution of psoriatic activity as assessed by clinical, histological and immunohistochemical criteria, and support the hypothesis that effective antipsoriatic activity may be achieved through modulation of PhK activity.

Hiller, K. (1995). “The pharmaceutical value of selected herbal teas.” Deutsche Apotheker Zeitung 135(16): 35-50.

Hill-Kapturczak, N., V. Thamilselvan, et al. (2001). “Mechanism of heme oxygenase-1 gene induction by curcumin in human renal.” American Journal of Physiology - Renal Physiology 281(5 50-5): F851-F859. Heme oxygenase-1 (HO-1) catalyzes the rate-limiting step in heme degradation, releasing iron, carbon monoxide, and biliverdin. Induction of HO-1 occurs as an adaptive and protective response to several inflammatory stimuli. The transcription factor activator protein-1 (AP-1) has been implicated in the activation of the HO-1 gene. To elucidate the molecular mechanism of HO-1 induction, we examined the effects of diferuloylmethane (curcumin), an inhibitor of the transcription factor AP-1. Surprisingly, curcumin by itself was a very potent inducer of HO-1. Curcumin has anti-inflammatory, antioxidant, and renoprotective effects. To evaluate the mechanism of curcumin-mediated induction of HO-1, confluent human renal proximal tubule cells were exposed to curcumin (1-8 muM). We observed a time- and dose-dependent induction of HO-1 mRNA that was associated with increased HO-1 protein. Coincubation of curcumin with actinomycin D completely blocked the upregulation of HO-1 mRNA. Blockade of nuclear factor-kappaB (NF-kappaB) with an IkappaBalpha phosphorylation inhibitor attenuated curcumin-mediated induction of HO-1 mRNA and protein. These data demonstrate that curcumin induces HO-1 mRNA and protein in renal proximal tubule cells. HO-1 induction by curcumin is mediated, at least in part, via transcriptional mechanisms and involves the NF-kappaB pathway.

Hishikawa, K. and T. Nakaki (2001). “NF-kappab as a therapeutic drug target.” Folia Pharmacologica Japonica 118(3): 197-202. The transcription factor NF-kappaB has attracted widespread interest based on its unusual regulation, the variety of stimuli that activate it, the diverse genes and biological responses that it controls, the striking evolutionary conservation of structure and function among family members, and its apparent involvement in a variety of human diseases. Recently NF-kappaB has been shown to be the target of new drug discovery. Here, we discuss the so-called NF-kappaB inhibitors and consider the development of new therapeutic agents.

Hozumi, A., Y. Nishimura, et al. (2001). “Induction of MMP-9 in normal human bronchial epithelial cells by.” American Journal of Physiology - Lung Cellular and Molecular Physiology 281(6 25-6): L1444-L1452. In this study, we determined whether the proinflammatory cytokines tumor necrosis factor (TNF)-alpha and interleukin-1beta

contribute to the regulation of matrix metalloproteinase (MMP)-9 in human bronchial epithelial cells and whether the induction of MMP-9 is regulated by the transcription factor nuclear factor (NF)-kappaB. We demonstrated that TNF-alpha induced MMP-9 at both the protein and mRNA levels in human bronchial epithelial cells and that interleukin-1beta did not. In contrast, induction of the tissue inhibitor of metalloproteinase-1 by TNF-alpha was less than that of interleukin-1beta. Increased expression of MMP-9 and NF-kappaB activation induced by TNF-alpha were inhibited by pyrrolidine dithiocarbamate and N-acetyl-L-cysteine but were not inhibited by curcumin. These results suggest that TNF-alpha induces the expression of MMP-9 in human bronchial epithelial cells and that this induction is mediated via the NF-kappaB-mediated pathway.

Hsu, H. Y. and M. H. Wen (2002). "Lipopolysaccharide-mediated reactive oxygen species and signal." Journal of Biological Chemistry 277(25): 22131-22139. Lipopolysaccharide (LPS) stimulates macrophages to release inflammatory cytokines, interleukin-1beta (IL-1), and tumor necrosis factor (TNF). LPS-induced TNF suppresses scavenger receptor functions in macrophages (van Lenten, B. J., and Fogelman, A. M. (1992) J. Immunol. 148, 112-116), which is regulated by TNF-mediated protein kinases (Hsu, H. Y., and Twu, Y. C. (2000) J. Biol. Chem. 275, 41035-41048). To examine the molecular mechanism for LPS induction of IL-1 in macrophages, we demonstrated that LPS quickly stimulated reactive oxygen species (ROS), and 3 h later induced prointerleukin-1beta (pro-IL-1, precursor of IL-1) production and IL-1 secretion. LPS stimulated pro-IL-1 message/protein between 3 and 10 h; however, there was a 40% reduction of pro-IL-1 in preincubation of the antioxidant, N-acetylcysteine (NAC). Moreover, NAC moderated LPS-induced IL-1 secretion partially via interleukin 1-converting enzyme. The maximal activity of LPS-induced ERK, JNK, and p38 was 12-(30 min), 5- (30 min), and 16-fold (15 min), respectively. In contrast, NAC reduced ERK activity to 60% and decreased p38 activity to the basal level, but JNK activity was induced 2-fold. Furthermore, the pharmacological antagonists LY294002, SB203580, curcumin, calphostin C, and PD98059 revealed the diverse roles of LPS-mediated protein kinases in pro-IL-1. On the other hand, NAC and diphenyleneiodonium chloride partially inhibited LPS-induced Rac activity and protein-tyrosine kinase (PTK), indicating that LPS-mediated ROS and NADPH oxidase correspond to Rac activation and IL-1 expression. Our findings establish for the first time that LPS-mediated PTK/phosphatidylinositol 3-kinase/Rac/p38 pathways play a more important role than pathways of PTK/PKC/MEK/ERK and of PTK/phosphatidylinositol 3-kinase/Rac/JNK in the regulation of pro-IL-1/IL-1. The findings also further elucidate the critical role of LPS-mediated ROS in signal transduction pathways. Our results suggest that understanding LPS-transduced signals in IL-1 induction upon the antibacterial action of macrophages should provide a therapeutic strategy for aberrant inflammatory responses leading to severe cellular injury or concurrent multiorgan septic damage.

Huang, H. C., T. R. Jan, et al. (1992). "Inhibitory effect of curcumin, an anti-inflammatory agent, on vascular." European Journal of Pharmacology 221(2-3): 381-384. The effects of curcumin, an anti-inflammatory agent from Curcuma longa, on the proliferation of blood mononuclear cells and vascular smooth muscle cells were studied.

Proliferative responses were determined from the uptake of tritiated thymidine. In human peripheral blood mononuclear cells, curcumin dose dependently inhibited the responses to phytohemagglutinin and mixed lymphocyte reaction at the dose ranges of 10sup -sup 6 to 3 x 10sup -sup 5 and 3 x 10sup -sup 6 to 3 x 10sup -sup 5 M, respectively. Curcumin (10sup -sup 6 to 10sup -sup 4 M) dose dependently inhibited the proliferation of rabbit vascular smooth muscle cells stimulated by fetal calf serum. Curcumin had a greater inhibitory effect on platelet-derived growth factor-stimulated proliferation than on serum-stimulated proliferation. Cinnamic acid, coumaric acid and ferulic acid were much less effective than curcumin as inhibitors of serum-induced smooth muscle cell proliferation, suggesting that the cinnamic acid and ferulic acid moieties alone are not sufficient for activity, and that the characteristics of the diferuloylmethane molecule itself are necessary for activity. Curcumin may be useful as a new template for the development of better remedies for the prevention of the pathological changes of atherosclerosis and restenosis.

Huang, M. T., N. Ma, et al. (1995). “Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and.” Carcinogenesis 16(10): 2493-2497. Commercial grade curcumin (~77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin) is widely used as a yellow coloring agent and spice in foods. In the present study topical application of commercial grade curcumin, pure curcumin or demethoxycurcumin had an equally potent inhibitory effect on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced increases in ornithine decarboxylase activity and TPA-induced tumor promotion in 7,12-dimethylbenz(a)anthracene-initiated mouse skin. Bisdemethoxycurcumin and tetrahydrocurcumin were less active. In additional studies we found that commercial grade curcumin, pure curcumin, demethoxycurcumin and bisdemethoxycurcumin had about the same potent inhibitory effect on TPA-induced inflammation of mouse ears, as well as TPA-induced transformation of cultured JB6 (Psup +) cells. Tetrahydrocurcumin was less active. The results indicate that pure curcumin and demethoxycurcumin (the major constituents of commercial grade curcumin) have the same potent inhibitory effects as commercial grade curcumin for inhibition of TPA-induced tumor promotion, but bisdemethoxycurcumin and tetrahydrocurcumin are less active.

Huang, M. T., T. Lysz, et al. (1991). “Inhibitory effects of curcumin on in vitro lipoxygenase and.” Cancer Research 51(3): 813-819. Topical application of curcumin, the yellow pigment in turmeric and curry, strongly inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity, DNA synthesis, and tumor promotion in mouse skin (Huang et al., Cancer Res., 48: 5941-5946, 1988). Chlorogenic acid, caffeic acid, and ferulic acid (structurally related dietary compounds) were considerably less active. In the present study, topical application of curcumin markedly inhibited TPA- and arachidonic acid-induced epidermal inflammation (ear edema) in mice, but chlorogenic acid, caffeic acid, and ferulic acid were only weakly active or inactive. The in vitro addition of 3, 10, 30, or 100 muM curcumin to cytosol from homogenates of mouse epidermis inhibited the metabolism of arachidonic acid to 5-hydroxyeicosatetraenoic acid (5-HETE) by 40, 60, 66, or 83%, respectively, and the metabolism of arachidonic acid to 8-HETE was inhibited by 40, 51, 77, or 85%, respectively (IC$D5inf 0 (concentration needed for 50% inhibition) = 5-10 muM).

Chlorogenic acid, caffeic acid, or ferulic acid (100 muM) inhibited the metabolism of arachidonic acid to 5-HETE by 36, 10, or 16%, respectively, and these hydroxylated cinnamic acid derivatives inhibited the metabolism of arachidonic acid to 8-HETE by 37, 20, or 10%, respectively (IC$D5inf 0 > 100 muM). The metabolism of arachidonic acid to prostaglandin Einf 2, prostaglandin F(2alpha), and prostaglandin Dinf 2 by epidermal microsomes was inhibited approximately 50% by the in vitro addition of 5-10 muM curcumin. Chlorogenic acid, caffeic acid, and ferulic acid (100 muM) were inactive. In vitro rat brain protein kinase C activity was not affected by 50-200 muM curcumin, chlorogenic acid, caffeic acid, or ferulic acid. The inhibitory effects of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on TPA-induced tumor promotion in mouse epidermis parallel their inhibitory effects on TPA-induced epidermal inflammation and epidermal lipoxygenase and cyclooxygenase activities.

Ireson, C., S. Orr, et al. (2001). “Characterization of metabolites of the chemopreventive agent curcumin.” Cancer Research 61(3): 1058-1064. Curcumin, the yellow pigment in turmeric, has been shown to prevent malignancies in a variety of tissues in rodents, especially in the intestinal tract. Pharmacological activities of curcumin in cells in situ germane to chemoprevention, such as inhibition of expression of cyclooxygenase-2 (COX-2), require drug concentrations in the 10SUP-5-10SUP-4 M range. The systemic bioavailability of curcumin is low, so that its pharmacological activity may be mediated, in part, by curcumin metabolites. To investigate this possibility, we compared curcumin metabolism in human and rat hepatocytes in suspension with that in rats in vivo. Analysis by high-performance liquid chromatography with detection at 420 and 280 nm permitted characterization of metabolites with both intact diferoylmethane structure and increased saturation of the heptatrienone chain. Chromatographic inferences were corroborated by mass spectrometry. The major metabolites in suspensions of human or rat hepatocytes were identified as hexahydrocurcumin and hexahydrocurcuminol. In rats, in vivo, curcumin administered i.v. (40 mg/kg) disappeared from the plasma within 1 h of dosing. After p.o. administration (500 mg/kg), parent drug was present in plasma at levels near the detection limit. The major products of curcumin biotransformation identified in rat plasma were curcumin giucuronide and curcumin sulfate whereas hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide were present in small amounts. To test the hypothesis that curcumin metabolites resemble their progenitor in that they can inhibit COX-2 expression, curcumin and four of its metabolites at a concentration of 20 muM were compared in terms of their ability to inhibit phorbol ester-induced prostaglandin ESUB2 (PGESUB2) production in human colonic epithelial cells. Curcumin reduced PGESUB2 levels to preinduction levels, whereas tetrahydrocurcumin, previously shown to be a murine metabolite of curcumin, hexahydrocurcumin, and curcumin sulfate, had only weak PGESUB2 inhibitory activity, and hexahydrocurcuminol was inactive. The results suggest that (a) the major products of curcumin biotransformation by hepatocytes occur only at low abundance in rat plasma after curcumin administration; and (b) metabolism of curcumin by reduction or conjugation generates species with reduced ability to inhibit COX-2 expression. Because the gastrointestinal tract seems to be exposed more prominently to unmetabolized curcumin than any other tissue, the results support the clinical evaluation of curcumin as a colorectal cancer chemopreventive agent.

Ishikawa, Y., H. Sugiyama, et al. (1999). “Bioflavonoid quercetin inhibits interleukin-1-induced transcriptional.” Journal of the American Society of Nephrology 10(11): 2290-2296. Flavonoids are semiessential food components that possess anti- inflammatory properties. This report describes a novel potential of bioflavonoid quercetin as an inhibitor of monocyte chemoattractant protein-1 (MCP-1) in glomerular cells. Cultured mesangial cells as well as isolated glomeruli expressed MCP-1 mRNA in response to interleukin-1beta (IL-1beta). Quercetin dramatically inhibited the cytokine-triggered MCP-1 expression. To explore the mechanisms involved, effects of quercetin on the putative transcriptional activators of MCP-1, nuclear factor-kappaB (NF-kappaB) and activator protein-1 (AP-1), were examined. Exposure of the cells to IL-1beta caused activation of NF-kappaB without significant upregulation of AP-1 activity. NF-kappaB inhibitor MG132 diminished the IL-1-induced expression of MCP-1 in mesangial cells and isolated glomeruli, whereas c-Jun/AP-1 inhibitor curcumin did not affect this process. Consistently, NF-kappaB-inactive mesangial cells expressing a super-repressor mutant of IkappaBalpha showed blunted expression of MCP-1 by IL-1beta. In contrast, AP-1-inactive mesangial cells expressing a dominant-negative mutant of c-Jun exhibited the same level of MCP-1 mRNA as that in control cells. These results suggest that: (1) quercetin has the ability to attenuate activation of NF-kappaB; and (2) it inhibits IL-1-triggered MCP-1 expression via suppression of NF-kappaB, but not AP-1, in glomerular cells.

Ishizaki, C., T. Oguro, et al. (1996). “Enhancing effect of ultraviolet A on ornithine decarboxylase induction.” Dermatology 193(4): 311-317. Background: Previous studies have demonstrated appreciable tumor induction in mouse skin by daily irradiation with high-power long-wavelength ultraviolet A (UVA). Object: The aim of the present study was to examine the enhancing effects of UVA on changes in mouse skin mediated by the tumor promoter 12-o-tetradecanoylphorbol-13-acetate (TPA) by measurement of ornithine decarboxylase (ODC) activity and morphometric analysis. In addition, we examined the inhibitory effects of curcumin, a component of turmeric, on these changes. Method: ODC activity in the epidermis of CD-1 mice was determined by the method of Russell and Snyder. Epidermal and dermal thickness, and the number of dermal infiltrating inflammatory cells were quantified using a computer-assisted image analyzer. Results: A combination of topical TPA application and UVA irradiation produced a greater increment of ODC activity at 4 h than TPA alone ($p < 0.05$). Histopathologically, TPA plus UVA tended to increase the dermal infiltrating inflammatory cells in contrast to TPA alone. Pretreatment of mice with curcumin significantly abrogated the TPA-induced changes in ODC activity and the dermal infiltrating inflammatory cells as well as the TPA plus UVA-mediated enhancement of these changes. Conclusion: Our data indicate that UVA irradiation (18.72 J/cmsup 2) significantly enhances ODC induction at an early stage (4-6 h) after topical application of TPA, and aggravates the dermatitis elicited by TPA. Pretreatment with curcumin significantly inhibits these enhancing effects.

Jaga, K. and H. Duvvi (2001). “Risk reduction for DDT toxicity and carcinogenesis through dietary.” Journal of The Royal Society for the Promotion of Health 121(2): 107-113. Organochlorine pesticides, including dichlorodiphenyl-trichloroethane (DDT), are

an environmental hazard due to their persistent nature and potential health effects. DDT and 1,1,dichloro-2,2,bis(p-chlorophenyl)ethylene (DDE) are lipid-soluble pesticides which accumulate in fatty tissues and are, therefore, more present in fat-containing foods such as meat, fish, milk, cheese and oil than in fruit, vegetables and grain. Scientists have for some time been concerned about the human exposure to DDT and the potential risk of breast cancer due to its oestrogenic activity. The introduction of foods containing chemopreventive agents in the diet could inhibit the oestrogenic effects of DDT and the risk of developing cancer. Phytooestrogens are weak oestrogens found in certain plants such as soybean. They compete with DDT for oestrogen receptors and inhibit the oestrogenic effect of DDT on cultured human breast (MCF) cells. Curcumin, a spice widely used in Indian dishes, has anti-carcinogenic and anti-inflammatory properties. It also inhibits the oestrogenic effects of DDT and is synergistic with phytooestrogens. Indole-3-carbinol, a compound naturally found in cruciferous vegetables, stimulates oestrogen metabolism towards 2-hydroxyoestrone which reduces the oestrogenic response in MCF cells and the risk of breast cancer. Since DDT is lipid soluble and accumulates in adipose tissue it could have a role in lipid metabolism. Would a low fat diet reduce DDT bioaccumulation? A reduction in calories can decrease oestrogen levels and possibly reduce the risk of breast cancer. A dietary modification with the introduction of soy products, curcumin, cruciferous vegetables and low fat could be beneficial in reducing the risk of developing cancer and possibly the effects of DDT.

Jaruga, E., A. Sokal, et al. (1998). “Apoptosis-independent alterations in membrane dynamics induced by.” Experimental Cell Research 245(2): 303-312. Curcumin is a well-known natural compound with antiinflammatory properties. Its antiproliferative effect and ability to modulate apoptotic response are considered essential in cancer therapy. The physicochemical properties of curcumin suggest membranous localization, which prompted an investigation of the mechanisms of membrane disturbances evoked by curcumin. We chose the erythrocyte as a convenient model for studying membrane effects of curcumin and showed its nonspecific, apoptosis-independent way of action. Curcumin was found to expand the cell membrane, inducing echinocytosis. Changes in cell shape were accompanied by transient exposure of phosphatidylserine. Membrane asymmetry was recovered by the action of aminophospholipid translocase, which remained active in the presence of curcumin. Lipids rearrangements and drug partitioning caused changes of lipid fluidity. Such nonspecific effects of curcumin on cellular membranes would produce artifacts of apoptosis measurement, since several methods are based on membrane changes.

Jobin, C., C. A. Bradham, et al. (1999). “Curcumin blocks cytokine-mediated NF-kappaB activation and.” Journal of Immunology 163(6): 3474-3483. NF-kappaB plays a critical role in the transcriptional regulation of proinflammatory gene expression in various cells. Cytokine-mediated activation of NF-kappaB requites activation of various kinases, which ultimately leads to the phosphorylation and degradation of IkappaB, the NF-kappaB cytoplasmic inhibitor. The food derivative curcumin has been shown to inhibit NF-kappaB activity in some cell types. In this report we investigate the mechanism of action of curcumin on cytokine-induced proinflammatory gene expression using intestinal epithelial cells (IEC). Curcumin inhibited IL-1beta-mediated ICAM-1 and IL-8 gene

expression in IEC-6, HT-29, and Caco-2 cells. Cytokine-induced NF-kappaB DNA binding activity, RelA nuclear translocation, IkappaBalpha degradation, IkappaB serine 32 phosphorylation, and bob kinase (IKK) activity were blocked by curcumin treatment. Wound-induced p38 phosphorylation was not inhibited by curcumin treatment. In addition, mitogen-activated protein kinase/ERK kinase kinase-1-induced IL-8 gene expression and 12-O-tetraphorbol 12-myristate 13- acetate-responsive element-driven luciferase expression were inhibited by curcumin. However, IkappaBalpha degradation induced by ectopically expressed NF- kappaB-inducing kinase or IKK was not inhibited by curcumin treatment. Therefore, curcumin blocks a signal upstream of NF-kappaB-inducing kinase and IKK. We conclude that curcumin patently inhibits cytokine-mediated NF-kappaB activation by blocking a signal leading to IKK activity.

Joe, B. and B. R. Lokesh (1994). “Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the.” Biochimica et Biophysica Acta - Molecular Cell Research 1224(2): 255-263. Reactive oxygen species (ROS) generated by activated macrophages play an important role in the initiation of inflammation. Ten different spice principles, some of which with known anti-inflammatory properties, were tested for their effect on generation of superoxide anions, hydrogen peroxide and nitrite radical generation by activated rat peritoneal macrophages. Preincubation of macrophages with 10 muM capsaicin (from red pepper) or 10 muM curcumin (from turmeric) completely inhibited the superoxide anions, hydrogen peroxide and nitrite radical production in vitro by macrophages. Higher concentrations (500 muM) of eugenol (from clove) and piperine (from pepper) were required to completely inhibit superoxide anion and hydrogen peroxide release by macrophages. Capsaicin and curcumin were then fed to rats which were on a diet containing 8 wt% of coconut oil or olive oil or peanut oil or cod liver oil for 8 weeks, by gavage for 2 weeks. The peritoneal macrophages isolated from these animals produced lower levels of ROS compared to the macrophages from the control groups fed with the oil alone. Macrophages from cod liver oil fed animals generated lower levels of superoxide anions (76%), hydrogen peroxide (70%) and nitrite radicals (88%) compared to those isolated from coconut oil fed animals. Peanut oil and olive oil feeding also lowered the extent of ROS generation in macrophages compared to those from coconut oil fed animals. Capsaicin and curcumin feeding further lowered the generation and release of ROS. It is concluded that capsaicin or curcumin in combination with dietary fatty acids differentially lowers the production of ROS in macrophages.

Joe, B. and B. R. Lokesh (1997). “Effect of curcumin and capsaicin on arachidonic acid metabolism and.” Lipids 32(11): 1173-1180. The inflammatory mediators secreted by macrophages play an-important role in autoimmune diseases. Spice components, such as curcumin from turmeric and capsaicin from red pepper, are shown to exhibit antiinflammatory properties. The influence of these spice components on arachidonic acid metabolism and secretion of lysosomal enzymes by macrophages was investigated. Rat peritoneal macrophages preincubated with 10 muM curcumin or capsaicin for 1 h inhibited the incorporation of arachidonic acid into membrane lipids by 82 and 76%: prostaglandin Einf 2 by 45 and 48%; leukotriene Binf 4 by 61 and 46%, and leukotriene Cinf 4 by 34 and 48%, respectively, but did not affect the release of arachidonic acid from macrophages stimulated by phorbol myristate acetate. However, the secretion of 6-

keto PG F(1alpha) was enhanced by 40 and 29% from macrophages preincubated with 10 muM curcumin or capsaicin, respectively, as compared to those produced by control cells. Curcumin and capsaicin also inhibited the secretion of collagenase, elastase, and hyaluronidase to the maximum extent of 57, 61, 66%, and 46, 69, 67%, respectively. These results demonstrated that curcumin and capsaicin can control the release of inflammatory mediators such as eicosanoids and hydrolytic enzymes secreted by macrophages and thereby may exhibit antiinflammatory properties.

Joe, B. and B. R. Lokesh (1997). “Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages.” Lipids 32(11): 1173-1180. The effect of curcumin and capsaicin on arachidonic acid metabolism and secretion of lysosomal enzymes by macrophages isolated from peritoneal exudates of adult male Wistar rats weighing approximately 250 g, was investigated. Macrophages preincubated with 10 micro M curcumin or capsaicin for 1 h inhibited the incorporation of arachidonic acid into membrane lipids by 82 and 76%, prostaglandin E2 by 45 and 48%; leukotriene B4 by 61 and 46%, and leukotriene C4 by 34 and 48%, respectively, but did not affect the release of arachidonic acid from macrophages stimulated by phorbol myristate acetate. However, the secretion of 6-keto PG F1alpha was increased by 40 and 29% from macrophages preincubated with 10 micro M curcumin or capsaicin, respectively, as compared to those produced by control cells. Curcumin and capsaicin also inhibited the secretion of collagenase, elastase and hyaluronidase to the maximum extent of 57, 61 and 66%, and 46, 69 and 67%, respectively. It is concluded that curcumin and capsaicin can control the release of inflammatory mediators such as eicosanoids and hydrolytic enzymes secreted by macrophages and thereby may exhibit antiinflammatory properties.

Joe, B. and B. R. Lokesh (1997). “Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages.” Lipids 32(11): 1173-1180. The effect of curcumin and capsaicin on arachidonic acid metabolism and secretion of lysosomal enzymes by macrophages isolated from peritoneal exudates of adult male Wistar rats weighing approximately 250 g, was investigated. Macrophages preincubated with 10 micro M curcumin or capsaicin for 1 h inhibited the incorporation of arachidonic acid into membrane lipids by 82 and 76%, prostaglandin E2 by 45 and 48%; leukotriene B4 by 61 and 46%, and leukotriene C4 by 34 and 48%, respectively, but did not affect the release of arachidonic acid from macrophages stimulated by phorbol myristate acetate. However, the secretion of 6-keto PG F1alpha was increased by 40 and 29% from macrophages preincubated with 10 micro M curcumin or capsaicin, respectively, as compared to those produced by control cells. Curcumin and capsaicin also inhibited the secretion of collagenase, elastase and hyaluronidase to the maximum extent of 57, 61 and 66%, and 46, 69 and 67%, respectively. It is concluded that curcumin and capsaicin can control the release of inflammatory mediators such as eicosanoids and hydrolytic enzymes secreted by macrophages and thereby may exhibit antiinflammatory properties.

Joe, B. and B. R. Lokesh (1997). “Prophylactic and therapeutic effects of n-3 polyunsaturated fatty.” Journal of Nutritional Biochemistry 8(7): 397-407. The

prophylactic and therapeutic effects of dietary n-3 polyunsaturated fatty acids and antiinflammatory spice principlcs - curcumin and capsaicin on adjuvant induced arthritis in rats were studied Rats fed codliver oil (1 mL/day/rat or 8 wt % in the diet) rich in n-3 fatty acids were found to have a decreased incidence of adjuvant induced arthritis as compared with those observed in coconut oil- or groundnut oil-fed animals. The inflammation in animals which developed adjuvant arthritis in codliver oil-fed animals was also significantly lower than that observed in the other two groups. Additional feeding of spice principles - capsaicin (from red pepper) (5 mg/kg bw/day) or curcumin (from turmeric) (30 mg/kg bw/day) along with dietary lipids delayed the onset of the disease and also lowered the extent of inflammation in arthritic rats. In addition, feeding of the codliver oil-containing diets to rats which have already developed arthritis arrested further progression of the disease. Curcumin and capsaicin feeding to arthritic rats also lowered paw inflammation. This beneficial effect of spice principles was observed irrespective of the nature of the dietary lipids fed to the rats. These studies indicated that the dietary n-3 polyunsaturated fatty acids, capsaicin, and curcumin can decrease the incidence, delay the onset and reduce the extent of inflammation of adjuvant-induced arthritis in rats.

Joe, B. and B. R. Lokesh (1997). “Prophylactic and therapeutic effects of n-3 polyunsaturated fatty acids, capsaicin, and curcumin on adjuvant induced arthritis in rats.” Journal of Nutritional Biochemistry 8(7): 397-407. Rats fed cod liver oil (1 ml/day or 8 weight% in the diet) had a decreased incidence of adjuvant induced arthritis compared with coconut oil- or groundnut oil-fed rats. Inflammation in arthritic cod liver oil-fed rats was significantly lower than that observed in the other 2 groups. Capsaicin (from red pepper) (5 mg/kg body weight daily) or curcumin (from turmeric) (30 mg/kg body weight daily) delayed the onset of arthritis and reduced the extent of inflammation. In addition, feeding cod liver oil-containing diets to rats which have already developed arthritis arrested further progression of the disease. Curcumin and capsaicin feeding to arthritic rats also lowered paw inflammation. The beneficial effects of curcumin and capsaicin were observed irrespective of the nature of the dietary lipids fed to the rats. It is concluded that n-3 polyunsaturated fatty acids, capsaicin and curcumin can decrease the incidence, delay the onset and reduce the extent of inflammation of adjuvant-induced arthritis in rats.

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concluded that n-3 polyunsaturated fatty acids, capsaicin and curcumin can decrease the incidence, delay the onset and reduce the extent of inflammation of adjuvant-induced arthritis in rats.

Joe, B. and B. R. Lokesh (2000). “Dietary n-3 fatty acids, curcumin and capsaicin lower the release of.” Molecular and Cellular Biochemistry 203(1-2): 153-161. Male Wistar rats (12 rats/group) were fed a diet containing 8 wt % coconut oil or groundnut oil or cod-liver oil for a total period of 8 weeks. The diets were also supplemented with 2 wt % groundnut oil for providing essential fatty acids. During the last 2 weeks, 6 rats form each group were additionally given curcumin (30 mg/kg body wt/day) or capsaicin (5 mg/kg body wt/day) in 1 ml groundnut oil. The peritoneal macrophages from rats fed cod- liver oil diet secreted lower levels of lysosomal enzymes collagenase, elastase and hyaluronidase as compared to those from rats fed coconut oil or groundnut oil diets. Curcumin and capsaicin significantly lowered the secretion of these lysosomal enzymes from macrophages in animals given coconut oil or groundnut oil diet. Macrophages from rats fed cod-liver oil secreted lower amounts of prostaglandin Einf 2, 6-keto PGF(1alpha), leukotrienes Binf 4 and Cinf 4 and also incorporated lesser amounts of [sup 3H]-arachidonic acid as compared to those given coconut oil or groundnut oil diets. Curcumin and capsaicin lowered the secretion of these eicosanoids and decreased the incorporation of [sup 3H]-arachidonic acid in macrophage lipids. However curcumin and capsaicin significantly increased the secretion of 6-keto PGF(1alpha) in all the groups of animals. These studies indicated that dietary cod-liver oil (rich in n-3 fatty acids), and spice principles curcumin and capsaicin can lower the secretory functions of macrophages in a beneficial manner.

Jones, E. A., A. Shahed, et al. (2000). “Modulation of apoptotic and inflammatory genes by bioflavonoids and.” Urology 56(2): 346-351. Objectives. Ureteral obstruction results in an injury response that can progress to irreversible renal fibrosis and tubular atrophy by apoptosis. The molecular events leading to apoptosis from obstruction are not well understood. We investigated the effect of bioflavonoids and angiotensin II inhibition on apoptotic and inflammatory gene expression in a model of unilateral ureteral obstruction (UUO). Methods. Complete UUO was produced in rats by ureteral ligation. The rats were treated with dimethyl sulfoxide (control), enalapril, losartan, curcumin, or quercetin. The animals were killed on day 7 and both obstructed and contralateral unobstructed kidneys were harvested. Expression of the inflammatory chemokine monocyte chemotactic protein-1, apoptosis effector genes Fas and Fas ligand, and oxidative stress gene HO-1 was evaluated by reverse transcriptase-polymerase chain reaction. Results. Ureteral obstruction was associated with a 6.3-fold increase in monocyte chemotactic protein-1 expression compared with sham-operated rats ($P = 0.01$). Monocyte chemotactic protein-1 expression was severely attenuated in all other treatment groups ($P < 0.05$). Similarly, Fas and Fas ligand expression were increased in control UUO kidneys compared with sham-operated ones ($P < 0.05$). Fas gene expression was significantly inhibited by quercetin but not enalapril, losartan, or curcumin compared with the control. The induction of Fas ligand was attenuated in all treatment groups ($P < 0.05$). HO-1 was expressed at low levels in both unobstructed and obstructed kidneys. Treatment with curcumin increased HO-1 expression fourfold ($P < 0.05$). Conclusions. The expression of

apoptotic and chemokine genes is significantly upregulated in UUO. Bioflavonoids and angiotensin inhibitors are able to attenuate the expression of these genes and thus may be beneficial in renal protection. Copyright (C) 2000 Elsevier Science Inc.

Kang, B. Y., Y. J. Song, et al. (1999). "Curcumin inhibits Th1 cytokine profile in CD4+ T cells by suppressing interleukin-12 production in macrophages." British Journal of Pharmacology 128(2): 380-384. The effects of curcumin, a natural product of plants obtained from Curcuma longa (turmeric), on interleukin-12 (IL-12) production by mouse splenic macrophages and the subsequent ability of these cells to regulate cytokine production by CD4+ T cells was evaluated. Pretreatment with curcumin significantly inhibited IL-12 production by macrophages stimulated with lipopolysaccharide (LPS) or head- killed Listeria monocytogenes (HKL). Curcumin-pretreated macrophages reduced their ability to induce interferon-gamma (IFN-gamma) and increased the ability to induce IL-4 in Ag-primed CD4+ T cells. Addition of recombinant IL-12 to cultures of curcumin-pretreated macrophages and CD4+ T cells restored IFN-gamma production in CD4+ T cells. The in vivo administration of curcumin inhibited IL-12 production by macrophages stimulated in vitro with LPS or HKL, leading to the inhibition of Th1 cytokine profile (decreased IFN- gamma and increased IL-4 production) in CD4+ T cells. It is concluded that curcumin may inhibit Th1 cytokine profile in CD4+ T cells by suppressing IL-12 production in macrophages, pointing to a possible therapeutic use of curcumin in the Th1-mediated immune diseases.

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Kang, B., S. Chung, et al. (1999). "Inhibition of interleukin-12 production in lipopolysaccharide- activated macrophages by curcumin." European Journal of Pharmacology 384(2/3): 191-195. Pharmacological control of interleukin-12 production may be a key therapeutic strategy for modulating immunological diseases dominated by type-1 cytokine responses. In this study we investigated the effects of curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6- heptadiene-3,5-dione) on the production of

interleukin-12 from mouse macrophages stimulated with lipopolysaccharide. Curcumin potently inhibited the production of interleukin-12 in a dose-dependent manner. The effect of curcumin on interleukin-12 p40 promoter activation was analysed by transfecting RAW264.7 monocytic cells with p40 promoter/reporter constructs. The repressive effect mapped to a region in the p40 promoter containing a binding site for nuclear factor kappaB (p40-kappaB). Furthermore, activation of macrophages by lipopolysaccharide resulted in markedly enhanced binding activity to the kappaB site, which significantly decreased upon addition of curcumin. These results suggest that curcumin-induced inhibition of interleukin-12 production in macrophages may explain some of the biological effects of curcumin including its antiinflammatory activity.

Kang, B., S. Chung, et al. (1999). “Inhibition of interleukin-12 production in lipopolysaccharide- activated macrophages by curcumin.” European Journal of Pharmacology 384(2/3): 191-195. Pharmacological control of interleukin-12 production may be a key therapeutic strategy for modulating immunological diseases dominated by type-1 cytokine responses. In this study we investigated the effects of curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6- heptadiene-3,5-dione) on the production of interleukin-12 from mouse macrophages stimulated with lipopolysaccharide. Curcumin potently inhibited the production of interleukin-12 in a dose-dependent manner. The effect of curcumin on interleukin-12 p40 promoter activation was analysed by transfecting RAW264.7 monocytic cells with p40 promoter/reporter constructs. The repressive effect mapped to a region in the p40 promoter containing a binding site for nuclear factor kappaB (p40-kappaB). Furthermore, activation of macrophages by lipopolysaccharide resulted in markedly enhanced binding activity to the kappaB site, which significantly decreased upon addition of curcumin. These results suggest that curcumin-induced inhibition of interleukin-12 production in macrophages may explain some of the biological effects of curcumin including its antiinflammatory activity.

Kawamori, T., R. Lubet, et al. (1999). “Chemopreventive effect of curcumin, a naturally occurring anti.” Cancer Research 59(3): 597-601. Curcumin, derived from the rhizome of Curcuma longa L. and having both antioxidant and anti-inflammatory properties, inhibits chemically induced carcinogenesis in the skin, forestomach, and colon when it is administered during initiation and/or postinitiation stages. This study was designed to investigate the chemopreventive action of curcumin when it is administered (late in the premalignant stage) during the promotion/progression stage of colon carcinogenesis in male F344 rats. We also studied the modulating effect of this agent on apoptosis in the tumors. At 5 weeks of age, groups of male F344 rats were fed a control diet containing no curcumin and an experimental AIN-76A diet with 0.2% synthetically derived curcumin (purity, 99.9%). At 7 and 8 weeks of age, rats intended for carcinogen treatment were given s.c. injections of azoxymethane (AOM) at a dose rate of 15 mg/kg body weight per week. Animals destined for the promotion/progression study received the AIN- 76A control diet for 14 weeks after the second AOM treatment and were then switched to diets containing 0.2 and 0.6% curcumin. Premalignant lesions in the colon would have developed by week 14 following AOM treatment. They continued to receive their respective diets until 52 weeks after carcinogen treatment and were then sacrificed. The results confirmed our earlier study in that administration of 0.2% curcumin during both

the initiation and postinitiation periods significantly inhibited colon tumorigenesis. In addition, administration of 0.2% and of 0.6% of the synthetic curcumin in the diet during the promotion/progression stage significantly suppressed the incidence and multiplicity of noninvasive adenocarcinomas and also strongly inhibited the multiplicity of invasive adenocarcinomas of the colon. The inhibition of adenocarcinomas of the colon was, in fact, dose dependent. Administration of curcumin to the rats during the initiation and postinitiation stages and throughout the promotion/progression stage increased apoptosis in the colon tumors as compared to colon tumors in the groups receiving AOM and the control diet. Thus, chemopreventive activity of curcumin is observed when it is administered prior to, during, and after carcinogen treatment as well as when it is given only during the promotion/progression phase (starting late in premalignant stage) of colon carcinogenesis.

Kelloff, G. J., C. C. Sigman, et al. (1999). “Cancer chemopreventionprogress and promise.” European Journal of Cancer 35(14): 2031-2038. Cancer chemoprevention is the use of agents to inhibit, delay or reverse carcinogenesis. The focus of chemoprevention research in the next millennium will include defining the genotypic and phenotypic (functional and histological) changes during carcinogenesis, the cancer risk conferred by these changes, their modulation in preclinical experimentation and randomised clinical trials by chemopreventive drugs, dietary agents and regimens and treatments resulting from early detection. The key elements of this research effort will be basic and translational risk evaluation programmes; chemopreventive and dietary agent drug discovery and development; development of transgenic animal models; required safety and pharmacology studies; well-designed phase I, II and III chemoprevention studies; and much expanded early detection programmes. The large number of chemoprevention research programmes now ongoing ensures that the promise of chemoprevention will continue to be realised in the next decade. Copyright (C) 1999 Elsevier Science Ltd.

Kelloff, G. J., C. W. Boone, et al. (1994). “Chemopreventive drug development: Perspectives and progress.” Cancer Epidemiology Biomarkers and Prevention 3(1): 85-98. Chemoprevention drug development has the goal of identifying safe and effective chemopreventive agents for clinical use. Several distinctive strategies are pursued in developing chemopreventive agents: (a) identifying and validating predysplastic and early dysplastic lesions that can be used instead of cancers as endpoints for measuring chemopreventive activity; (b) identifying and testing candidate agents based on considerations of mechanisms of action; (c) evaluating combinations of agents with potential for maximizing efficacy and minimizing toxicity; and (d) applying a systematic methodology for identifying and ranking candidate agents at each stage of development to ensure discovery of the best agents and most effective use of available resources. This article discusses 22 drugs and three drug combinations which have reached an advanced stage of development as chemopreventive agents. The first generation of drugs are the most advanced, now being in Phase II and Phase III clinical trials. These drugs include several retinoids (vitamin A, 13-cis-retinoic acid, all-trans-N-(4-hydroxyphenyl)retinamide), calcium, beta-carotene, tamoxifen, and finasteride. The second generation drugs are those in Phase I clinical trials. From most to least advanced,

these drugs are 2-difluoromethylornithine, sulindac, piroxicam, oltipraz, N-acetyl-l-cysteine, aspirin, ibuprofen, carbenoxolone, 18beta-glycyrrhetinic acid, and the combination of 2-difluoromethylornithine with piroxicam. The third generation includes agents with significant evidence of chemopreventive activity in animal models. These agents are now in preclinical toxicity testing. They are S-allyl-l-cysteine, phenhexyl isothiocyanate, curcumin, ellagic acid, fumaric acid, fluasterone, and the combinations of all-trans-N-(4-hydroxyphenyl)retinamide with oltipraz and all-trans-N-(4-hydroxyphenyl) retinamide with tamoxifen.

Kelloff, G. J., C. W. Boone, et al. (1996). "New agents for cancer chemoprevention." Journal of Cellular Biochemistry 63(SUPPL. 26): 1-28. Clinical chemoprevention trials of more than 30 agents and agent combinations are now in progress or being planned. The most advanced agents are well known and are in large Phase III chemoprevention intervention trials or epidemiological studies. These drugs include several retinoids (e.g., retinol, retinyl palmitate, all-trans-retinoic acid, and 13-cis-retinoic acid), calcium, betacarotene, vitamin E, tamoxifen, and finasteride. Other newer agents are currently being evaluated in or being considered for Phase II and early Phase III chemoprevention trials. Prominent in this group are all-trans-N-(4-hydroxy phenyl)retinamide (4-HPR) (alone and in combination with tamoxifen), 2-difluoromethylomithine (DFMO), nonsteroidal antiinflammatory drugs (aspirin, piroxicam, sulindac), oltipraz, and dehydroepiandrostenedione (DHEA). A third group is new agents showing chemopreventive activity in animal models, epidemiological studies, or in pilot clinical intervention studies. They are now in preclinical toxicology testing or Phase I safety and pharmacokinetics trials preparatory to chemoprevention efficacy trials. These agents include S-allyl-l-cysteine, curcumin, DHEA analog 8354 (fluasterone), genistein, ibuprofen, indole-3- carbinol, perillyl alcohol, phenethyl isothiocyanate, 9-cis-retinoic acid, sulindac sulfone, tea extracts, ursodiol, vitamin D analogs, and p-xylyl selenocyanate. A new generation of agents and agent combinations will soon enter clinical chemoprevention studies based primarily on promising chemopreventive activity in animal models and in mechanistic studies. Among these agents are more efficacious analogs of known chemopreventive drugs including novel carotenoids (e.g., alpha-carotene and lutein). Also included are safer analogs which retain the chemopreventive efficacy of the parent drug such as vitamin Dinf 3 analogs. Other agents of high interest are aromatase inhibitors (e.g., (+)-vorozole), and protease inhibitors (e.g., Bowman-Birk soybean trypsin inhibitor). Combinations are also being considered, such as vitamin E with l-selenomethionine. Analysis of signal transduction pathways is beginning to yield classes of potentially active and selective chemopreventive drugs. Examples are ras isoprenylation and epidermal growth factor receptor inhibitors.

Kelloff, G. J., J. A. Crowell, et al. (1996). "Clinical development plans for cancer chemopreventive agents." Journal of Cellular Biochemistry 63(SUPPL. 26): 72-315.

Kelloff, G. J., J. A. Crowell, et al. (1999). "Progress in cancer chemoprevention." Annals of the New York Academy of Sciences 889(-): 1-13. More than 40 promising agents and agent combinations are being evaluated clinically as chemopreventive drugs for major cancer targets. A few have been in vanguard, large-scale intervention trials - for example,

the studies of tamoxifen and fenretinide in breast, 13-cis-retinoic acid in head and neck, vitamin E and selenium in prostate, and calcium in colon. These and other agents are currently in phase II chemoprevention trials to establish the scope of their chemopreventive efficacy and to develop intermediate biomarkers as surrogate end points for cancer incidence in future studies. In this group are fenretinide, 2-difluoromethylornithine, and oltipraz. Nonsteroidal anti-inflammatories (NSAID) are also in this group because of their colon cancer chemopreventive effects in clinical intervention, epidemiological, and animal studies. New agents are continually considered for development as chemopreventive drugs. Preventive strategies with antiandrogens are evolving for prostate cancer. Anti-inflammatories that selectively inhibit inducible cyclooxygenase (COX)-2 are being investigated in colon as alternatives to the NSAID, which inhibit both COX-1 and COX-2 and derive their toxicity from COX-1 inhibition. Newer retinoids with reduced toxicity, increased efficacy, or both (e.g., 9-cis-retinoic acid) are being investigated. Promising chemopreventive drugs are also being developed from dietary substances (e.g., green and black tea polyphenols, soy isoflavones, curcumin, phenethyl isothiocyanate, sulforaphane, lycopene, indole-3-carbinol, perillyl alcohol). Basic and translational research necessary to progress in chemopreventive agent development includes, for example, (1) molecular and genomic biomarkers that can be used for risk assessment and as surrogate end points in clinical studies, (2) animal carcinogenesis models that mimic human disease (including transgenic and gene knockout mice), and (3) novel agent treatment regimens (e.g., local delivery to cancer targets, agent combinations, and pharmacodynamically guided dosing).

Key, N. S. and R. R. Bach (2001). “Tissue factor as a therapeutic target.” Thrombosis and Haemostasis 85(3): 375-376.

Khanna, N. M. (1999). “Turmeric - nature's precious gift.” Current Science 76(10): 1351-1356. Turmeric (Curcuma longa) is a well-known indigenous herbal medicine. Its constituents and their biological activities are reviewed. The major constituents, such as curcumin, various curcuminoids, curcuma oil (particularly DL-ar-turmerone) exhibit a wide range of biological activities: antibacterial, antiinflammatory, hypolipaemic, hepatoprotective, and enzyme (lipoxygenase, cyclooxygenase, protease and lipid peroxidase) inhibitory activities, and are effective active oxygen species scavengers.

Khanna, N. M. (1999). “Turmeric - nature's precious gift.” Current Science 76(10): 1351-1356. Turmeric (Curcuma longa) is a well-known indigenous herbal medicine. Its constituents and their biological activities are reviewed. The major constituents, such as curcumin, various curcuminoids, curcuma oil (particularly DL-ar-turmerone) exhibit a wide range of biological activities: antibacterial, antiinflammatory, hypolipaemic, hepatoprotective, and enzyme (lipoxygenase, cyclooxygenase, protease and lipid peroxidase) inhibitory activities, and are effective active oxygen species scavengers.

Kim, E. S., W. K. Hong, et al. (2002). “Chemoprevention of aerodigestive tract cancers.” Annual Review of Medicine 53(-): 223-243. Epithelial cancers are a major worldwide health problem. Since the mid-1970s, advances in multidisciplinary cancer therapeutics have only slightly improved the mortality rate from epithelial malignancies.

Chemoprevention is the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent progression to invasive cancer. Chemopreventive medicine is based on translating basic biologic research into clinical chemical interventions, thus attempting to impede carcinogenesis. Its principles build on the concepts of field cancerization (diffuse epithelial injury that results from carcinogen exposure) and multistep carcinogenesis (a stepwise accumulation of cellular and genetic alterations that progress to cancer). Chemoprevention targets the carcinogenic process at earlier and potentially more reversible stages, focusing on the inhibition of one or many steps in the progression towards cancer. Strategies of chemoprevention include primary prevention in groups at high risk, reversal of premalignant lesions, and prevention of second primary tumors.

Kolars, J. C. and C. L. Kurth (1998). "Influence of diet, vitamins and chemotherapeutic agents on." Journal of Gastroenterology and Hepatology 13(SUPPL. NOV.): S173-S177. Dietary influences play a major role in the pathogenesis of most gastrointestinal malignancies. However, it has been difficult to define which dietary components will be most significant for any given individual. In this article we discuss the methodological challenges to research in this field as well as recent observations that have been made on the role of dietary factors in specific digestive tract neoplasms.

Krishnan, K. and D. E. Brenner (1996). "Chemoprevention of colorectal cancer." Gastroenterology Clinics of North America 25(4): 821-858. This review summarizes the principles of cancer chemoprevention and discusses the evidence from epidemiologic and experimental studies and preclinical and clinical trials of potential colorectal chemopreventive agents. The putative mechanisms of action of the drugs in chemoprevention and their potential to reduce the incidence and mortality rate of colorectal neoplasms are discussed. The future of colorectal chemoprevention will depend on important new insights into molecular carcinogenesis of colorectal cancer, application of molecular markers as surrogate endpoints, and ultimately on therapeutic targets of prevention in clinical trials.

Krishnan, K. and D. E. Brenner (1997). "Nonsteroidal anti-inflammatory drugs (NSAIDS) in colorectal cancer." Cancer Journal 10(1): 10-16. Colorectal carcinoma is an important, feasible and attractive target for chemoprevention because a) it is a major cause of mortality in the United States and in other developed countries worldwide, b) there is a high mortality associated with advanced disease, c) there is a well described molecular carcinogenesis pathway and d) recent advances in molecular genetics will improve the ability to identify high-risk subjects. Epidemiological data, colonoscopic screening and advances in molecular genetics has made possible the identification and selection of subjects at increased risk of developing colorectal cancer. Due to this new information it may be possible to impede malignant cellular transformation with drugs. Such intervention with relatively simple maneuvers, such as a low daily dose of aspirin, can potentially reduce mortality from colorectal cancer. Prospective trials need to confirm experimental and epidemiological data supporting the efficacy of aspirin and other NSAID as chemopreventive agents before they can be used in the general population at risk. To use cancer chemopreventives effectively and safely in an asymptomatic population, the risks should be minimized and the benefits maximized by determination

of optimal dose, schedule and chemopreventive mechanism of the NSAID. By linking the putative mechanism of drug action to effect endpoints, we expect to know whether the chemopreventive intervention is likely to be effective in a given individual.

Krishnan, K., I. M. T. Ruffin, et al. (1998). “Cancer chemoprevention: A new way to treat cancer before it happens.” Primary Care - Clinics in Office Practice 25(2): 361-379. Cancer chemoprevention uses noncytotoxic drugs or nutrients to prevent, retard, or delay carcinogenesis. The future of cancer chemoprevention depends on understanding key cellular growth and proliferation-controlling events, developing markers of molecular carcinogenesis, surrogate endpoint biomarkers, and targeted chemopreventive approaches.

Krishnan, K., I. M. T. Ruffin, et al. (1998). “Clinical models of chemoprevention for colon cancer.” Hematology/Oncology Clinics of North America 12(5): 1079-1113. Colon cancer is a common malignancy in the westernized world and is incurable in its advanced stages. This article summarizes the currently available information on colorectal cancer chemoprevention. A brief outline of the incidence and etiologic factors is followed by a discussion of the evidence on which chemopreventive strategies for colon cancer are modeled. This includes a description of the development of surrogate endpoint biomarkers and experimental models to study colorectal cancer chemopreventives, a review of the promising colorectal cancer chemopreventives, and a discussion of the issues to be addressed in the design of future chemoprevention trials. The article concludes with an emphasis on the development and validation of biomarkers and selection of high-risk cohorts using genetic and epidemiologic tools as the main goals of future colon cancer chemoprevention trials before large-scale, risk-reduction trials are conducted.

Kumar, V., S. A. Lewis, et al. (2002). “Biodegradable microspheres of curcumin for treatment of inflammation.” Indian Journal of Physiology and Pharmacology 46(2): 209-217. Curcumin, a natural constituent of Curcuma longa (turmeric, CAS 458-37-7) was formulated as prolonged release biodegradable microspheres for treatment of inflammation. Natural biodegradable polymers, namely, bovine serum albumin and chitosan were used to encapsulate curcumin to form a depot forming drug delivery system. Microspheres were prepared by emulsion-solvent evaporation method coupled with chemical cross-linking of the natural polymers. Curcumin could be encapsulated into the biodegradable carriers upto an extent of 79.49 and 39.66% respectively with albumin and chitosan. Different drug:polymer ratios did not affect the mean particle size or particle size distribution significantly. However, the concentration of the crosslinking agent had remarkable influence on the drug release. In-vitro release studies indicated a biphasic drug release pattern, characterized by a typical burst-effect followed by a slow release which continued for several days. Evaluation of antiinflammatory activity using Freund's adjuvant induced arthritic model in Wistar rats revealed significant difference between both the formulations, albumin microspheres and chitosan microspheres as well as against control. It was evident from the present study that the curcumin biodegradable microspheres could be successfully employed as prolonged release drug delivery system

for better therapeutic management of inflammation as compared to oral or subcutaneous route.

Kushwah, A., M. K. P. Amma, et al. (1978). “Effect of some anti-inflammatory agents on lysosomal & testicular.” Indian Journal of Experimental Biology 16(2): 222-224.

Lal, B., A. K. Kapoor, et al. (2000). “Role of curcumin in idiopathic inflammatory orbital pseudotumours.” Phytotherapy Research 14(6): 443-447. The present report, describes for the first time the clinical efficacy of curcumin, the active constituent of rhizomes of Curcuma longa, in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumours. Curcumin was administered orally at a dose of 375 mg/3 times/day orally for a period of 6-22 months in eight patients. They were followed up for a period of 2 years at 3 monthly intervals. Five patients completed the study, out of which four recovered completely and in one patient the swelling regressed completely but some limitation of movement persisted. No side effect was noted in any patient and there was no recurrence. It is suggested that curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours. (C) 2000 John Wiley and Sons, Ltd.

Lal, B., A. K. Kapoor, et al. (2000). “Role of curcumin in idiopathic inflammatory orbital pseudotumours.” Phytotherapy Research 14(6): 443-447. The present report describes for the first time the clinical efficacy of curcumin, the active constituent of rhizomes of Curcuma longa, in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumours. Curcumin was administered orally at a dose of 375 mg 3 times a day for a period of 6-22 months in 8 patients (5 males and 3 females; age = 6-54 years) from Uttar Pradesh, India. All patients were followed up for a period of 2 years at 3-monthly intervals. Five patients completed the study, out of which 4 recovered completely and in one patient the swelling regressed completely but some limitation of movement persisted. Response to curcumin was faster in younger than in aged patients. No side effect was noted in any patient and there was no recurrence. It is suggested that curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours.

Lal, B., A. K. Kapoor, et al. (2000). “Role of curcumin in idiopathic inflammatory orbital pseudotumours.” Phytotherapy Research 14(6): 443-447. The present report describes for the first time the clinical efficacy of curcumin, the active constituent of rhizomes of Curcuma longa, in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumours. Curcumin was administered orally at a dose of 375 mg 3 times a day for a period of 6-22 months in 8 patients (5 males and 3 females; age = 6-54 years) from Uttar Pradesh, India. All patients were followed up for a period of 2 years at 3-monthly intervals. Five patients completed the study, out of which 4 recovered completely and in one patient the swelling regressed completely but some limitation of movement persisted. Response to curcumin was faster in younger than in aged patients. No side effect was noted in any patient and there was no recurrence. It is suggested that curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours.

Leu, T. H. and M. C. Maa (2002). “The molecular mechanisms for the antitumorigenic effect of curcumin.” Current Medicinal Chemistry - Anti-Cancer Agents 2(3): 357-370. Curcumin, an active yellow pigment of turmeric and curry, possesses anti-inflammatory, antioxidative and anticarcinogenic properties. Analysis of its structure revealed the presence of beta-diketone moiety and phenolic hydroxy groups that were believed to contribute to antioxidation. And vanillin, ferulic acid and a dimer of curcumin were identified as the curcumin-derived radical reaction products. In addition to antioxidation, curcumin could also induce apoptosis by targeting mitochondria, affecting p53-related signaling and blocking NF-kappaB activation. To further dissect its anticarcinogenic mechanisms, a number of curcumin targets were identified. These included the aryl hydrocarbon receptor, cytochrome P450, glutathione S-transferase, serine/threonine kinases, transcription factors, cyclooxygenase, ornithine decarboxylase, nitric oxide synthase, matrix metalloproteinases and tyrosine kinases. This review will summarize our current knowledge on how these important proteins are affected by curcumin, and hopefully, may provide a whole picture illustrating how the chemopreventive and antitumorigenic effect of curcumin is achieved.

Levi, M. S., R. F. Borne, et al. (2001). “A review of cancer chemopreventive agents.” Current Medicinal Chemistry 8(11): 1349-1362. In the late 20th century, the treatment of cancer began to include its prevention. Today, compounds exist that will lower the risk of developing certain types of cancer. This has been demonstrated in studies where chemically induced tumor growth has been slowed or reversed. Anti-inflammatory compounds having chemopreventive activity are piroxicam, sulindac, aspirin, celecoxib and curcumin. The selective estrogen receptor modulators, tamoxifen and raloxifene, are beneficial in the prevention of estrogendependent tumors. Retinoids, vitamin A derivatives, such as targretin and fenretinide are useful in the prevention of tumors. Compounds containing sulfur, such as sulforaphane and oltipraz, are even useful as radioprotective agents. The steroid dehydroepiandosterone can inhibit experimental carcinogenesis. All of these chemical classes provide a start for the medicinal chemist to design more effective chemopreventive agents. The biomarkers used to determine the chemopreventive activity of new compounds are quite often activities of enzymes. The identification of those individuals at high risk is still in its infancy and presents a troubling dilemma.

Lim, G. P., T. Chu, et al. (2001). “The curry spice curcumin reduces oxidative damage and amyloid pathology.” Journal of Neuroscience 21(21): 8370-8377. Inflammation in Alzheimer's disease (AD) patients is characterized by increased cytokines and activated microglia. Epidemiological studies suggest reduced AD risk associates with long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs). Whereas chronic ibuprofen suppressed inflammation and plaque-related pathology in an Alzheimer transgenic APPSw mouse model (Tg2576), excessive use of NSAIDs targeting cyclooxygenase I can cause gastrointestinal, liver, and renal toxicity. One alternative NSAID is curcumin, derived from the curry spice turmeric. Curcumin has an extensive history as a food additive and herbal medicine in India and is also a potent polyphenolic antioxidant. To evaluate whether it could affect Alzheimer-like pathology in the APPSw mice, we tested

a low (160 ppm) and a high dose of dietary curcumin (5000 ppm) on inflammation, oxidative damage, and plaque pathology. Low and high doses of curcumin significantly lowered oxidized proteins and interleukin-1beta, a proinflammatory cytokine elevated in the brains of these mice. With low-dose but not high-dose curcumin treatment, the astrocytic marker GFAP was reduced, and insoluble beta-amyloid (Abeta), soluble Abeta, and plaque burden were significantly decreased by 43-50%. However, levels of amyloid precursor (APP) in the membrane fraction were not reduced. Microgliosis was also suppressed in neuronal layers but not adjacent to plaques. In view of its efficacy and apparent low toxicity, this Indian spice component shows promise for the prevention of Alzheimer's disease.

Lin, J. K., M. H. Pan, et al. (2000). “Recent studies on the biofunctions and biotransformations of curcumin.” BioFactors 13(1-4): 153-158. Curcumin is a major component of Curcuma species, which is commonly used as a yellow coloring and flavoring agent in foods. Curcumin has shown anti-carcinogenic activity in animals as indicated by its ability to block colon tumor initiation by azoxymethane and skin tumor promotion induced by phorbol ester TPA. Curcumin possesses anti-inflammatory activity and is a potent inhibitor of reactive oxygen-generating enzymes such as lipoxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase and inducible nitric oxide synthase. Curcumin is also a potent inhibitor of protein kinase C, EGF-receptor tyrosine kinase and IkappaB kinase. Subsequently, curcumin inhibits the activation of NFkappaB and the expressions of c-jun, c-fos, c-myc and iNOS. It is proposed that curcumin may suppress tumor promotion through blocking signal transduction pathways in the target cells. Curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and that these compounds subsequently were converted to monoglucuronide conjugates. These results suggest that curcumin-glucuronide, dihydro-curcumin-glucuronide, tetrahydrocurcumin-glucuronide and tetrahydrocurcumin are major metabolites of curcumin in mice.

Lippman, S. M., S. E. Benner, et al. (1994). “Cancer chemoprevention.” Journal of Clinical Oncology 12(4): 851-873. Purpose: To review the most important recent advances in clinical trials and biologic studies within the growing field of chemoprevention. Methods: The most critical methods issue concerns the definitive end point of phase III trials, which is now cancer incidence. This end point usually needs thousands of subjects monitored for 5 to 10 or more years to determine efficacy. Biologic markers of potential intermediate end points are under intensive study and may one day replace cancer incidence. Validated intermediate end point biomarkers could greatly reduce phase III trial populations, durations, and costs. Results: Randomized clinical trials over the last 5 years have produced significant activity in reversing oral, skin, colon, and cervical premalignancy; in preventing primary skin and stomach cancer; and in preventing second primary tumors associated with head and neck and lung cancer. These clinical advances have been paralleled at the basic science level by elegant molecular studies of premalignant carcinogenesis and of chemopreventive agents' mechanisms of action. One major laboratory advance is the discovery of nuclear retinoic acid receptors and strong evidence of their roles both in carcinogenic progression and in its response to retinoids. Conclusion: Chemoprevention has matured greatly in recent

years with the significant reversal or suppression of premalignancy by chemopreventive agents in several sites. The future of chemoprevention will be determined largely by several ongoing phase III trials, including trials of retinoids, beta-carotene, and alpha-tocopherol in the aerodigestive tract, of tamoxifen and fenretinide in the breast, and of finasteride in the prostate.

Literat, A., F. Su, et al. (2001). “Regulation of pro-inflammatory cytokine expression by curcumin in.” Life Sciences 70(3): 253-267. Persistent expression of pro-inflammatory cytokines is believed to play a major role in the pathogenesis of chronic lung disease (CLD) in premature infants. Inhibition of pro-inflammatory cytokine production in the lungs of preterm newborns may result in the attenuation of CLD. Curcumin is a naturally occurring phenolic compound derived from the food spice tumeric with broad based in vitro anti-inflammatory properties. In this study lung inflammatory cells from preterm newborns at risk for the development of CLD were derived via modified broncho-alveolar lavage and stimulated ex vivo with lipopolysaccharide (LPS) (10ng/ml). Curcumin was added to these cultures at 0, 0.5 and 20 uM concentrations. Pro-inflammatory cytokine, TNFalpha, IL-1beta and IL-8 protein was measured from the culture supernatants 12 hours post culture. For control, adult peripheral blood mononuclear cells (PBMC) were cultured under the same conditions. Both neonatal lung inflammatory cells and adult PBMC produced high levels of pro-inflammatory cytokines in response to LPS. Curcumin produced significant inhibition of IL-1beta and IL-8 but minimal inhibition of TNFalpha expression by preterm lung inflammatory cells at 20 uM concentrations. Adult PBMC expression of IL-8 was significantly inhibited by curcumin at 20 uM concentrations. Therefore, curcumin inhibits pro-inflammatory cytokine production (TNFalpha, IL-1beta and IL-8) by lung inflammatory cells ex vivo. Pathways involved with curcumin regulation of these cytokines are developmentally intact and functional in premature infants. Curcumin may be effective as a therapeutic agent in the attenuation of CLD. (c) 2001 Elsevier Science Inc. All rights reservsed.

Liu, Y. (1997). Curcumin: an ingredient that reduces platelet aggregation and hyperlipidemia, and enhances antioxidant and immune functions. Washington: American Chemical Society, USA, American Chemical Society. Curcumin is isolated from the plant Curcuma longa which is a medicinal plant widely used in China. It has been shown to have anti- inflammatory and antioxidant properties. The effects of curcumin and flavone of Matricaria chamomilla [Chamomilla recutita] on blood platelet aggregation, hyperlipaemia, lipid peroxidation and immune function were studied in rats. Curcumin reduced platelet aggregation and the effect was enhanced in the presence of M. chamomilla flavone. Blood cholesterol levels of rats fed 1% cholesterol was elevated 284%. Supplementation with curcumin and curcumin plus M. chamomilla flavone to the cholesterol diet depressed this elevation significantly. Total lipid levels, free and total cholesterol and triacylglycerol levels in the liver decreased significantly when curcumin and M. chamomilla flavone were added to the hypercholesterolaemic diet, but free cholesterol levels in the liver were not affected. Curcumin increased immune function in cyclophosphane-induced immunosuppressed rats.

Liu, Y. (1997). Curcumin: an ingredient that reduces platelet aggregation and hyperlipidemia, and enhances antioxidant and immune functions. Washington: American Chemical Society, USA, American Chemical Society. Curcumin is isolated from the plant Curcuma longa which is a medicinal plant widely used in China. It has been shown to have anti- inflammatory and antioxidant properties. The effects of curcumin and flavone of Matricaria chamomilla [Chamomilla recutita] on blood platelet aggregation, hyperlipaemia, lipid peroxidation and immune function were studied in rats. Curcumin reduced platelet aggregation and the effect was enhanced in the presence of M. chamomilla flavone. Blood cholesterol levels of rats fed 1% cholesterol was elevated 284%. Supplementation with curcumin and curcumin plus M. chamomilla flavone to the cholesterol diet depressed this elevation significantly. Total lipid levels, free and total cholesterol and triacylglycerol levels in the liver decreased significantly when curcumin and M. chamomilla flavone were added to the hypercholesterolaemic diet, but free cholesterol levels in the liver were not affected. Curcumin increased immune function in cyclophosphane-induced immunosuppressed rats.

Luft, F. C. (2002). “Proinflammatory effects of angiotensin II and endothelin: Targets for.” Current Opinion in Nephrology and Hypertension 11(1): 59-66. Angiotensin II and endothelin-1 can both be regulated by nuclear factor-kappaB. They are to varying degrees also capable of activating nuclear factor-kappaB and increasing the expression of nuclear factor-kappaB dependent genes. Angiotensin II related vascular effects are in part mediated by endothelin-1. Nitric oxide synthase inhibition facilitates angiotensin II related effects, which can be inhibited both by angiotensin II type 1 receptor blockers and by endothelin system inhibitors. This supports the notion that a combined therapeutic strategy of inhibiting angiotensin II and endothelin-1 generation or blocking their effects at the receptor level would be superior to either strategy alone. Animal studies are encouraging but not without conflicting results. Angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers have a superb track record in experimental animal models and in a host of clinical studies. Selective and nonselective blockers of the endothelin-1 receptors are important research tools and are also undergoing clinical trials. Inhibitors of the endothelin converting enzyme have been developed. The recent elucidation of the endothelin converting enzyme's physical structure should facilitate the development of still more novel compounds to inhibit endothelin-1 generation. (c) 2002 Lippncott WiLliams & Wilkins.

Maiwald, L. and P. A. Schwantes (1991). “Curcuma xanthorrhiza Roxb.” Zeitschrift fur Phytotherapie 12(2): 35-45.

Majumdar, A. P. N. (2000). “Role of nutrition in gastric and colon cancers.” International Review of Allergology and Clinical Immunology 6(4): 145-149. It is estimated that environmental factors are responsible for 80 to 90% of all cancers and that one-third of all cancers may be related to diet. The digestive organs account for more cancer than any other organ system. Dietary factors play a key role in the development and progression of gastrointestinal tumors. Thus, manipulations of diet, gut flora and the gastrointestinal milieu are likely targets to modify the risk of cancer in the gastrointestinal tract. Gastric and colon cancers are two of the most common gastrointestinal tumors. Colon cancer is

the second most common cancer in the United States and other Western countries with about 100.000 newly diagnosed cases per year in the United States alone. Although gastric cancer rates have declined in the United States in recent decades, it still represents a leading cause of death in other parts of the world, particularly in Japan where deaths from gastric cancer was found to be as high as 40.000 per year. Nutrition plays an important role in the development, course, treatment, and eventual outcome of gastrointestinal cancers. Epidemiological studies have strongly suggested a relationship between increased intake of fat and risk of developing colon cancer, while a number of nutrients, particularly folic acid and calcium appear to be chemopreventive. Although a clear-cut relationship between a particular nutrient(s) and the development of gastric cancer has not been established, certain food items and additives, including starch, benzpyrene and nitrosamines have been shown to augment the risk of developing gastric cancer. Consumption of fresh vegetables, particularly those that are rich in vitamin C has been shown to be protective against gastric cancer. Recent data from this laboratory also suggest a chemopreventive role for folic acid in gastric cancer. Studies are in progress to evaluate the chemopreventive role of a number of dietary factors in colon cancer, which, among others include green tea extracts, curcumin, mono-and dihydroxy vitamin DSUB3, selenium and dietary flavanoids.

Manson, M. M., A. Gescher, et al. (2000). “Blocking and suppressing mechanisms of chemoprevention by dietary.” Toxicology Letters 112-113(-): 499-505. Many dietary constituents are chemopreventive in animal models, and experiments with cultured cells are revealing various potential mechanisms of action. Compounds classified as blocking agents can prevent, or greatly reduce, initiation of carcinogenesis, while suppressing agents affect later stages of the process by reducing cell proliferation. Many compounds have both types of activity. Blocking mechanisms include alteration of drug metabolising activities and scavenging of reactive oxygen species. Mechanisms which suppress tumorigenesis often involve modulation of signal transduction pathways, leading to altered gene expression, cell cycle arrest or apoptosis. As our knowledge of how these dietary components affect cell biochemistry improves, so the likelihood of success in chemoprevention trials and in provision of dietary advice to the general population to optimise the chances of preventing disease is increased. Copyright (C) 2000 Elsevier Science Ireland Ltd.

Mariadason, J. M., G. A. Corner, et al. (2000). “Genetic reprogramming in pathways of colonic cell maturation induced by.” Cancer Research 60(16): 4561-4572. The short-chain fatty acid butyrate, produced by microbial fermentation of dietary fiber in the large intestine, is a physiological regulator of major pathways of colonic epithelial cell maturation: Cell cycle arrest, lineage-specific differentiation, and apoptosis. Microarray analysis of 8,063 sequences demonstrated a complex cascade of reprogramming of SW620 colonic epithelial cells upon treatment with butyrate characterized by the progressive recruitment of gene sets as a function of time. Comparison with the effects of trichostatin A, in conjunction with differences in the kinetics of alteration of histone acetylation induced by butyrate and trichostatin A, identified subsets of induced and repressed genes likely coordinately regulated by altered histone acetylation. The butyrate response was also compared in detail with that of sulindac, a nonsteroidal anti-

inflammatory drug with significant chemopreventive activity for colon cancer, and curcumin, a component of mustard and curry structurally and functionally related to sulindac that also has chemopreventive activity. Although gene clusters were identified that showed similar responses to butyrate and sulindac, the data were characterized by the extensive differences in the effects of the two agents. This was striking for functional classes of genes involved in signaling pathways and in cell cycle progression, although butyrate and sulindac induce a similar GSUB0-GSUB1 arrest, elevation of beta-catenin-Tcf signaling, and apoptotic cascade. As regards cell cycle arrest, the underlying mechanism in response to butyrate was most similar to that of the Caco-2 cell line that had spontaneously undergone a GSUB0-GSUB1 arrest and least similar to the GSUB2-M arrest stimulated by curcumin. Thus, high-throughput microarray analysis of gene expression profiles can be used to characterize and distinguish the mechanisms of response of colonic epithelial cells to physiological and pharmacological inducers of cell maturation. This has important implications for characterization of chemopreventive agents and recognition of potential toxicity and synergies. The data bases, gene clusters, and analyses are available at http://sequence.aecom.yu.edu/genome/.

Masuda, T. and A. Jitoe (1994). “Antioxidative and antiinflammatory compounds from tropical gingers: isolation, structure determination, and activities of cassumunins A, B, and C, new complex curcuminoids from Zingiber cassumunar.” Journal of Agricultural and Food Chemistry 42(9): 1850-1856. Three new compounds, cassumunins A-C, having both antioxidant and antiinflammatory activities, were isolated following activity-guided fractionation from the acetone extract of rhizomes of a tropical ginger, Z. cassumunar (collected from Indonesia). Antioxidant activity and antiinflammatory activity were measured using a thiocyanate method and a 12-O-tetradecanoylphorbol 13-acetate-induced method on mouse ear, respectively. The structures of cassumunins A-C were elucidated from spectral data. The antioxidant and antiinflammatory activities of cassumunins A-C were stronger than those of curcumin. It is suggested that the substituted group at the 5'-position of curcumin increased the antioxidant and antiinflammatory activities of cassumunins.

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Masuda, T., A. Jitoe, et al. (1993). “Anti-oxidative and anti-inflammatory curcumin-related phenolics from rhizomes of Curcuma domestica.” Phytochemistry 32(6): 1557-1560. Four known curcuminoids (including curcumin), and 2 new neutral phenolics were isolated from the rhizomes of C. domestica [C. longa] (collected from Iriomote Island, Japan). The structures of the phenolics were determined to be 1,5-bis(4-hydroxy-3-methoxyphenyl)- penta-(1E,4E)-1,4-dien-3-one, and 1-(4-hydroxy-3-methoxyphenyl)-5-(4- hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one, respectively, from spectral and chemical analyses. The phenolics exhibited strong antioxidant activities (inhibited the autoxidation of linoleic acid in a water-alcohol system). All 6 compounds exhibited antiinflammatory activities against TPA (12-O-tetradecanoylphorbol- 13-acetate)-induced oedema in mice.

Masuda, T., A. Jitoe, et al. (1993). “Anti-oxidative and anti-inflammatory curcumin-related phenolics from rhizomes of Curcuma domestica.” Phytochemistry 32(6): 1557-1560. Four known curcuminoids (including curcumin), and 2 new neutral phenolics were isolated from the rhizomes of C. domestica [C. longa] (collected from Iriomote Island, Japan). The structures of the phenolics were determined to be 1,5-bis(4-hydroxy-3-methoxyphenyl)- penta-(1E,4E)-1,4-dien-3-one, and 1-(4-hydroxy-3-methoxyphenyl)-5-(4- hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one, respectively, from spectral and chemical analyses. The phenolics exhibited strong antioxidant activities (inhibited the autoxidation of linoleic acid in a water-alcohol system). All 6 compounds exhibited antiinflammatory activities against TPA (12-O-tetradecanoylphorbol- 13-acetate)-induced oedema in mice.

Matsuguchi, T., T. Musikacharoen, et al. (2000). “Gene expressions of Toll-like receptor 2, but not Toll-like receptor 4.” Journal of Immunology 165(10): 5767-5772. Toll-like receptors (TLRs) are a family of mammalian homologues of Drosophila Toll and play important roles in host defense. Two of the TLRs, TLR2 and TLR4, mediate the responsiveness to LPS. Here the gene expression of TLR2 and TLR4 was analyzed in mouse macrophages. Mouse splenic macrophages responded to an intraperitoneal injection or in vitro treatment of LPS by increased gene expression of TLR2, but not TLR4. Treatment of a mouse macrophage cell line with LPS, synthetic lipid A, IL-2, IL-15, IL-1beta, IFN-gamma, or TNF-alpha significantly increased TLR2 mRNA expression, whereas TLR4 mRNA expression remained constant. TLR2 mRNA increase in response to synthetic lipid A was severely impaired in splenic macrophages isolated from TLR4-mutated C3H/HeJ mice, suggesting that TLR4 plays an essential role in the process. Specific inhibitors of mitogen-activated protein/extracellular signal-regulated kinase kinase and p38 kinase did not significantly inhibit TLR2 mRNA up-regulation by LPS. In contrast, LPS-mediated TLR2 mRNA induction was abrogated by pretreatment with a high concentration of curcumin, suggesting that NF-kappaB activation may be essential for the process. Taken together, our results indicate that TLR2, in contrast to TLR4, can be induced in macrophages in response to bacterial infections and may accelerate the innate immunity against pathogens.

Mays-Holland, T. (2000). “Chemoprevention of colorectal cancer.” Hospital Pharmacy 35(9): 964-979. Goal - After completing this CE program, the participant will understand

the basic concepts of chemoprevention and will be able to discuss the currently existing clinical data for the chemoprevention of colorectal cancer. Objectives - At the completion of this program the participant will be able to:' 1. Discuss the complicating factors in performing chemoprevention trials. 2. Describe the dietary modifications to prevent colorectal cancer. 3. Describe the proposed mechanisms of action of agents used in the chemoprevention of colorectal cancer. 4. Discuss, and where appropriate recommend, appropriate agents for use in the chemoprevention of colorectal cancer. 5. Describe the differences between familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), and spontaneous adenoma formation.

Meletis, C. D. (2000). “Natural relief for inflammation of sprains, strains, and arthritis.” Alternative and Complementary Therapies 6(3): 141-144. There is no better treatment approach when dealing with inflammation than prevention. The inflammatory process that presents with pain and swelling is a natural response to injury. With that said, there is no question that glucosamine sulfate and other nutrients that help to maintain the integrity of the musculoskeletal system top the list of preventive steps that can be taken to minimize the effects of wear and tear. These same nutrients are also critical for supporting the healing process. Regardless of the preventive measures that may have been taken, injury and degenerative processes can still arise and require immediate and strong anti-inflammatory agents. Among some of the most potent natural anti-inflammatories are Lyprinol (derived from Perna canaliculus), bromelain (derived from the pineapple plant), curcumin, and various flavonoids. Preventing and limiting inflammation can speed the process of healing from musculoskeletal injuries dramatically.

Mendez-Samperio, P., J. Palma, et al. (2001). “Roles of intracellular calcium and NF-kappaB in the Bacillus.” Cellular Immunology 211(2): 113-122. Interleukin (IL)-8 secretion contributes to the early host response against mycobacterial infection by increasing local inflammation and recruiting professional phagocytes. Because the mechanisms through which Mycobacterium bovis Calmette-Guerin (BCG) induces IL-8 secretion are unknown, the aim of the present study was to characterize the nature of IL-8 production induced by BCG in human monocytes. In this study, we found that the induction of IL-8 synthesis was dose- and time-dependent after stimulation with BCG. This IL-8 secretion was not attributed to LPS contamination or the presence of TNF-alpha. We also determined that BCG-induced IL-8 secretion occurs through a mechanism that requires intracellular calcium and likely involves a calmodulin-sensitive step. Interestingly, BCG-induced secretion of IL-8 from human monocytes resulted from transcriptional up-regulation of the IL-8 gene. Moreover, we present evidence that BCG activates nuclear translocation of the transcription factor NF-kappaB, since pretreatment of monocytes with sulfasalazine, a inhibitor of NF-kappaB activity, blocked the ability of BCG to induce IL-8 secretion in a dose-dependent manner, producing 92.5% inhibition at a concentration of 2 mM. These results were further supported by the fact that treatment of cells with curcumin, another well-described inhibitor of NF-kappaB activity with a different mechanism of action, significantly diminished the effect of BCG on IL-8 secretion. Together, these studies are the first to demonstrate that BCG-induced IL-8 secretion by human monocytes appears to be mediated by intracellular CaSUP2+ and is

NF-kappaB-dependent and at the same time suggest that production of IL-8 in response to M. bovis BCG can contribute to the initial local and systemic inflammatory response in human tuberculosis. (c) 2001 Academic Press.

Mio, K. and R. Stern (2002). “Inhibitors of the hyaluronidases.” Matrix Biology 21(1): 31-37. The inhibitors of hyaluronidase present in mammalian sera, first described half a century ago, have remained uncharacterized. Because of increased interest in hyaluronidases and their hyaluronan substrate, a study of these inhibitors was undertaken recently. The predominant serum inhibitor is magnesium-dependent and is eliminated by protease or chondroitinase digestion, and by heat. Kinetics of inhibition are similar against hyaluronidases from testis, snake and bee venom. The inhibitor has no effect on Streptomyces hyaluronidase; indicating inhibition is not through protection of the hyaluronan substrate. Circulating inhibition levels are increased in mice following carbon tetrachloride or interleukin-1 injection, inducers of the acute-phase response. Reverse hyaluronan gel zymography reveals a predominant band of 120 kDa relative molecular size. Additional studies indicate that the inhibitor resembles a member of the Kunitz type inter-alpha-inhibitor family. Inhibition of hyaluronidase activity is observed using purified inter-alpha-inhibitor and is reversed by antibodies specific for inter-alpha-inhibitor. This molecule, found in the hyaluronan-rich cumulus mass surrounding mammalian ova and the pericellular coat of fibroblasts and mesothelial cells, may function to stabilize such matrices by protecting against hyaluronidase degradation. Turnover of circulating hyaluronan is extraordinarily rapid, with a half-life of two to five min. Prompt increases in levels of serum hyaluronan occur in patients with shock, septicemia or massive burns, increases that may be partly attributed to suppression by these acute phase reactants of the constant and rapid rates of hyaluronan degradation by hyaluronidase. A literature survey of other hyaluronidase inhibitors is also presented. Copyright (c) 2002 Elsevier Science B.V./International Society of Matrix Biology.

Miquel, J., A. Bernd, et al. (2002). “The curcuma antioxidants: Pharmacological effects and prospects for.” Archives of Gerontology and Geriatrics 34(1): 37-46. In agreement with the predictions of the oxygen-stress theory of aging and age-related degenerative diseases, diet supplementation with a number of phenolic or thiolic antioxidants has been able to increase the life span of laboratory animals, protect against senescent immune decline and preserve the respiratory function of aged mitochondria. In addition to the above, more recent data reviewed here suggest that the polyphenolic compound curcumin and related non-toxic antioxidants from the rhizome of the spice plant Curcuma longa have a favorable effect on experimental mouse tumorigenesis as well as on inflammatory processes such as psoriasis and ethanol-caused hepatic injury. Our own research has focused on the effects of diet supplementation with an antioxidant-rich hydroalcoholic extract of the curcuma rhizome on key risk factors of atherogenesis and related cardiovascular disease. Our reviewed data show that, in human healthy subjects, the daily intake of 200 mg of the above extract results in a decrease in total blood lipid peroxides as well as in HDL and LDL-lipid peroxidation. This anti-atherogenic effect was accompanied by a curcuma antioxidant-induced normalization of the plasma levels of fibrinogen and of the apo B/apo A ratio, that may also decrease the cardiovascular risk. The reviewed literature indicates that curcumin and related plant co-antioxidants are

powerful anti-inflammatory agents. Further, since they potentiate the anti-atherogenic effect of alpha-tocopherol, more extensive clinical testing of their probable usefulness in cardiovascular risk reduction seems justified. Copyright (c) 2002 .

Motterlini, R., R. Foresti, et al. (2000). “Curcumin, an antioxidant and anti-inflammatory agent, induces heme.” Free Radical Biology and Medicine 28(8): 1303-1312. Curcumin, a widely used spice and coloring agent in food, has been shown to possess potent antioxidant, antitumor promoting and anti-inflammatory properties in vitro and in vivo. The mechanism(s) of such pleiotropic action by this yellow pigment is unknown; whether induction of distinct antioxidant genes contributes to the beneficial activities mediated by curcumin remains to be investigated. In the present study we examined the effect of curcumin on endothelial heme oxygenase-1 (HO-1 or HSP32), an inducible stress protein that degrades heme to the vasoactive molecule carbon monoxide and the antioxidant biliverdin. Exposure of bovine aortic endothelial cells to curcumin (5-15 muM) resulted in both a concentration- and time-dependent increase in HO-1 mRNA, protein expression and heme oxygenase activity. Hypoxia (18 h) also caused a significant ($P < 0.05$) increase in heme oxygenase activity which was markedly potentiated by the presence of low concentrations of curcumin (5 muM). Interestingly, prolonged incubation (18 h) with curcumin in normoxic or hypoxic conditions resulted in enhanced cellular resistance to oxidative damage; this cytoprotective effect was considerably attenuated by tin protoporphyrin IX, an inhibitor of heme oxygenase activity. In contrast, exposure of cells to curcumin for a period of time insufficient to up-regulate HO-1 (1.5 h) did not prevent oxidant-mediated injury. These data indicate that curcumin is a potent inducer of HO-1 in vascular endothelial cells and that increased heme oxygenase activity is an important component in curcumin-mediated cytoprotection against oxidative stress. Copyright (C) 2000 Elsevier Science Inc.

Mukhopadhyay, A., N. Basu, et al. (1982). “Anti-inflammatory and irritant activities of curcumin analogues in rats.” Agents and Actions 12(4): 508-515.

Nakayama, K., A. Furusu, et al. (2001). “Unexpected transcriptional induction of monocyte chemoattractant.” Journal of Immunology 167(3): 1145-1150. Proteasome inhibitors, the well-known inhibitors of NF-kappaB, are recently considered therapeutic agents for inflammation. However, the anti-inflammatory properties of these agents have not been fully evaluated. In this report we describe a novel effect of proteasome inhibitors on the expression of monocyte chemoattractant protein 1 (MCP-1) in mesangial cells. We found that proteasome inhibitor MG132 dose-dependently induced expression of MCP-1 at the transcriptional level. The stimulatory effect was similarly observed with other proteasome inhibitors (proteasome inhibitor 1 and lactacystin) and in other cell types (NRK fibroblasts). The 5prime-flanking region of the MCP-1 gene contains multiple AP-1 sites. To explore the mechanisms involved, we examined the effects of proteasome inhibition on the AP-1 pathway. Northern blot analysis showed that MG132 rapidly induced the expression of c-jun, but not c-fos. Immunoblot analysis showed that MG132 prevented degradation of c-Jun protein. Kinase assay revealed that c-Jun N-terminal kinase (JNK) was rapidly activated by MG132. Consistent with these results, a reporter assay showed that AP-1 activity was up-regulated after treatment with

MG132. Curcumin, a pharmacological inhibitor of the JNK-AP-1 pathway, abrogated the induction of MCP-1 by MG132. Similarly, stable transfection with a dominant-negative mutant of c-Jun attenuated both MG132-induced activation of AP-1 and expression of MCP-1. The transcriptional activation by proteasome inhibitors was observed not only in MCP-1, but also in other AP-1-dependent genes, including stromelysin and mitogen-activated protein kinase phosphatase 1. These data revealed that proteasome inhibition triggered the expression of MCP-1 and other genes via the multistep induction of the JNK-c-Jun/AP-1 pathway.

Natarajan, C. and J. J. Bright (2002). "Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through janus kinase-STAT pathway in T lymphocytes." Journal of Immunology 168(12): 6506-6513. Experimental allergic encephalomyelitis (EAE) is a CD4+ Th1 cell- mediated inflammatory demyelinating autoimmune disease of the central nervous system (CNS) that serves as an animal model for multiple sclerosis (MS). IL-12 is a pro-inflammatory cytokine that plays a crucial role in the induction of neural Ag-specific Th1 differentiation and pathogenesis of CNS demyelination in EAE and MS. Curcumin (1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5- dione) is a naturally occurring polyphenolic phytochemical isolated from the rhizome of the medicinal plant Curcuma longa. It has profound antiinflammatory activity and has been traditionally used to treat inflammatory disorders. In this study we have examined the effect and mechanism of action of curcumin on the pathogenesis of CNS demyelination in EAE. In vivo treatment of SJL/J mice with curcumin significantly reduced the duration and clinical severity of active immunization and adoptive transfer EAE. Curcumin inhibited EAE in association with a decrease in IL-12 production from macrophage/microglial cells and differentiation of neural Ag-specific Th1 cells. In vitro treatment of activated T cells with curcumin inhibited IL-12-induced tyrosine phosphorylation of Janus kinase 2, tyrosine kinase 2, and STAT3 and STAT4 transcription factors. The inhibition of Janus kinase-STAT pathway by curcumin resulted in a decrease in IL-12-induced T cell proliferation and Th1 differentiation. These findings highlight the fact that curcumin inhibits EAE by blocking IL-12 signalling in T cells and suggest its use in the treatment of MS and other Th1 cell-mediated inflammatory diseases.

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Nurfina, A. N., M. S. Reksohadiprodjo, et al. (1997). “Synthesis of some symmetrical curcumin derivatives and their.” European Journal of Medicinal Chemistry 32(4): 321-328. Curcumin is not only a frequently used food additive, but it is also a well-known constituent of Indonesian traditional medicines. Several beneficial effects are ascribed to curcumin, eg, its antiinflammatory properties. In order to study the antiinffammatory activity, a series of curcumin derivatives were prepared and the inhibition of the carrageenin-induced oedema by these compounds was established. It appeared that the para hydroxy groups in curcumin are important for antiinflammatory activity. This activity is enhanced when, in combination with the para hydroxy groups, the meta positions are occupied with alkyl groups. Since the methyl derivatives are more active than the corresponding ethyl and tert-butyl derivatives, it is suggested that sterical hindrance is involved.

Okada, K., C. Wangpoengtrakul, et al. (2001). “Curcumin and especially tetrahydrocurcumin ameliorate oxidative.” Journal of Nutrition 131(8): 2090-2095. Protective effects of curcumin (U1), one of the major yellow pigments in turmeric and its derivative, tetrahydrocurcumin (THU1), against ferric nitrilotriacetate (Fe-NTA)-induced oxidative renal damage were studied in male ddY mice. Single Fe-NTA treatment (5 mg Fe/kg body intraperitoneally) transiently causes oxidative stress, as shown by the accumulation of lipid peroxidation products and 8-hydroxy-2prime-deoxyguanosine in the kidney. Mice were fed with a diet containing 0.5 g/100 g U1 or THU1 for 4 wk. THU1 significantly inhibited 2-thiobarbituric acid reactive substances and 4-hydroxy-2-nonenal-modified proteins and 8-hydroxy-2prime-deoxyguanosine formation in the kidney; U1 inhibited only 4-hydroxy-2-nonenal-modified protein formation. To elucidate the mechanisms of protection by U1 and THU1, the pharmacokinetics and radical-scavenging capacities of U1 and THU1 were investigated by HPLC and electron spin resonance spin trapping with 5,5-dimethyl-1-pyrroline-N-oxide, respectively. Induction of antioxidant enzymes was also investigated. The amounts of THU1 and its conjugates (as sulfates and glucuronides) in the liver and serum were larger in the THU1 group than in the U1 group. The amounts of U1 and its conjugates were small even in the U1 group. These results suggest that THU1 is more easily absorbed from the gastrointestinal tract than U1. Furthermore, THU1 induced antioxidant enzymes, such as glutathione peroxidase, glutathione S-transferase and NADPH: quinone reductase, as well as or better than U1 and scavenged Fe-NTA-induced free radicals in vitro better than U1. These

results suggest that U1 is converted to THU1 in vivo and that THU1 is a more promising chemopreventive agent.

Opletalova, V. (1995). “Constituents of the plants of the Zingiberaceae family and their.” Ceska a Slovenska Farmacie 44(6): 305-307. The compounds contained in various plants of the Zingiberaceae family, e.g. zingeron, gingerols and curcumin, as well as their naturally occurring and synthetic analogues showed interesting anti-inflammatory properties. The present paper briefly deals with the structures and activities of these compounds.

Pal, S., T. Choudhuri, et al. (2001). “Mechanisms of curcumin-induced apoptosis of Ehrlich's ascites carcinoma.” Biochemical and Biophysical Research Communications 288(3): 658-665. Curcumin, the active ingredient from the spice turmeric (Curcuma longa Linn), is a potent antioxidant and anti-inflammatory agent. It has been recently demonstrated to possess discrete chemopreventive activities. However, the molecular mechanisms underlying such anticancer properties of curcumin still remain unrealized, although it has been postulated that induction of apoptosis in cancer cells might be a probable explanation. In the current study, curcumin was found to decrease the Ehrlich's ascites carcinoma (EAC) cell number by the induction of apoptosis in the tumor cells as evident from flow-cytometric analysis of cell cycle phase distribution of nuclear DNA and oligonucleosomal fragmentation. Probing further into the molecular signals leading to apoptosis of EAC cells, we observed that curcumin is causing tumor cell death by the up-regulation of the proto-oncoprotein Bax, release of cytochrome c from the mitochondria, and activation of caspase-3. The status of Bcl-2 remains unchanged in EAC, which would signify that curcumin is bypassing the Bcl-2 checkpoint and over-riding its protective effect on apoptosis. (c) 2001 Academic Press.

Pan, M. H., S. Y. Lin-Shiau, et al. (2000). “Comparative studies on the suppression of nitric oxide synthase by.” Biochemical Pharmacology 60(11): 1665-1676. Nitric oxide (NO) plays an important role in inflammation and in the multiple stages of carcinogenesis. In this study, we investigated the inhibitory effects of curcumin and its metabolites, tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin, on the induction of NO synthase (NOS) in RAW 264.7 cells activated with lipopolysaccharide (LPS). Western blotting and northern blotting analyses demonstrated that curcumin strongly reduced 130-kDa protein and 4.5-kb mRNA levels of iNOS in LPS-activated macrophages compared with its metabolites, tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin. Moreover, electrophoretic mobility shift assay (EMSA) experiments indicated that curcumin blocked the LPS-induced binding of nuclear factor-kappaB (NFkappaB), a transcription factor necessary for iNOS induction to its sup 3sup 2P-labeled double-stranded oligonucleotide probe. The inhibition of NFkappaB activation occurred through the prevention of inhibitor kappaB (IkappaB) degradation. Transient transfection experiments also showed that curcumin inhibited NFkappaB-dependent transcriptional activity. Curcumin blocked the disappearance of inhibitory kappaBalpha (IkappaBalpha) and p65 from the cytosolic fraction, and inhibited the phosphorylation of IkappaBalpha. Furthermore, we showed that curcumin could inhibit the IkappaB kinase 1 (IKK1) and IkappaB kinase 2 (IKK2) activities induced by LPS, but

tetrahydrocurcumin, hexahydrocurcumin, and octahydrocurcumin were less active. These results suggest that curcumin may exert its anti-inflammatory and anti-carcinogenic properties by suppressing the activation of NFkappaB through inhibition of IKK activity. (C) 2000 Elsevier Science Inc.

Parka, K. K., K. S. Chun, et al. (1998). “Inhibitory effects of [6]-gingerol, a major pungent principle of.” Cancer Letters 129(2): 139-144. A wide array of phytochemicals have been shown to possess potential cancer chemopreventive properties. Ginger contains pungent phenolic substances with pronounced antioxidative and antiinflammatory activities. In the present study, we have determined the antitumor promotional activity of [6]-gingerol, a major pungent principle of ginger, using a two-stage mouse skin carcinogenesis model. Topical application of [6]-gingerol onto shaven backs of female ICR mice prior to each topical dose of 12-O-tetradecanoylphorbol-13-acetate (TPA) significantly inhibited 7,12-dimethylbenz[a]anthracene-induced skin papillomagenesis. The compound also suppressed TPA-induced epidermal ornithine decarboxylase activity and inflammation.

Pendurthi, U. R., J. T. Williams, et al. (1997). “Inhibition of tissue factor gene activation in cultured endothelial.” Arteriosclerosis, Thrombosis, and Vascular Biology 17(12): 3406-3413. Binding of plasma factor VII(a) to tissue factor (TF) initiates the coagulation cascade. In health, TF is not expressed in endothelial cells. However, endothelial cells express TF in response to lipopolysaccharide (LPS), tumor necrosis factor-alpha (TNFalpha), and other biological stimuli. TF expression by endothelial cells is implicated in thrombotic disorders in patients with a variety of clinical disorders. In the present study, we demonstrate that curcumin (diferulolylmethane), a known anticarcinogenic and anti-inflammatory agent, inhibited phorbol 12-myristate 13-acetate (PMA), LPS, TNFalpha, and thrombin-induced TF activity and TF gene transcription in human endothelial cells. The present data show that curcumin prevented the activation of c-Rel/p65, which is essential for TF gene activation in endothelial cells, by impairing the proteolytic degradation inhibitor protein, IkappaBalpha. The data also show that curcumin downregulated AP-1 binding activity. The present studies are the first to demonstrate that PMA, but not LPS, TNFalpha, and thrombin, induced Egr-1 binding to the second serum- responsive region (SRR-2) of TF promoter and that curcumin inhibited the PMA- induced Egr-1 binding to SRR-2. Overall, the data suggest that the anticarcinogenic and anti-inflammatory properties of curcumin may be related to its ability to inhibit cellular gene expression regulated by transcription factors NF-kappaB, AP-1, and Egr-1.

Perkins, S., R. D. Verschoyle, et al. (2002). “Chemopreventive efficacy and pharmacokinetics of curcumin in the min/+.” Cancer Epidemiology Biomarkers and Prevention 11(6): 535-540. Curcumin, the major yellow pigment in turmeric, prevents the development of adenomas in the intestinal tract of the C57BI/6J Min/+ mouse, a model of human familial APC. To aid the rational development of curcumin as a colorectal cancer-preventive agent, we explored the link between its chemopreventive potency in the Min/+ mouse and levels of drug and metabolites in target tissue and plasma. Mice received dietary curcumin for 15 weeks, after which adenomas were enumerated. Levels of

curcumin and metabolites were determined by high-performance liquid chromatography in plasma, tissues, and feces of mice after either long-term ingestion of dietary curcumin or a single dose of [SUP14C]curcumin (100 mg/kg) via the i.p. route. Whereas curcumin at 0.1% in the diet was without effect, at 0.2 and 0.5%, it reduced adenoma multiplicity by 39 and 40%, respectively, compared with untreated mice. Hematocrit values in untreated Min/+ mice were drastically reduced compared with those in wild-type C57BI/6J mice. Dietary curcumin partially restored the suppressed hematocrit. Traces of curcumin were detected in the plasma. Its concentration in the small intestinal mucosa, between 39 and 240 nmol/ g of tissue, reflects differences in dietary concentration. [SUP14C]Curcumin disappeared rapidly from tissues and plasma within 2-8 h after dosing. Curcumin may be useful in the chemoprevention of human intestinal malignancies related to Apc mutations. The comparison of dose, resulting curcumin levels in the intestinal tract, and chemopreventive potency suggests tentatively that a daily dose of 1.6 g of curcumin is required for efficacy in humans. A clear advantage of curcumin over nonsteroidal anti-inflammatory drugs is its ability to decrease intestinal bleeding linked to adenoma maturation.

Pilz, R. (1975). “Therapeutic effect of Aristochol in chronic cholecystopathies.” Medizinische Welt 26(29-30): 1385-1388.

Piwocka, K., E. Jaruga, et al. (2001). “Effect of glutathione depletion on caspase-3 independent apoptosis.” Free Radical Biology and Medicine 31(5): 670-678. Curcumin, a yellow pigment from Curcuma longa, exhibits anti-inflammatory, antitumor, and antioxidative properties. Although its precise mode of action has not been elucidated so far, numerous studies have shown that curcumin may induce apoptosis in normal and cancer cells. Previously, we showed that in Jurkat cells curcumin induced nontypical apoptosis-like pathway, which was independent of mitochondria and caspase-3. Now we show that the inhibition of caspase-3 by curcumin, which is accompanied by attenuation of internucleosomal DNA fragmentation, may be due to elevation of glutathione, which increased in curcumin-treated cells to 130% of control. We have demonstrated that glutathione depletion does not itself induce apoptosis in Jurkat cells; though, it can release cytochrome c from mitochondria and caspase-3 from inhibition by curcumin, as shown by Western blot. The level of Bcl-2 protein was not affected by glutathione depletion even upon curcumin treatment. Altogether, our results show that in Jurkat cells curcumin prevents glutathione decrease, thus protecting cells against caspase-3 activation and oligonucleosomal DNA fragmentation. On the other hand, it induces nonclassical apoptosis via a still-unrecognized mechanism, which leads to chromatin degradation and high-molecular-weight DNA fragmentation. (c) 2001 Elsevier Science Inc.

Pulla Reddy Ch, A. and B. R. Lokesh (1994). “Studies on anti-inflammatory activity of spice principles and dietary.” Annals of Nutrition and Metabolism 38(6): 349-358. The antioxidant spice principles curcumin and eugenol when given by gavage lowered the carrageenan-induced edema in the foot pads of rats. This lowering effect was dependent on the concentration, the time gap between the administration of spice principles and the induction of inflammation by carrageenan. Dietary lipids also influenced the extent of inflammation. Animals fed 10% cod liver oil (containing n-3 polyunsaturated fatty acids

(PUFA)) for 10 weeks showed a significantly lower inflammation compared to that observed in animals fed diets supplemented with 10% groundnut oil (rich in n-6 PUFA) or 10% coconut oil (rich in medium-chain saturated fatty acids). Supplementation of diets with 1 weight% of curcumin did not affect the inflammatory responses of animals to carrageenan injection. However, supplementation of diets with 0.17 weight% eugenol further lowered inflammation by 16, 32 and 30% in animals fed coconut oil, groundnut oil and cod liver oil, respectively. Therefore, combinations of dietary lipids with spice principles like eugenol can help in lowering inflammation.

Pulla Reddy, A. C., E. Sudharshan, et al. (1999). “Interaction of curcumin with human serum albumin - A spectroscopic.” Lipids 34(10): 1025-1029. Curcumin (diferuloyl methane) has a wide range of physiological and pharmacological actions. Curcumin interaction with human serum albumin (HSA) has been followed by fluorescence quenching and circular dichroism (CD) measurements. Based on fluorescence measurements, the equilibrium constant for the interaction is 2.0 +/- 0.2 x 10sup 5 Msup -sup 1. Binding of curcumin to HSA induces an extrinsic CD band in the visible region. From the induced CD band measurements, the equilibrium constant has a value of 2.1 +/- 0.3 x 10sup 4 Msup - sup 1. Thus, HSA has two kinds of affinity sites for curcumin, one with high affinity and the other with lower affinity. Job's plot indicated a binding stoichiometry of 1:1 for the high-affinity site. The equilibrium constant was invariant with temperature in the range of 15 to 45degreeC, suggesting the role of hydrophobic interactions in the binding of curcumin to HSA. Curcumin does not change the conformation of the HSA molecule. These measurements have implications in the understanding of the curcumin transport under physiological conditions.

Punithavathi, D., N. Venkatesan, et al. (2000). “Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats.” British Journal of Pharmacology 131(2): 169-172. Curcumin, an anti-inflammatory, antioxidant, was evaluated for its ability to suppress bleomycin (BLM)-induced pulmonary fibrosis in rats. A single intratracheal instillation of BLM (0.75 U 100sup -sup 1 g, sacrificed 3, 5, 7, 14 and 28 days post-BLM) resulted in significant increases in total cell numbers, total protein, and angiotensin-converting enzyme (ACE), and alkaline phosphatase (AKP) activities in bronchoalveolar lavage fluid. Animals with fibrosis had a significant increase in lung hydroxyproline content. Alveolar macrophages from BLM-administered rats elaborated significant increases in tumour necrosis factor (TNF)-alpha release, and superoxide and nitric oxide production in culture medium. Interestingly, oral administration of curcumin (300 mg kgsup -sup 1 10 days before and daily thereafter throughout the experimental time period) inhibited BLM-induced increases in total cell counts and biomarkers of inflammatory responses in BALF. In addition, curcumin significantly reduced the total lung hydroxyproline in BLM rats. Furthermore, curcumin remarkably suppressed the BLM-induced alveolar macrophage production of TNF-alpha, superoxide and nitric oxide. These findings suggest curcumin as a potent anti-inflammatory and anti-fibrotic agent against BLM-induced pulmonary fibrosis in rats.

Rafiee, P., C. P. Johnson, et al. (2002). “Cyclosporine A enhances leukocyte binding by human intestinal.” Journal of Biological Chemistry 277(38): 35605-35615. The

calcineurin inhibitor cyclosporine A (CsA) modulates leukocyte cytokine production but may also effect nonimmune cells, including microvascular endothelial cells, which regulate the inflammatory process through leukocyte recruitment. We hypothesized that CsA would promote a proinflammatory phenotype in human intestinal microvascular endothelial cells (HIMEC), by inhibiting inducible nitric-oxide synthase (iNOS, NOS2)-derived NO, normally an important mechanism in limiting endothelial activation and leukocyte adhesion. Primary cultures of HIMEC were used to assess CsA effects on endothelial activation, leukocyte interaction, and the expression of iNOS as well as cell adhesion molecules. CsA significantly increased leukocyte binding to activated HIMEC, but paradoxically decreased endothelial expression of cell adhesion molecules (E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule-1). In contrast, CsA completely inhibited the expression of iNOS in tumor necrosis factor-alpha/lipopolysaccharide-activated HIMEC. CsA blocked p38 MAPK phosphorylation in activated HIMEC, a key pathway in iNOS expression, but failed to inhibit NFkappaB activation. These studies demonstrate that CsA exerts a proinflammatory effect on HIMEC by blocking iNOS expression. CsA exerts a proinflammatory effect on the microvascular endothelium, and this drug-induced endothelial dysfunction may help explain its lack of efficacy in the long-term treatment of chronically active inflammatory bowel disease.

Ramsewak, R. S., D. L. Dewitt, et al. (2000). “Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins.” Phytomedicine 7(4): 303-308. Curcumin I, curcumin II (monodemethoxycurcumin) and curcumin III (bisdemethoxycurcumin) from Curcuma longa were assayed for their cytotoxicity, antioxidant and anti-inflammatory activities. These compounds showed activity against leukemia, colon, CNS, melanoma, renal, and breast cancer cell lines. The inhibition of liposome peroxidation by curcumins I-III at 100 mug/ml were 58, 40 and 22 %, respectively. The inhibition of COX-I and COX-II enzymes by the curcumins was observed. Curcumins I-III were active against COX-I enzyme at 125 mug/ml and showed 32, 38.5 and 39.2 % inhibition of the enzyme, respectively. Curcumins I-III also showed good inhibition of the COX-II enzyme at 125 mg/ml with 89.7, 82.5 and 58.9 % inhibition of the enzyme, respectively.

Ramsewak, R. S., D. L. DeWitt, et al. (2000). “Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from Curcuma longa.” Phytomedicine 7(4): 303-308. Curcumin I, curcumin II (monodemethoxycurcumin) and curcumin III (bisdemethoxycurcumin) from Curcuma longa were assayed for their cytotoxicity, antioxidant and antiinflammatory activities. These compounds showed activity against leukaemia, colon, CNS, melanoma, renal, and breast cancer cell lines. The inhibition of liposome peroxidation by curcumins I-III at 100 micro g/ml were 58, 40 and 22%, respectively. The inhibition of cyclooxygenase (COX)-I and COX- II enzymes by the curcumins was observed. Curcumins I-III were active against COX-I enzyme at 125 micro g/ml and showed 32, 38.5 and 39.2% inhibition of the enzyme, respectively. Curcumins I-III also showed good inhibition of the COX-II enzyme at 125 mg/ml with 89.7, 82.5 and 58.9% inhibition of the enzyme, respectively.

Ramsewak, R. S., D. L. DeWitt, et al. (2000). “Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from Curcuma longa.” Phytomedicine 7(4): 303-308. Curcumin I, curcumin II (monodemethoxycurcumin) and curcumin III (bisdemethoxycurcumin) from Curcuma longa were assayed for their cytotoxicity, antioxidant and antiinflammatory activities. These compounds showed activity against leukaemia, colon, CNS, melanoma, renal, and breast cancer cell lines. The inhibition of liposome peroxidation by curcumins I-III at 100 micro g/ml were 58, 40 and 22%, respectively. The inhibition of cyclooxygenase (COX)-I and COX- II enzymes by the curcumins was observed. Curcumins I-III were active against COX-I enzyme at 125 micro g/ml and showed 32, 38.5 and 39.2% inhibition of the enzyme, respectively. Curcumins I-III also showed good inhibition of the COX-II enzyme at 125 mg/ml with 89.7, 82.5 and 58.9% inhibition of the enzyme, respectively.

Razga, Z. and M. Gabor (1995). “Effects of curcumin and nordihydroguaiaretic acid on mouse ear oedema.” Pharmazie 50(2): 156-157.

Razga, Z. and M. Gabor (1995). “Effects of curcumin and nordihydroguaiaretic acid on mouse ear oedema induced by Croton oil or dithranol.” Pharmazie 50(2): 156-157. Curcumin (dipheruloylmethane), a yellow pigment contained in the rhizome of Curcuma longa, is widely used in Indian folk medicine for its antiinflammatory, antithrombotic and platelet antiaggregation properties. Its molecular structure is similar to that of nordihydroguaiaretic acid, a lipoxygenase inhibitor. In an experiment with CFLP mice, administration of curcumin at 10 mg/kg, i.p., or of nordihydroguaiaretic acid at 5 mg/kg, i.p., significantly reduced Croton oil-induced ear oedema. A lower dose of curcumin (5 mg/kg) but a higher dose of nordihydroguaiaretic acid (10 mg/kg) significantly reduced dithranol-induced ear oedema. The antiinflammatory activity of curcumin is likely to be related to its known inhibitory effect on leukotriene B4 formation.

Razga, Z. and M. Gabor (1995). “Effects of curcumin and nordihydroguaiaretic acid on mouse ear oedema induced by Croton oil or dithranol.” Pharmazie 50(2): 156-157. Curcumin (dipheruloylmethane), a yellow pigment contained in the rhizome of Curcuma longa, is widely used in Indian folk medicine for its antiinflammatory, antithrombotic and platelet antiaggregation properties. Its molecular structure is similar to that of nordihydroguaiaretic acid, a lipoxygenase inhibitor. In an experiment with CFLP mice, administration of curcumin at 10 mg/kg, i.p., or of nordihydroguaiaretic acid at 5 mg/kg, i.p., significantly reduced Croton oil-induced ear oedema. A lower dose of curcumin (5 mg/kg) but a higher dose of nordihydroguaiaretic acid (10 mg/kg) significantly reduced dithranol-induced ear oedema. The antiinflammatory activity of curcumin is likely to be related to its known inhibitory effect on leukotriene B4 formation.

Reddy, A. C. P. and B. R. Lokesh (1994). “Studies on anti-inflammatory activity of spice principles and dietary n-3 polyunsaturated fatty acids on carrageenan-induced inflammation in rats.” Annals of Nutrition and Metabolism 38(6): 349-358. Curcumin and eugenol given by gavage lowered the carrageenan-induced oedema in the foot pads of rats. This effect was dependent on the concentration and on the time gap between administration of the spice principles and the induction of inflammation by carrageenan.

Dietary lipids also influenced the extent of inflammation. Rats fed on 10% cod liver oil for 10 weeks showed significantly less inflammation compared with that observed in rats fed on diets supplemented with 10% groundnut oil or 10% coconut oil. Supplementation of diets with curcumin (1% by weight) did not affect the inflammatory responses of rats to carrageenan injection. However, supplementation of diets with eugenol (0.17% by weight) further lowered inflammation by 16, 32 and 30% in rats fed on coconut oil, groundnut oil and cod liver oil, respectively. Therefore, combinations of dietary lipids with spice principles such as eugenol can help in reducing inflammation.

Reddy, A. C. P. and B. R. Lokesh (1994). "Studies on anti-inflammatory activity of spice principles and dietary n-3 polyunsaturated fatty acids on carrageenan-induced inflammation in rats." Annals of Nutrition and Metabolism 38(6): 349-358. Curcumin and eugenol given by gavage lowered the carrageenan-induced oedema in the foot pads of rats. This effect was dependent on the concentration and on the time gap between administration of the spice principles and the induction of inflammation by carrageenan. Dietary lipids also influenced the extent of inflammation. Rats fed on 10% cod liver oil for 10 weeks showed significantly less inflammation compared with that observed in rats fed on diets supplemented with 10% groundnut oil or 10% coconut oil. Supplementation of diets with curcumin (1% by weight) did not affect the inflammatory responses of rats to carrageenan injection. However, supplementation of diets with eugenol (0.17% by weight) further lowered inflammation by 16, 32 and 30% in rats fed on coconut oil, groundnut oil and cod liver oil, respectively. Therefore, combinations of dietary lipids with spice principles such as eugenol can help in reducing inflammation.

Reddy, B. S. (2000). "Novel approaches to the prevention of colon cancer by nutritional." Cancer Epidemiology Biomarkers and Prevention 9(3): 239-247. Large bowel cancer is one of the most common human malignancies in Western countries including North America. This report details the preventive strategies aimed at reducing the incidence and mortality of large bowel cancer by nutritional manipulation and chemopreventive agents. During recent decades, multidisciplinary research in epidemiology and laboratory animal model studies have contributed much to our understanding of the etiology of this cancer; more importantly, it has enabled us to approach cancer prevention. An impressive body of data thus far accumulated has provided important concepts about dietary factors such as fat and fiber as key modulators of large bowel cancer. Compelling experimental evidence indicates that certain dietary lipids and fibers influence tumorigenesis in the colon. Data obtained in metabolic epidemiological and laboratory animal model studies are sufficiently convincing in showing the enhancement of colon cancer by certain types of fat and protection against it by certain dietary fibers. Our approach to the primary prevention of large bowel cancer is to translate the findings from clinical epidemiological and laboratory studies into sound advice for patients and for the public at large to reduce fat intake and increase fiber intake, specifically cereals and grains. Preclinical efficacy studies have provided scientifically sound evidence as to how several phytochemicals and their synthetic analogues act to retard, block, or reverse carcinogenesis. Equally exciting are opportunities for effective chemoprevention with nonsteroidal anti-inflammatory agents, both synthetic and naturally occurring, or selective cyclooxygenase-2 inhibitors. Our exploration of the multistep process of carcinogenesis has provided substantial

insights into the mechanisms by which chemopreventive agents modulate these events. Growing knowledge in this area has brought about an innovative combination of agents with different modes of action as a means of increasing efficacy and minimizing toxicity. There is growing optimism for the view that realization of preventive concepts in large bowel cancer will also serve as a model for preventing malignancies such as cancer of the prostate and breast.

Reddy, B. S. and C. V. Rao (2002). "Novel approaches for colon cancer prevention by cyclooxygenase-2." Journal of Environmental Pathology, Toxicology and Oncology 21(2): 155-164. During recent years, multidisciplinary studies in epidemiology and molecular biology, as well as preclinical studies, have contributed much to our understanding of the etiology of colorectal cancer; more importantly they have enabled us to approach its prevention. An impressive body of epidemiological data suggests an inverse relationship between colorectal cancer risk and regular use of nonsteroidal antiinflammatory drugs (NSAIDs), including aspirin. Clinical trials with NSAIDs have demonstrated that NSAID treatment caused regression of preexisting colon adenomas in patients with familial adenomatous polyposis. Preclinical efficacy studies have provided compelling evidence that several phytochemicals with antiinflammatory properties and NSAIDs act to retard, block, or reverse colon carcinogenesis. Equally exciting are opportunities for effective chemoprevention with selective cyclooxygenase-2 (COX-2) inhibitors including celecoxib and rofecoxib in a variety of preclinical models of colon cancer. Naturally occurring COX-2 inhibitors such as curcumin and certain phytosterols have been proven to be effective as chemopreventive agents against colon carcinogenesis with minimal gastrointestinal toxicity. Multistep process of carcinogenesis has provided substantial insights into the mechanisms by which naturally occurring and synthetic antiinflammatory agents modulate these events leading to suppression of tumorigenesis. Growing knowledge in this area has brought about innovative approaches using a combination of agents with different modes of action as a means of increasing efficacy and minimizing toxicity. The natural history of colorectal cancer, from dysplastic aberrant crypts to adenomas and adenocarcinomas, offers multiple opportunities for assessment and intervention. Of further importance would be to identify molecular targets that are critical in the growth and survival of the malignant colorectal cell and are modulated by NSAIDs and COX-2 inhibitors.

Reuter, H. D. (1995). "Medicinal plants in the therapy of gallbladder diseases. Part I. Joint." Zeitschrift fur Phytotherapie 16(1): 13-14+17-20.

Santoro, M. G. and S. M. Roberts (1999). "Search for novel cytoprotective and antiviral prostanoids." Drug News and Perspectives 12(7): 395-400. Prostaglandins (PGs) function as intracellular signal mediators in the regulation of numerous physiological and pathological processes. Although after their discovery some PGs of the E and F series were developed for clinical use, initial hopes for PG-based therapies have largely been unfulfilled. Until the early 1980s, relatively little attention was paid to the preparation of the conjugated cyclopentenone prostaglandins PGAinf 2 and PGJinf 2; however, following a serendipitous discovery, natural and synthetic cyclopentenone PGs (cyPGs) were found to express antiviral activity in a variety of experimental models. CyPGs

inhibit virus replication by turning on an intracellular defense response, which involves the induction of cytoprotective heat shock proteins (HSPs) and the control of nuclear factor- kappaB (NF-kappaB) activation. These molecules represent an interesting model for the development of novel cytoprotective and antiviral drugs that can affect different targets during the virus life cycle. CyPGs are in fact potent inhibitors of NF-kappaB activation in human cells, and simultaneously induce the synthesis of hsp70 in human cells via activation of the heat shock transcription factor HSF. Recent observations encourage the search for novel prostanoids and indicate that a new class of molecules, characterized by the ability to activate HSF while inhibiting NF-kappaB and devoid of the pleiotropic effects of natural PGs, could be designed, opening new perspectives for therapeutic intervention in inflammatory and infectious diseases.

Sartor, L., E. Pezzato, et al. (2002). “Inhibition of matrix-proteases by polyphenols: Chemical insights for.” Biochemical Pharmacology 64(2): 229-237. Flavanols - a class of plant polyphenols abundant in tea leaves and grape seeds and skins - have been found to inhibit some matrix-proteases instrumental in inflammation and cancer invasion, such as leukocyte elastase (LE) and gelatinases. In order to establish the relationship between chemical structure and activity, 27 different flavonoids (antocyanidins, dihydrochalcones, dihydroflavonols, flavanolignans, flavanols, flavones, flavonols and isoflavones) and other compounds with anti-oxidant properties were evaluated for their potential in blocking LE and gelatinase activities. LE activity was measured using a chromogenic substrate: from comparison of the different levels of inhibition, it was deduced that a crucial role in inhibition might be played by a galloyl moiety or hydroxyl group at C3, three hydroxyl groups at B ring, one hydroxyl group at C4prime, and a 2,3-double bond. Gelatinase activity was measured using the gelatin-zymography assay, and its inhibition showed that three hydroxyl groups at the A or B ring, or, for non-planar molecules, a galloyl moiety at C3 could be determinant. This comparative study is proposed as a basis for designing new molecules with enhanced anti-proteolytic activities, and no or reduced side-effects, for use in hindering inflammation, cancer invasion and angiogenesis.

Satoskar, R. R., S. J. Shah, et al. (1986). “Evaluation of anti-inflammatory property of curcumin (diferuloyl.” International Journal of Clinical Pharmacology Therapy and Toxicology 24(12): 651-654.

Scapagnini, G., R. Foresti, et al. (2002). “Caffeic acid phenethyl ester and curcumin: A novel class of heme.” Molecular Pharmacology 61(3): 554-561. Heme oxygenase-1 (HO-1) is a redox-sensitive inducible protein that provides efficient cytoprotection against oxidative stress. Curcumin, a polyphenolic natural compound that possesses anti-tumor and anti-inflammatory properties, has been reported recently to induce potently HO-1 expression in vascular endothelial cells (Free Rad Biol Med 28:1303-1312, 2000). Here, we extend our previous findings by showing that caffeic acid phenethyl ester (CAPE), another plant-derived phenolic agent, markedly increases heme oxygenase activity and HO-1 protein in astrocytes. The effect seems to be related to the peculiar chemical structures of curcumin and CAPE, because analogous antioxidants containing only portions of these two molecules were totally ineffective. At a final concentration of 30

muM, both curcumin and CAPE maximally up-regulated heme oxygenase activity while promoting marked cytotoxicity at higher concentrations (50-100 muM). Similar results were obtained with Curcumin-95, a mixture of curcuminoids commonly used as a dietary supplement. Incubation of astrocytes with curcumin or CAPE at concentrations that promoted maximal heme oxygenase activity resulted in an early increase in reduced glutathione followed by a significant elevation in oxidized glutathione contents. A curcumin-mediated increase in heme oxygenase activity was not affected by the glutathione precursor and thiol donor N-acetyl-L-cysteine. These data suggest that regulation of HO-1 expression by polyphenolic compounds is evoked by a distinctive mechanism which is not necessarily linked to changes in glutathione but might depend on redox signals sustained by specific and targeted sulfydryl groups. This study identifies a novel class of natural substances that could be used for therapeutic purposes as potent inducers of HO-1 in the protection of tissues against inflammatory and neurodegenerative conditions.

Schuhbaum, H., J. Burgermeister, et al. (2000). “Anti-inflammatory activity of Zingiber officinale extracts.” Pharmaceutical and Pharmacological Letters 10(2): 82-85. The anti-inflammatory effect of ginger root extracts could be substantiated with an acetonic extract containing essential oil components and the ginger specific compounds such as gingerols, shogaols and minor compounds like gingerenone A, [6]-gingerdiol, hexahydrocurcumin and zingerone. The total extract, in the HET-CAM-test showed a pronounced dose dependent anti-inflammatory overall effect. However, testing the purified individual components of the acetonic extract, a close relationship with a single substance or a series of homologues could not be established. It is obvious that the optimal anti-inflammatory effect is correlated with the genuine extract composition where individual components might have synergistic effects. Comparable results were obtained in the Griess-assay, where the ability to reduce the liberation of NO by the iNOS is examined. The total extract showed a dose dependent effect in this in vitro assay. Individual purified components however could not be closely correlated to the NO-interfering activity. In conclusion, the acetonic ginger extract is an interesting substrate for formulations of the appropriate anti-inflammatory remedies.

Seiler, N., C. L. Atanassov, et al. (1998). “Polyamine metabolism as target for cancer chemoprevention (Review).” International Journal of Oncology 13(5): 993-1006. The natural polyamines putrescine, spermidine and spermine are intimately involved in growth-related processes. More and more evidence indicates that the excessive accumulation of putrescine and spermidine favors malignant transformation of cells. Selective depletion of putrescine has been shown to restore in some transformed cells the normal phenotype. Inhibition of polyamine formation appears, therefore, a rational target in chemoprevention. Clinical trials with 2-(difluoromethyl)ornithine, a selective inactivator of ornithine decarboxytase, a key enzyme of polyamine biosynthesis, are promising. Structural analogs of the polyamines with polyamine-mimetic or antagonist properties, and calmodulin antagonists are other types of drugs which affect several key reactions of polyamine metabolism, and appear to be candidates for the prevention of carcinogenesis especially of the gastrointestinal tract.

Sener, B. and F. Bingol (1988). “Screening of natural sources for antiinflammatory activity (Review).” International Journal of Crude Drug Research 26(4): 197-207.

Shamon, L. A., C. Chen, et al. (1994). “A correlative approach for the identification of antimutagens that.” Anticancer Research 14(5 A): 1775-1778. Seventy natural and synthetic compounds were tested for potential to inhibit mutation induced by 7,12-dimethylbenz(a)anthracene (DMBA) in Salmonella typhimurium strain TM677. Results were compared with their ability to inhibit DMBA-induced preneoplastic lesions in a mouse mammary gland organ culture system. The response mediated by fifty-five of the test compounds was either positive or negative in both test systems, indicating that the combined use of these assays should aid in the discovery of antimutagenic agents that have cancer chemopreventive potential.

Sharma, R. A., A. J. Gescher, et al. (2001). “Familiar drugs may prevent cancer.” Postgraduate Medical Journal 77(910): 492-497. Despite positive results in large scale chemoprevention trials, many physicians are unaware of the potential cancer preventive properties of drugs in common usage. The antioestrogen tamoxifen and the selective cyclo-oxygenase-2 inhibitor celecoxib have been licensed in the USA for the chemoprevention of breast and colorectal cancers respectively in selected high risk individuals. Similarly, folate and retinol have been shown to decrease the incidence of colorectal cancer and squamous cell carcinoma of the skin respectively in large scale intervention trials. Other retinoids have proved efficacious in the tertiary chemoprevention of cancers of the breast and head/neck. Epidemiological evidence also exists in favour of aspirin, nonsteroidal anti-inflammatory drugs, and angiotensin converting enzyme inhibitors preventing certain cancers. Phytochemicals may represent less toxic alternatives to these agents. Although some of these drugs are available without prescription and most are not yet licensed for use in cancer chemoprevention, physicians and students of medicine should be aware of this accumulating evidence base. Practitioners should be amenable to patient referral to discuss complex issues such as risk estimation or potential benefit from intervention.

Shih, C. A. and J. K. Lin (1993). “Inhibition of 8-hydroxydeoxyguanosine formation by curcumin in mouse.” Carcinogenesis 14(4): 709-712. Curcumin, a pigment responsible for the yellow color of curry, has been shown to be an anti-inflammation agent, an antioxidant and an antipromoter. 8-Hydroxydeoxyguanosine (8-OH-dG), an oxidized nucleoside, may be responsible for a genetic event of tumor promotion in carcinogenesis. 8-OH-dG can be detected selectively and sensitively at the fmol level by HPLC-electrochemical detection at an applied potential of +0.8 V versus Ag/AgCl. Phorbol-12-myristate-13-acetate (PMA), a potent tumor promoter, induces lipid peroxidation and 8-OH-dG formation. Curcumin can strongly scavenge the hydroxyl radical (OH-) to prevent 8-OH-dG formation from dG (deoxyguanosine) in vitro and reduce the production of PMA-induced lipid peroxidation and 8-OH-dG in mouse fibroblast cells. These results suggest that curcumin inhibits the PMA-induced tumor promotion by functioning as an OH- radical scavenger to prevent 8-OH-dG formation within the DNA molecule.

Shureiqi, I., P. Reddy, et al. (2000). “Chemoprevention: General perspective.” Critical Reviews in Oncology/Hematology 33(3): 157-167. Chemoprevention is the use of natural or synthetic compounds to block, reverse, or prevent the development of invasive cancers. Cellular carcinogenesis forms the biologic basis for the identification of chemopreventives, assessment of their activity, and ultimately the success or failure of a chemopreventive. Chemopreventive agents undergo multistep evaluations to assess efficacy that are similar in concept but vastly different in practice to standard ablative oncologic therapeutics. In vitro assessments of potential anticarcinogenesis efficacy include measurements of an agent's antioxidant activity, induction of phase II metabolizing enzymes and effects upon cellular proliferation and apoptotic control pathways. In vivo efficacy is assessed primarily in rodent models of carcinogenesis that are specific for a given organ target. The role of genetically modified animal models in the in vivo assessment of chemoprevention agents remains unclear. Clinical assessment of chemopreventive agent efficacy consists of a multistep process of identification of an optimal chemopreventive agent (phase 1), demonstration of efficacy in humans through the modulation of reversal of a tissue, biochemical, and molecular surrogates for neoplastic transformation and invasion (phase 2) and cancer risk reduction in large cohort trials (phase 3). Opportunities and future needs include the development of reliable, predictive in vivo models of carcinogenesis, careful exploration of the preventive pharmacology of therapeutic agents being used for non-cancer prevention indications, and the incorporation of genetic risk cohorts to define cancer chemopreventive efficacy. Copyright (C) 2000 Elsevier Science Ireland Ltd.

Shylesh, B. S. and J. Padikkala (1999). “Antioxidant and anti-inflammatory activity of Emilia sonchifolia.” Fitoterapia 70(3): 275-278. Fresh juice and methanolic extract of Emilia sonchifolia leaves were found to be potent inhibitors of hydroxyl radical formation and superoxide radical generation in vitro. The methanolic extract inhibited the carrageenan-induced oedema.

Sidhu, G. S., H. Mani, et al. (1999). “Curcumin enhances wound healing in streptozotocin induced diabetic rats.” Wound Repair and Regeneration 7(5): 362-374. Tissue repair and wound healing are complex processes that involve inflammation, granulation and tissue remodeling. Interactions of different cells, extracellular matrix proteins and their receptors are involved in wound healing, and are mediated by cytokines and growth factors. Previous studies from our laboratory have shown that curcumin (diferuloylmethane), a natural product obtained from the rhizomes of Curcuma longa, enhanced cutaneous wound healing in rats and guinea pigs. In this study, we have evaluated the efficacy of curcumin treatment by oral and topical applications on impaired wound healing in diabetic rats and genetically diabetic mice using a full thickness cutaneous punch wound model. Wounds of animals treated with curcumin showed earlier re-epithelialization, improved neovascularization, increased migration of various cells including dermal myofibroblasts, fibroblasts, and macrophages into the wound bed, and a higher collagen content. Immunohistochemical localization showed an increase in transforming growth factor-beta1 in curcumin-treated wounds compared to controls. Enhanced transforming growth factor-beta1 mRNA expression in treated wounds was confirmed by in situ hybridization, and laser scan cytometry. A delay in the apoptosis

patterns was seen in diabetic wounds compared to curcumin treated wounds as shown by terminal deoxynucleotidyl transferase- mediated deoxyuridyl triphosphate nick end labeling analysis. Curcumin was effective both orally and topically. These results show that curcumin enhanced wound repair in diabetic impaired healing, and could be developed as a pharmacological agent in such clinical settings.

Siegers, C. P., M. Deters, et al. (1997). "Choleretic properties of different curcuminoids in the rat bile-fistula." Pharmaceutical and Pharmacological Letters 7(2-3): 87-89. Curcumin is, besides other components, the main pharmacological principle in Curcuma xanthorrhiza Rhizomes (Zingiberaceae) while the compound bisdesmethoxycurcumin was detected only in Curcuma domestica Rhizoma. The traditional use of extracts of Curcuma is the treatment of diseases of the gastrointestinal tract, in particular of the liver and the bile-duct system. Curcumin is believed to exert choleretic properties whereas bisdesmethoxycurcumin was found to inhibit the bile flow in rats. To test these former observations, we performed experiments in the well-known bile-fistula model in male wistar rats under methane anaesthesia. Different curcuminoids were given intravenously at a dose of 25 mg/kg b.w. Bile flow and excretion of bile acids were measured over a period of 2 hrs. Both curcumin and bisdesmethoxycurcumin showed pronounced choleretic effects increasing the bile flow by 80% (curcumin) and 120% (bisdesmethoxycurcumin), respectively.

Siegfried, J. M. (1998). "Biology and chemoprevention of lung cancer." Chest 113(1 SUPPL.): 40S-45S. Advances in cell and molecular biology have increased our understanding of the multiple events that lead to the development of lung cancer. The field cancerization theory suggests that multiple genetic abnormalities occur throughout the respiratory epithelium as a result of long-term carcinogen exposure. Because of this diffuse injury throughout the lung, systemic therapy that could halt or reverse the development of cancerous changes may be effective in preventing lung cancer. This article summarizes the chemoprevention agents that have been used in clinical trials to prevent lung cancer of the head and neck. Biomarkers that have been suggested as intermediate end points in evaluating the effectiveness of chemoprevention agents are also discussed.

Singh, S. and B. B. Aggarwal (1995). "Activation of transcription factor NF-kappaB is suppressed by curcumin." Journal of Biological Chemistry 270(42): 24995-25000. When activated, NF-kappaB, a ubiquitous transcription factor, binds DNA as a heterodimeric complex composed of members of the Rel/NF-kappaB family of polypeptides. Because of its intimate involvement in host defense against disease, this transcription factor is an important target for therapeutic intervention. In the present report we demonstrate that curcumin (diferuloylmethane), a known anti-inflammatory and anticarcinogenic agent, is a potent inhibitor of NF-kappaB activation. Treatment of human myeloid ML-1a cells with tumor necrosis factor (TNF) rapidly activated NF-kappaB, which consists of p50 and p65 subunits, and this activation was inhibited by curcumin. AP-1 binding factors were also found to be down-modulated by curcumin, whereas the Sp1 binding factor was unaffected. Besides TNF, curcumin also blocked phorbol ester- and hydrogen peroxide-mediated activation of NF-kappaB. The TNF-dependent phosphorylation and degradation

of IkappaBalpha was not observed in curcumin-treated cells; the translocation of p65 subunit to the nucleus was inhibited at the same time. The mechanism of action of curcumin was found to be different from that of protein tyrosine phosphatase inhibitors. Our results indicate that curcumin inhibits NF-kappaB activation pathway at a step before IkappaBalpha phosphorylation but after the convergence of various stimuli.

Sreejayan and M. N. A. Rao (1997). “Nitric oxide scavenging by curcuminoids.” Journal of Pharmacy and Pharmacology 49(1): 105-107. Because curcumin, a compound with anti-inflammatory and anticancer activity, inhibits induction of nitric oxide synthase in activated macrophages and has been shown to be a potent scavenger of free radicals we have investigated whether it can scavenge nitric oxide directly. Curcumin reduced the amount of nitrite formed by the reaction between oxygen and nitric oxide generated from sodium nitroprusside. Other related compounds, e.g. demethoxycurcumin, bisdemethoxycurcumin and diacetylcurcumin were as active as curcumin, indicating that the methoxy and the phenolic groups are not essential for the scavenging activity. The results indicate curcumin to be a scavenger of nitric oxide. Because this compound is implicated in inflammation and cancer, the therapeutic properties of curcumin against these conditions might be at least partly explained by its free-radical scavenging properties, including those toward nitric oxide.

Sreejayan and M. N. A. Rao (1997). “Nitric oxide scavenging by curcuminoids.” Journal of Pharmacy and Pharmacology 49(1): 105-107. Curcumin is the major pigment of Curcuma longa rhizomes and has been shown to be a potent scavenger of free radicals. Curcumin (25 micro M) reduced the amount of nitrite formed by the reaction between oxygen and nitric oxide generated from sodium nitroprusside. Other related compounds (e.g. demethoxycurcumin, bisdemethoxycurcumin and diacetylcurcumin) were as active as curcumin, indicating that the methoxy and phenolic groups are not essential for the nitric oxide scavenging activity. Because nitric oxide is implicated in inflammation and cancer, the therapeutic properties of curcumin against these conditions might be at least partly explained by its free-radical scavenging properties, including those toward nitric oxide.

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Sreejayan, N. and M. N. A. Rao (1996). “Free radical scavenging activity of curcuminoids.” Arzneimittel-Forschung/Drug Research 46(2): 169-171. Three natural

curcuminoids (curcumin (CAS 458-37-7), demethoxycurcumin, bisdemethoxycurcumin) and acetylcurcumin were compared for their ability to scavenge superoxide radicals and to interact with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radicals. The results showed that curcumin is the most potent scavenger of superoxide radicals followed by demethoxycurcumin and bisdemethoxycurcumin. Acetylcurcumin was inactive. Interaction with DPPH showed a similar activity profile. The study indicates that the phenolic group is essential for the free radical scavenging activity and presence of methoxy group further increases the activity.

Srihari Rao, T., N. Basu, et al. (1982). "Anti-inflammatory activity of curcumin analogues." Indian Journal of Medical Research 75(4): 574-578.

Srimal, R. C. (1997). "Turmeric: A brief review of medicinal properties." Fitoterapia 68(6): 483-493. Turmeric has been attributed a number of medicinal properties in the traditional system of medicine and its internal as well local use has been advocated. The major claims have been for use as antiseptic, cure for poisoning, eliminating body waste products, for dyspesia, respiratory disorders and cure for a number of skin diseases including promotion of wound healing. Recent studies have confirmed some of the older claims and brought out several new useful properties. Curcumin, curcuminoids and essential oils are the major active constituents. The main activities have been found to be anti-inflammatory, hepatoprotective, antimicrobial, wound healing, anticancer, antitumor and antiviral. Discovery of antiviral properties in curcumin, particularly against HIV, is interesting and needs proper evaluation. The review highlights some of the newer researches which may explain the multifaceted activity of this natural product. Different extracts of turmeric and also curcumin have been tried clinically in several diseased conditions with gratifying results.

Srimal, R. C. and B. N. Dhawan (1973). "Pharmacology of diferuloyl methane (curcumin), a non steroidal anti." Journal of Pharmacy and Pharmacology 25(6): 447-452.

Srivastava, K. and P. K. Mehrotra (1978). "Effect of some nonsteroidal anti-inflammatory agents on IUCD induced." Indian Journal of Pharmacology 10(1): 21-25.

Steele, V. E., R. C. Moon, et al. (1994). "Preclinical efficacy evaluation of potential chemopreventive agents in." Journal of Cellular Biochemistry 56(SUPPL. 20): 32-54. In the NCI, Chemoprevention Branch drug development program, potential chemopreventive agents are evaluated for efficacy against chemical carcinogen-induced tumors in ainmal models. This paper summarizes the results of 144 agents in 352 tests using various animal efficacy models. Of these results, 146 were positive, representing 85 different agents. The target organs selected for the animals model are representative of high-incidence human cancers. The assays include inhibition of tumors induced by MNU in hamster trachea, DEN in hamster lung, AOM in rat colon (including inhibition of AOM-induced aberrant crypts), MAM in mouse colon, DMBA and MNU in rat mammary glands, DMBA promoted by TPA in mouse skin, and OH-BBN in mouse bladder. The agents tested may be classified into various pharmacological and chemical

structural categories that are relevant to their chemopreventive potential. These categories include antiestrogens, antiinflammatories (e.g., NSAIDs), antioxidants, arachidonic acid metabolism inhibitors, GST and GSH enhancers, ODC inhibitors, protein kinase C inhibitors, retinoids and carotenoids, organosulfur compounds, calcium compounds, vitamin Dinf 3 and analogs, and phenolic compounds (e.g., flavonoids). The various categories of compounds have different spectra of efficacy in animal models. In hamster lung, GSH-enhancing agents and antioxidants appear to have high potential for inhibiting carcinogenesis. In the colon, NSAIDs and other antiinflammatory agents appear particularly promising. Likewise, NSAIDs are very active in mouse bladder. In rat mammary glands, retinoids and antiestrogens (as would be expected) are efficacious. Several of the chemicals evaluated also appear to be promising chemopreventive agents based on their activity in several of the animal models. Particularly, the ODC inhibitor DFMO was active in the colon, mammary glands, and bladder models, while the dithiolthione, oltipraz, was efficacious in all the models listed above (i.e., lung, colon, mammary glands, skin, and bladder).

Stoner, G. D. and H. Mukhtar (1995). “Polyphenols as cancer chemopreventive agents.” Journal of Cellular Biochemistry 58(SUPPL. 22): 169-180. This article summarizes available data on the chemopreventive efficacies of tea polyphenols, curcumin and ellagic acid in various model systems. Emphasis is placed upon the anticarcinogenic activity of these polyphenols and their proposed mechanism(s) of action. Tea is grown in about 30 countries and, next to water, is the most widely consumed beverage in the world. Tea is manufactured as either green, black, or oolong; black tea represents approximately 80% of tea products. Epidemiological studies, though inconclusive, suggest a protective effect of tea consumption on human cancer. Experimental studies of the antimutagenic and anticarcinogenic effects of tea have been conducted principally with green tea polyphenols (GTPs). GTPs exhibit antimutagenic activity in vitro, and they inhibit carcinogen-induced skin, lung, forestomach, esophagus, duodenum and colon tumors in rodents. In addition, GTPs inhibit TPA-induced skin tumor promotion in mice. Although several GTPs possess anticarcinogenic activity, the most active is (-)- epigallocatechin-3-gallate (EGCG), the major constituent in the GTP fraction. Several mechanisms appear to be responsible for the tumor-inhibitory properties of GTPs, including enhancement of antioxidant (glutathione peroxidase, catalase and quinone reductase) and phase II (glutathione-S- transferase) enzyme activities; inhibition of chemically induced lipid peroxidation; inhibition of irradiation- and TPA-induced epidermal ornithine decarboxylase (ODC) and cyclooxygenase activities; inhibition of protein kinase C and cellular proliferation; antiinflammatory activity; and enhancement of gap junction intercellular communication. Curcumin is the yellow coloring agent in the spice turmeric. It exhibits antimutagenic activity in the Ames Salmonella test and has anticarcinogenic activity, inhibiting chemically induced preneoplastic lesions in the breast and colon and neoplastic lesions in the skin, forestomach, duodenum and colon of rodents. In addition, curcumin inhibits TPA-induced skin tumor promotion in mice. The mechanisms for the anticarcinogenic effects of curcumin are similar to those of the GTPs. Curcumin enhances glutathione content and glutathione- S-transferase activity in liver; and it inhibits lipid peroxidation and arachidonic acid metabolism in mouse skin, protein kinase C activity in TPA- treated NIH 3T3 cells, chemically induced ODC and tyrosine protein

kinase activities in rat colon, and 8-hydroxyguanosine formation in mouse fibroblasts. Ellagic acid is a polyphenol found abundantly in various fruits, nuts and vegetables. Ellagic acid is active in antimutagenesis assays, and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents, and TPA-induced tumor promotion in mouse skin. Ellagic acid functions through a variety of mechanisms, including inhibition of microsomal P-450 enzymes, stimulation of glutathione-S-transferase, scavenging the reactive metabolites of carcinogens, and direct binding to DNA, thus potentially masking sites that would normally interact with ultimate carcinogens. GTP, curcumin and ellagic acid exhibit potent antioxidant effects. This property, coupled with their other effects, make them effective chemopreventives against both the initiation and promotion/progression stages of carcinogenesis.

Sun, S. Y. (2001). "Apoptosis induction by chemopreventive agents." Drug News and Perspectives 14(2): 75-80. The goals of cancer chemoprevention are to inhibit the induction or suppress the progression of preneoplastic lesions to invasive cancer by using specific natural or synthetic chemical agents. Numerous studies have demonstrated that suppression of apoptosis or defects in apoptotic pathways contribute to expansion of initiated or aberrant clones leading to cancer development. Therefore, agents that can eliminate aberrant clones by induction of apoptosis rather than merely slowing down their proliferation may have chemopreventive potential. The increased understanding of apoptosis pathways has directed attention to components of these pathways as potential targets for not only chemotherapeutic but also chemopreventive agents. Indeed, an increasing number of previously identified chemopreventive agents including retinoids, vitamin DSUB3 analogues, triterpenoids, butyroids, monoterpenoids, nonsteroidal antiinflammatory agents and others were found recently to enhance apoptosis in a variety of premalignant or malignant cell types in vitro and in a few animal models in vivo and in clinical trials. Further understanding of the effects of potential chemopreventive agents on specific components of the pathways that lead to apoptosis may provide a rational approach to use such agents alone or in combination with other agents to enhance apoptosis as a strategy for effective chemoprevention of cancer. (c) 2001 Prous Science.

Surh, Y. (1999). "Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances." Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 428(1/2): 305-327. A review. Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. A wide array of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of these naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Capsaicin (trans-8- methyl-N-vanillyl-6-nonenamide), a pungent ingredient of hot chilli pepper, protects against experimentally-induced mutagenesis and tumorigenesis. It also induces apoptosis in various immortalized or malignant cell lines. Plants of ginger family (Zingiberaceae) have been frequently and widely used as spices and also, in traditional oriental medicine. Curcumin, a yellow ingredient from turmeric (Curcuma longa L., Zingiberaceae), has been extensively

investigated for its cancer chemopreventive potential. Yakuchinone A [1-(4'- hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanone] and yakuchinone B [1- (4'-hydroxy-3'-methoxyphenyl)-7-phenylhept-1-en-3-one] present in Alpinia oxyphylla Miquel (Zingiberaceae) have inhibitory effects on phorbol ester-induced inflammation and skin carcinogenesis in mice, and oxidative stress in vitro. These diarylheptanoids suppress phorbol ester-induced activation of ornithine decarboxylase and production of tumor necrosis factor-alpha or interleukin-1alpha and their mRNA expression. They also nullified the phorbol ester- stimulated induction of activator protein 1 (AP-1) in cultured human promyelocytic leukemia (HL-60) cells. In addition, both yakuchinone A and B induced apoptotic death in HL-60 cells. Ginger (Zingiber officinale Roscoe, Zingiberaceae) contains such pungent ingredients as [6]-gingerol and [6]-paradol, which also have anti-tumor promotional and antiproliferative effects. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin found in grapes and other dietary and medicinal plants, and (-)-epigallocatechin gallate, a major antioxidative green tea polyphenol, exert striking inhibitory effects on diverse cellular events associated with multi-stage carcinogenesis. In addition, these compounds have ability to suppress proliferation of human cancer cells via induction of apoptosis.

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Surh, Y. J., K. K. Park, et al. (1999). “Anti-tumor-promoting activities of selected pungent phenolic substances.” Journal of Environmental Pathology, Toxicology and Oncology 18(2): 131-139. Ginger (Zingiber officinale Roscoe, Zingiberaceae) has been widely used as a dietary spice, as well as in traditional oriental medicine. The rhizome of ginger contains pungent vanillyl ketones, including [6]-gingerol and [6]paradol, and has been reported to possess a strong anti-inflammatory activity. These pungent substances

have a vanilloid structure found in other chemopreventive phytochemicals, including curcumin. In our study, we found anti-tumor-promoting properties of [6]-gingerol and [6]-paradol. Thus, topical application of [6]-gingerol or [6]-paradol 30 min prior to 12-O-tetradecanoylphorbol-13-acetate (TPA) attenuated the skin papillomagenesis initiated by 7,12-dimethylbenz[a]anthracene in female ICR mice. These substances also significantly inhibited the tumor-promoter-stimulated inflammation, TNF-alpha production, and activation of epidermal ornithine decarboxylase in mice. In another study, [6]-gingerol and [6]-paradol suppressed the superoxide production stimulated by TPA in differentiated HL- 60 cells. Taken together, these findings suggest that pungent vanilloids found in ginger possess potential chemopreventive activities.

Surh, Y. J., K. S. Chun, et al. (2001). “Molecular mechanisms underlying chemopreventive activities of.” Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 480-481(-): 243-268. A wide array of phenolic substances, particularly those present in edible and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Cyclooxygenase-2 (COX-2) inducible and nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory processes. Improper up-regulation of COX-2 and/or iNOS has been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. Examples are curcumin, a yellow pigment of turmeric (Curcuma longa L., Zingiberaceae), the green tea polyphenol epigallocatechin gallate (EGCG), and resveratrol from grapes (Vitis vinifera, Vitaceae) that strongly suppress tumor promotion. Recent studies have demonstrated that eukaryotic transcription factor nuclear factor-kappa B (NF-kappaB) is involved in regulation of COX-2 and iNOS expression. Several chemopreventive phytochemicals have been shown to inhibit COX-2 and iNOS expression by blocking improper NF-kappaB activation. Multiple lines of compelling evidence indicate that extracellular-regulated protein kinase and p38 mitogen-activated protein kinase are key elements of the intracellular signaling cascades responsible for NF-kappaB activation in response to a wide array of external stimuli. Curcumin, EGCG and resveratrol have been shown to suppress activation of NF-kappaB. One of the plausible mechanisms underlying inhibition of NF-kappaB activation by aforementioned phytochemicals involves repression of degradation of the inhibitory unit IkappaBalpha, which hampers subsequent nuclear translocation of the functionally active subunit of NF-kappaB.

Surh, Y., K. Chun, et al. (2001). “Molecular mechanisms underlying chemopreventive activities of anti- inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappaB activation.” Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 480(481): 243-268. A wide array of phenolic substances, particularly those present in edible and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of

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Surh, Y., K. Chun, et al. (2001). “Molecular mechanisms underlying chemopreventive activities of anti- inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappaB activation.” Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 480(481): 243-268. A wide array of phenolic substances, particularly those present in edible and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory processes. Improper up-regulation of COX-2 and/or iNOS has been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Since inflammation is closely linked to tumour promotion, substances with potent anti-inflammatory activities are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. Examples are curcumin, a yellow pigment of turmeric (Curcuma longa), the green tea polyphenol epigallocatechin gallate (EGCG) and resveratrol from grapes (Vitis vinifera) that strongly suppress tumour promotion. Recent studies have demonstrated that eukaryotic transcription factor nuclear factor-kappa B (NF-kappaB) is involved in regulation of COX-2 and iNOS expression. Several chemopreventive phytochemicals have been shown to inhibit COX-2 and iNOS expression by blocking improper NF-kappaB activation. Multiple lines of compelling evidence indicate that extracellular-regulated protein kinase and p38 mitogen-activated protein kinase are key elements of the intracellular signalling cascades responsible for NF- kappaB activation in response to a wide array of external

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Suto, M. J. and L. J. Ransone (1997). “Novel approaches for the treatment of inflammatory diseases: Inhibitors.” Current Pharmaceutical Design 3(5): 515-528. The transcription factors (TF's) AP-1 and NF-kappaB play a critical role in the regulation of several proinflammatory cytokines and related proteins and have thus been implicated in a number of immunoinflammatory and proliferative diseases. Recently, a great deal of work has been done to elucidate the regulatory pathways involved in the activation of NF-kappaB and AP-1. In addition, several antiarthritic drugs have been shown to block the activation of these transcription factors. These observations have led to drug discovery efforts aimed toward selectively modulating these targets. A variety of compounds have been shown to block these TF's, however none has shown enough selectivity to warrant further clinical development. Recently, two new series of compounds were identified that block the activation of both NF-kappaB and AP-1 selectively in T-cells. Subsequently, they were shown to be active in several animal models of inflammation and immunosuppression. Current work is focused on identifying orally-active analogs to be used in the treatment of chronic diseases associated with an overproduction of NF-kappaB and AP-1.

Tamimi, R. M., P. Lagiou, et al. (2002). “Prospects for chemoprevention of cancer.” Journal of Internal Medicine 251(4): 286-300. The recent progress in molecular biology and pharmacology has increased the likelihood that cancer prevention will rely increasingly on interventions collectively termed 'chemoprevention'. Cancer chemoprevention is the use of agents to inhibit, delay or reverse carcinogenesis. A number of potential targets for chemoprevention have recently been identified. Many classes of agents including antioestrogens, anti-inflammatories, antioxidants and other diet-derived agents have shown a great deal of promise. In this review, we will begin by describing the general classes of chemopreventive agents and the mechanisms by which these agents act. We will then describe the opportunities that presently exist for chemoprevention of specific cancers.

Tanaka, T. (1997). “Chemoprevention of human cancer: Biology and therapy.” Critical Reviews in Oncology/Hematology 25(3): 139-174.

Thampatty, B. P. and H. S. Rosenkranz (2002). “Structural concepts in cancer prevention.” European Journal of Cancer Prevention 11(SUPPL. 2): S76-S85. The notion of developing cancer preventative strategies is attractive both from a public health and from a health economic viewpoint. However, as currently visualized, this may involve dietary supplementation of publicly available foods or the ingestion of specific supplements for prolonged periods of time. In view of the fact that the outcome of such preventative strategies may as yet not be known, it is essential that the strategy is devoid of risks. Structure-activity relationship (SAR) concepts can be of use in identifying

possible health hazards associated with chemoprevention. Overall, SAR can be used (1) to predict the chemopreventative potential of a chemical and to understand its mechanism of action, (2) to evaluate the toxicological liabilities of such agents and (3) to design molecules with enhanced chemopreventative potency and decreased (or abolished) toxicity. While SAR techniques currently available are appropriate to achieve these aims, the primary block to their widespread deployment is lack of sufficient experimental data of acceptable quality to perform SAR modeling. The present report analyzes the current applicability of SAR methods to cancer chemoprevention. (c) 2002 Lippincott Williams & Wilkins.

Thesen, R., M. Schulz, et al. (1993). “Totally or partly 'negative' drugs.” Pharmazeutische Zeitung 138(15): 40+42+45-46.

Thun, M. J., S. J. Henley, et al. (2002). “Nonsteroidal anti-inflammatory drugs as anticancer agents: Mechanistic.” Journal of the National Cancer Institute 94(4): 252-266. Numerous experimental, epidemiologic, and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promise as anticancer agents. NSAIDs restore normal apoptosis in human adenomatous colorectal polyps and in various cancer cell lines that have lost adenomatous polyposis coli gene function. NSAIDs also inhibit angiogenesis in cell culture and rodent models of angiogenesis. Many epidemiologic studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and, to some extent, other cancers. Two NSAIDs, sulindac and celecoxib, have been found to inhibit the growth of adenomatous polyps and cause regression of existing polyps in randomized trials of patients with familial adenomatous polyposis (FAP). However, unresolved questions about the safety, efficacy, optimal treatment regimen, and mechanism of action of NSAIDs currently limit their clinical application to the prevention of polyposis in FAP patients. Moreover, the development of safe and effective drugs for chemoprevention is complicated by the potential of even rare, serious toxicity to offset the benefit of treatment, particularly when the drug is administered to healthy people who have low annual risk of developing the disease for which treatment is intended. This review considers generic approaches to improve the balance between benefits and risks associated with the use of NSAIDs in chemoprevention. We critically examine the published experimental, clinical, and epidemiologic literature on NSAIDs and cancer, especially that regarding colorectal cancer, and identify strategies to overcome the various logistic and scientific barriers that impede clinical trials of NSAIDs for cancer prevention. Finally, we suggest research opportunities that may help to accelerate the future clinical application of NSAIDs for cancer prevention or treatment.

Turini, M. E. and R. N. DuBois (2002). “Primary prevention: Phytoprevention and chemoprevention of colorectal.” Hematology/Oncology Clinics of North America 16(4): 811-840. Considering the various stages of carcinogenesis and the numerous tumor types and available chemoprevention agents, knowledge of the etiology and the type of cancer to be treated, or possibly prevented, and understanding of the mechanisms by which agents exert their chemoprevention benefits may provide for improved strategy in

designing therapeutic regimens. Because cancer usually develops over a 10- to 20-year period, it may be necessary for some agents to be provided before or early in the initiation steps of carcinogenesis to have beneficial effects. On the other hand, some agents may be more suitable for CRC prevention if provided at a later stage of carcinogenesis. Gene array, genomics, and proteomics are useful tools in advancing our understanding of the molecular events involved in carcinogenesis and in identifying markers of risk and surrogate end-points for colorectal cancer progression. These techniques may also serve for screening, identifying, and providing treatment targets for high-risk patient populations. Treatment could be developed depending on a patient's individual needs and genomic tumor profile. Clinical markers and surrogate end-points should be considered, together with molecular measurements, to more accurately assess risk. NSAIDs and COXIBs are clinically recognized as chemoprevention agents, and clinical trials evaluating their efficacy are ongoing. Treatment protocols, including dose and timing, remain to be determined, however. DFMO may best be used in combination with other chemoprevention agents. Dietary fiber and calcium supplements, as part of an overall low-fat diet, may decrease CRC risk. Long-term compliance with this regimen may be necessary to effect a beneficial outcome. Folate holds promise but needs further investigation, especially because its beneficial effects may depend on cancer type. Phytochemicals have been identified as strong candidates for use as agents to prevent colorectal cancer in cell culture and in rodent models of carcinogenesis. Their potential as chemoprevention agents must be demonstrated in clinical trials. In vitro and animal studies indicate that combination therapy may be a promising strategy over the monotherapy approach; clinical trials addressing the safety and efficacy of some combinations (DFMO/sulindac, fiber/calcium) are underway. The gastrointestinal tract and other organs are constantly exposed to a mixture of potentially toxic compounds and molecules considered favorable to health. Homeostasis between stress-mediated by toxic compounds and defensive mechanisms, is key for the maintenance of health and the prevention of disease. Whereas aggressive pharmacologic treatment may be necessary for patients at high risk for cancer, dietary supplements may be useful for populations at normal risk. The message for cancer prevention in the general population may well remain: keep a balanced healthy diet, eating a variety from all food groups, as part of a healthy lifestyle that includes moderate exercise.

Umar, A., J. L. Viner, et al. (2002). “Chemoprevention of colorectal carcinogenesis.” International Journal of Clinical Oncology 7(1): 2-26. The field of cancer prevention is advancing rapidly, largely owing to post-genomic technology that has revolutionized our ability to identify and characterize molecular profiles for cancer. Advances in colorectal cancer screening (e.g., endoscopy, fecal occult blood testing, and mutational analysis) have made the detection and eradication of preinvasive neoplastic lesions the standard of care. Basic and translational sciences are building on these advances, and continue to expose molecular hallmarks of carcinogenesis that can be exploited as targets for molecularly targeted preventive interventions (i.e., chemoprevention). These targets will help identify more effective and better tolerated preventive agents. Carcinogenesis is now recognized as a disease in itself and has become the target of an ever-expanding array of preventive interventions.

Vaillend, C., C. Rampon, et al. (2002). “Gene control of synaptic plasticity and memory formation: Implications.” Current Molecular Medicine 2(7): 613-628. There has been nearly a century of interest in the idea that information is stored in the brain as changes in the efficacy of synaptic connections between neurons that are activated during learning. The discovery and detailed report of the phenomenon generally known as long-term potentiation opened a new chapter in the study of synaptic plasticity in the vertebrate brain, and this form of synaptic plasticity has now become the dominant model in the search for the cellular and molecular bases of learning and memory. Accumulating evidence suggests that the rapid activation of the genetic machinery is a key mechanism underlying the enduring modification of neural networks required for the laying down of memory. Here we briefly review these mechanisms and illustrate with a few examples of animal models of neurological disorders how new knowledge about these mechanisms can provide valuable insights into identifying the mechanisms that go awry when memory is deficient, and how, in turn, characterisation of the dysfunctional mechanisms offers prospects to design and evaluate molecular and biobehavioural strategies for therapeutic prevention and rescue.

Van Baar, B. L. M., J. Rozendal, et al. (1998). “Electron ionization mass spectrometry of curcumin analogues: An olefin.” Journal of Mass Spectrometry 33(4): 319-327. The natural compound curcumin, used in cosmetics, traditional medicines and as a spice in food, is known as a multi-factorial anti-inflammatory agent. To study the anti-inflammatory activity of curcumin derivatives, 24 analogues were synthesized and their structures were confirmed by sup 1H NMR and electron ionization (EI) mass spectrometry. Most signals in the EI mass spectra can be attributed to commonly known fragmentations, but the formation of ring-substituted 1,2-diphenylethene (stilbene)-type radical cations, observed in the spectra of all compounds investigated and resulting in the base peak for some compounds, requires a peculiar rearrangement. Metastable ion spectra and sup 1sup 3C labelling studies show that the stilbene-type ions are formed directly from the molecular ions and contain the two original aryl groups and the 1 and 7 carbon atoms of the olefinic system. It is proposed that the formation of stilbene-type ions results from an intramolecular olefin metathesis reaction; this suggestion is supported by semi-empirical (MNDO/PM3) calculations.

Venkatesan, N. (1999). “Pulmonary protective effects of curcumin against paraquat toxicity.” Life Sciences 66(2): L-21-PL-28. An early feature of paraquat (PQ) toxicity is the influx of inflammatory cells, releasing proteolytic enzymes and oxygen free radicals, which can destroy the lung epithelium and result in pulmonary fibrosis. Therefore, the ability to suppress early lung injury seems to be an appropriate therapy of pulmonary damage before the development of irreversible fibrosis. Here I show curcumin confers remarkable protection against PQ lung injury. A single intraperitoneal injection of PQ (50 mg/kg) resulted in a significant rise in the levels of protein, angiotensin converting enzyme (ACE), alkaline phosphatase (AKP), N-acetyl-beta-D-glucosaminidase (NAG) and thiobarbituric acid reactive substances (TBARS), and neutrophils in the bronchoalveolar lavage fluid (BALF), while a decrease in glutathione levels. In paraquat rats bronchoalveolar lavage (BAL) cell TBARS concentration was increased with a simultaneous decrease in glutathione content. In addition, the data also demonstrated that

PQ caused a decrease in ACE and glutathione levels and an increase in levels of TBARS and myeloperoxidase (MPO) activity in the lung. Interestingly, curcumin prevented the general toxicity and mortality induced by PQ and blocked the rise in BALF protein, ACE, AKP, NAG TBARS and neutrophils. Similarly, curcumin prevented the rise in TBARS content in both BAL cell and lung tissue and MPO activity of the lung. In addition, PQ induced reduction in lung ACE and BAL cell and lung glutathione levels was abolished by curcumin treatment. These findings indicate that curcumin has important therapeutic implications in facilitating the early suppression of PQ lung injury.

Venkatesan, N. and G. Chandrakasan (1995). "Modulation of cyclophosphamide-induced early lung injury by curcumin." Molecular and Cellular Biochemistry 142(1): 79-87. Cyclophosphamide causes lung injury in rats through its ability to generate free radicals with subsequent endothelial and epithelial cell damage. In order to observe the protective effects of a potent anti- inflammatory antioxidant, curcumin (diferuloyl methane) on cyclophosphamide- induced early lung injury, healthy, pathogen free male Wistar rats were exposed to 20 mg/ 100 g body weight of cyclophosphamide, intraperitoneally as a single injection. Prior to cyclophosphamide intoxication oral administration of curcumin was performed daily for 7 days. At various time intervals (2, 3, 5 and 7 days post insult) serum and lung samples were analyzed for angiotensin converting enzyme, lipid peroxidation, reduced glutathione and ascorbic acid. Bronchoalveolar lavage fluid was analyzed for biochemical constituents. The lavage cells were examined for lipid peroxidation and glutathione content. Excised lungs were analyzed for antioxidant enzyme levels. Biochemical analyses revealed time course increases in lavage fluid total protein, albumin, angiotensin converting enzyme (ACE), lactate dehydrogenase, N-acetyl-beta-D-glucosaminidase, alkaline phosphatase, acid phosphatase, lipid peroxide levels and decreased levels of glutathione (GSH) and ascorbic acid 2, 3, 5 and 7 days after cyclophosphamide intoxication. Increased levels of lipid peroxidation and decreased levels of glutathione and ascorbic acid were seen in serum, lung tissue and lavage cells of cyclophosphamide groups. Serum angiotensin converting enzyme activity increased which coincided with the decrease in lung tissue levels. Activities of antioxidant enzymes were reduced with time in the lungs of cyclophosphamide groups. However, a significant reduction in lavage fluid biochemical constituents, lipid peroxidation products in serum, lung and lavage cells with concomitant increase in antioxidant defense mechanisms occurred in curcumin fed cyclophosphamide rats. Therefore, our results suggest that curcumin is effective in moderating the cyclophosphamide induced early lung injury and the oxidant-antioxidant imbalance was partly abolished by restoring the glutathione (GSH) with decreased levels of lipid peroxidation.

Venkatesan, N., V. Punithavathi, et al. (1997). "Curcumin protects bleomycin-induced lung injury in rats." Life Sciences 61(6): L51-PL58. The present study was designed to determine the protective effects of curcumin against bleomycin (BLM)-induced inflammatory and oxidant lung injury. The data indicate that BLM-mediated lung injury resulted in increases in lung lavage fluid biomarkers such as total protein, angiotensin-converting enzyme (ACE), lactate dehydrogenase (LDH), N-acetyl-beta-D-glucosaminidase (NAG), lipid peroxidation (LPO) products, superoxide dismutase (SOD) and catalase. Bleomycin administration also resulted in increased levels of

malondialdehyde (MDA) in bronchoalveolar lavage fluid (BALF) and bronchoalveolar lavage (BAL) cells and greater amounts of alveolar macrophage (AM) superoxide dismutase activity. By contrast, lower levels of reduced glutathione (GSH) were observed in lung lavage fluid, BAL cells and AM. Stimulated superoxide anion and hydrogen peroxide release by AM from BLM rats were found to be higher. Curcumin treatment resulted in a significant reduction in lavage fluid biomarkers. In addition, curcumin treatment resulted in the restoration of antioxidant status in BLM rats. These data suggest that curcumin treatment reduces the development of BLM-induced inflammatory and oxidant activity. Therefore, curcumin offers the potential for a novel pharmacological approach in the suppression of drug or chemical-induced lung injury.

Wang, H. K. (2000). "The therapeutic potential of flavonoids." Expert Opinion on Investigational Drugs 9(9): 2103-2119. Four most widely investigated flavonoids, flavopiridol, catechins, genistein and quercetin are reviewed in this article. Flavopiridol is a novel semisynthetic flavone analogue of rohitukine, a leading anticancer compound from an Indian tree. Flavopiridol inhibits most cyclin-dependent kinases and displays unique anticancer properties. It is the first cyclin-dependent kinase inhibitor to be tested in Phase II clinical trials. Catechin and its gallate are major ingredients in green tea and their anti-oxidant and cancer preventive effects have been widely investigated. A Phase I study of green tea extract GTE-TP91 has been conducted in adult patients with solid tumours. Similarly, genistein is a major ingredient in soybean and has been shown to prevent cancer and have antitumour, anti-oxidant and anti-inflammatory effects. Two antibody-genistein conjugates, B43-genistein and EGF-genistein, are currently in clinical development for the treatment of acute lymphoblastic leukaemia and breast cancer, respectively. Finally, most recent updates of quercetin are briefly described.

Ward, C., E. R. Chilvers, et al. (1999). "NF-kappaB activation is a critical regulator of human granulocyte." Journal of Biological Chemistry 274(7): 4309-4318. During beneficial inflammation, potentially tissue-damaging granulocytes undergo apoptosis before being cleared by phagocytes in a non-phlogistic manner. Here we show that the rate of constitutive apoptosis in human neutrophils and eosinophils is greatly accelerated in both a rapid and concentration-dependent manner by the fungal metabolite gliotoxin, but not by its inactive analog methylthiogliotoxin. This induction of apoptosis was abolished by the caspase inhibitor zVAD-fmk, correlated with the inhibition of nuclear factor-kappa B (NF-kappaB), and was mimicked by a cell permeable inhibitory peptide of NF-kappaB, SN-50; other NF-kappaB inhibitors, curcumin and pyrrolidine dithiocarbamate; and the proteasome inhibitor, MG-132. Gliotoxin also augmented dramatically the early (2-6 h) pro-apoptotic effects of tumor necrosis factor-alpha (TNF-alpha) in neutrophils and unmasked the ability of TNF- alpha to induce eosinophil apoptosis. In neutrophils, TNF-alpha caused a gliotoxin- inhibitable activation of an inducible form of NF-kappaB, a response that may underlie the ability of TNF-alpha to delay apoptosis at later times (12-24 h) and limit its early killing effect. Furthermore, cycloheximide displayed a similar capacity to enhance TNF-alpha induced neutrophil apoptosis even at time points when cycloheximide alone had no proapoptotic effect, suggesting that NF-kappaB may regulate the production of protein(s) which protect neutrophils from the cytotoxic effects of TNF-alpha. These data shed light on the

biochemical and molecular mechanisms regulating human granulocyte apoptosis and, in particular, indicate that the transcription factor NF-kappaB plays a crucial role in regulating the physiological cell death pathway in granulocytes.

Wargovich, M. J. (2001). “Herbals and cancer.” Advances in Experimental Medicine and Biology 492(-): 195-202.

Wargovich, M. J., C. D. Chen, et al. (1996). “Aberrant crypts as a biomarker for colon cancer: Evaluation of.” Cancer Epidemiology Biomarkers and Prevention 5(5): 355-360.

We assessed the effects of 41 potential chemopreventive agents in the F344 rat using the inhibition of carcinogen-induced aberrant crypt foci (ACF) in the colon as the measure of efficacy. ACF were induced by the carcinogen azoxymethane in F344 rats by two sequential weekly injections at a dose of 15 mg/kg. Two weeks after the last azoxymethane injection, animals were evaluated for the number of aberrant crypts detected in methylene blue- stained whole mounts of rat colon. The 41 agents were derived from a priority listing that was based on reports of chemopreventive activity in the literature and/or efficacy data from in vitro models of carcinogenesis. The list of agents included representative examples of phytochemicals, vitamins, minerals, inhibitors of proliferation, inducers of Phase 1 and Phase 2 metabolism systems, nonsteroidal anti-inflammatory agents, and differentiation agents. Eighteen agents were positive in the assay, significantly reducing the incidence of ACF at least in one of two doses tested. As a chemical class, the nonsteroidal anti-inflammatory drugs, which included ibuprofen, ketoprofen, piroxicam, and indomethacin, were most active; other less potent agents were arginine, butylated hydroxyanisole, curcumin, diallyl sulfide, difluoromethylornithine, 18beta-glycyrrhetinic acid, indole-3-carbinol, oltipraz, purpurin, rutin, and the sodium salts of butyrate, selenite, and thiosulfate. Twenty-three agents did not inhibit ACF; included among these were several agents that promoted the development of ACF at one or both doses tested: benzyl isothiocyanate, calcium glucarate, catechin, dihydroepiandosterone, fluocinolone acetonide, folio acid, levamisole, 2-mercaptoethanesulfonic acid, nordihydroguiaretic acid, potassium glucarate, propyl gallate, beta-sitosterol, sodium cromolyn, sodium molybdate, and sulfasalazine. The aberrant crypt assay demonstrates reasonable specificity and sensitivity in predicting which agents are likely to prevent colon cancer.

Wargovich, M. J., C. Harris, et al. (1992). “Growth kinetics and chemoprevention of aberrant crypts in the rat colon.” Journal of Cellular Biochemistry 50(SUPPL. G): 51-54.

Single and multiple colonic crypts exhibiting dysplasia that are detectable in situ by staining of rat colon with methylene blue are called aberrant crypts (AC) and may serve as an intermediate marker for colon cancer. In a characterization study, we have established the kinetics of AC growth and development over a period of 20 d following injection of rats with the carcinogen azoxymethane (AOM). AC are not present at 5 d post-injection, but are a constant feature at 10 d and thereafter. Multiple AC, presumably clonal, begin to evolve at 10 d and are consistent by 20 d, forming incipient microadenomata. We have examined 20 candidate chemopreventive agents for inhibition of AC. All agents were given in AIN-76 diet, at two dose levels, with injections of AOM. AC were measured after 5 weeks of growth. Among the most active AC-inhibiting agents were BHA, DFMO, quercetin, diallyl sulfide, 18beta-glycyrrhetinic acid, and ascohbyl

palmitate. In a postinitiation study, the differentiating agent sodium butyrate was ineffective, but piroxicam was highly effective in modulating AC growth. Further, piroxicam inhibited AC development at all stages of growth from single to polycryptal clusters of AC. The AC assay shows marked sensitivity and specificity for screening agents for chemoprevention of colon cancer.

Wu, M. and C. Huang (2001). "Inhibition of prostaglandin E2 production of a macrophage cell line by some phytochemicals." Food Science and Agricultural Chemistry 3(2): 59-71. Prostaglandin E2 is a well-known proinflammatory mediator and promoter of some tumours. To test the potentially anti-inflammatory activity of some phytochemicals commonly found in foods, an in vitro cell culture model using a macrophage cell line RAW264.7 was employed. The PGE2 production in RAW264.7 cells stimulated by LPS was dose-dependently inhibited by apigenin, curcumin, resveratrol, naringenin and quercetin with IC50s of 1.9, 9.7, 13.9, 36.7 and 23.3 micro M, respectively. Some green tea polyphenols, including, (-)epicatechin (-EC), (plus or minus)catechin, (-)epigallocatechin gallate (-EGCG) and (-)epicatechin gallate (-ECG), also inhibited PGE2 production of RAW264.7 cells stimulated by LPS, while (+)EC and (-)epigallocatechin (-EGC) did not. The IC50s were 11.3 micro M for (-)EGCG, 39.1 micro M for (-)ECG, 151 micro M for (-)EC and 321 micro M for (plus or minus)catechin. Ascorbic acid exerted a weak inhibition while beta-carotene was not effective. The inhibitory effect was not accompanied by a significant change in COX-2 protein expression, as revealed by Western blot analysis. The water (WE) and ethyl acetate (EAE) extract of onion, a rich source of quercetin, also showed an inhibitory effect on PGE2 production. On the other hand, WE of celery, a rich source of apigenin, enhanced PGE2 production in the absence of LPS, and EAE of celery also enhanced PGE2 production in the presence of LPS. The anti-inflammatory activity of phytochemicals in foods merits further studies to help those suffering from chronic inflammation to ameliorate their inflammatory conditions by a proper selection of foods.

Wu, M. and C. Huang (2001). "Inhibition of prostaglandin E2 production of a macrophage cell line by some phytochemicals." Food Science and Agricultural Chemistry 3(2): 59-71. Prostaglandin E2 is a well-known proinflammatory mediator and promoter of some tumours. To test the potentially anti-inflammatory activity of some phytochemicals commonly found in foods, an in vitro cell culture model using a macrophage cell line RAW264.7 was employed. The PGE2 production in RAW264.7 cells stimulated by LPS was dose-dependently inhibited by apigenin, curcumin, resveratrol, naringenin and quercetin with IC50s of 1.9, 9.7, 13.9, 36.7 and 23.3 micro M, respectively. Some green tea polyphenols, including, (-)epicatechin (-EC), (plus or minus)catechin, (-)epigallocatechin gallate (-EGCG) and (-)epicatechin gallate (-ECG), also inhibited PGE2 production of RAW264.7 cells stimulated by LPS, while (+)EC and (-)epigallocatechin (-EGC) did not. The IC50s were 11.3 micro M for (-)EGCG, 39.1 micro M for (-)ECG, 151 micro M for (-)EC and 321 micro M for (plus or minus)catechin. Ascorbic acid exerted a weak inhibition while beta-carotene was not effective. The inhibitory effect was not accompanied by a significant change in COX-2 protein expression, as revealed by Western blot analysis. The water (WE) and ethyl acetate (EAE) extract of onion, a rich source of quercetin, also showed an inhibitory effect on

PGE2 production. On the other hand, WE of celery, a rich source of apigenin, enhanced PGE2 production in the absence of LPS, and EAE of celery also enhanced PGE2 production in the presence of LPS. The anti-inflammatory activity of phytochemicals in foods merits further studies to help those suffering from chronic inflammation to ameliorate their inflammatory conditions by a proper selection of foods.

Xu, Y. X., K. R. Pindolia, et al. (1997). "Curcumin, a compound with anti-inflammatory and anti oxidant." Experimental Hematology 25(5): 413-422. Chemotactic cytokines or chemokines play an important role in the regulation of myelopoiesis. Since the production of chemokines and colony stimulating factors (CSFs) by bone marrow stromal cells requires inflammatory conditions, we investigated the effect of curcumin, an agent with anti-inflammatory and anti-oxidant activities, on the expression of monocyte chemoattractant protein-1 (MCP-1 or MCP-1/JE) and interferon inducible protein-10kD (IP-10) in mouse bone marrow stromal cell line +/+-1.LDA11. Both chemokines are readily expressed in stromal cells after stimulation with pro-inflammatory interleukin-1alpha (IL-1alpha), interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha), and endotoxin lipopolysaccharide (LPS). Curcumin attenuates the levels of MCP-1/JE and IP-10 mRNA expression by all of these stimulatory agents. A detailed analysis of the regulatory effects of curcumin on chemokine expression by IL-1alpha was performed. Curcumin inhibits both chemokine mRNAs in a dose- and time-dependent manner. The suppressive effect of curcumin on both mRNAs is reversible with complete recovery from suppression within 24 hours after removal of curcumin. The suppression of mRNA by curcumin is dependent on de novo synthesis of an intermediary protein(s), since suppression is abrogated by concomitant treatment with cycloheximide (CHX). Destabilization of mRNA transcripts is not the mechanism by which curcumin lowers the levels of mRNA; however, transcripts formed in the presence of curcumin are more stable, as indicated by their slower degradation kinetics. Run-on transcriptional assays demonstrate that curcumin inhibits the transcriptional activity of both genes. Finally, the attenuation of chemokine gene expression is associated with decreased production of chemotactic activity. Together, these findings indicate that while curcumin may post-transcriptionally stabilize mRNA transcripts formed in its presence, the overall reduction in mRNA levels by curcumin is mediated by inhibition of the transcription of chemokine genes.

Yamamoto, H., K. Hanada, et al. (1997). "Inhibitory effect of curcumin on mammalian phospholipase D activity." FEBS Letters 417(2): 196-198. Curcumin, the major yellow pigment of turmeric (Curcuma longa), has strong anticarcinogenic and antiinflammatory activities. The effects of curcumin on enzyme activities of the following phospholipases were examined in a cell-free system: G protein-mediated phospholipase D (PLD), phosphatidylinositol-specific phospholipase C and phospholipase A2 from mouse macrophage-like cell line J774.1 cells, sphingomyelinase from bovine brain, and phosphatidylcholine- phospholipase C from Bacillus cereus. Curcumin inhibited several types of phospholipases, most effectively PLD among those tested. It also showed dose-dependent inhibition of PLD activation by 12-O- tetradecanoylphorbol-13-acetate in intact J774.1 cells. These results suggest that the antiinflammatory and anticarcinogenic action of curcumin is partly due to the inhibition of PLD.

Yamamoto, H., K. Hanada, et al. (1997). “Inhibitory effect of curcumin on mammalian phospholipase D activity.” FEBS Letters 417(2): 196-198. Curcumin, the major yellow pigment of turmeric (Curcuma longa), has strong anticarcinogenic and antiinflammatory activities. The effects of curcumin on enzyme activities of the following phospholipases were examined in a cell-free system: G protein-mediated phospholipase D (PLD), phosphatidylinositol-specific phospholipase C and phospholipase A2 from mouse macrophage-like cell line J774.1 cells, sphingomyelinase from bovine brain, and phosphatidylcholine- phospholipase C from Bacillus cereus. Curcumin inhibited several types of phospholipases, most effectively PLD among those tested. It also showed dose-dependent inhibition of PLD activation by 12-O- tetradecanoylphorbol-13-acetate in intact J774.1 cells. These results suggest that the antiinflammatory and anticarcinogenic action of curcumin is partly due to the inhibition of PLD.

Yamamoto, S. (1993). “tumor promotion and arachidonic acid cascades.” Folia Pharmacologica Japonica 101(6): 349-361.

Yegnanarayan, R., A. P. Saraf, et al. (1976). “Comparison of anti inflammatory activity of various extracts of Curcuma.” Indian Journal of Medical Research 64(4): 601-608.

Zhang, F., N. K. Altorki, et al. (1999). “Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and.” Carcinogenesis 20(3): 445-451. We investigated whether curcumin, a chemopreventive agent, inhibited chenodeoxycholate (CD)- or phorbol ester (PMA)-mediated induction of cyclooxygenase-2 (COX-2) in several gastrointestinal cell lines (SK-GT-4, SCC450, IEC-18 and HCA-7). Treatment with curcumin suppressed CD- and PMA-mediated induction of COX-2 protein and synthesis of prostaglandin Einf 2. Curcumin also suppressed the induction of COX-2 mRNA by CD and PMA. Nuclear run-offs revealed increased rates of COX-2 transcription after treatment with CD or PMA and these effects were inhibited by curcumin. Treatment with CD or PMA increased binding of AP-1 to DNA. This effect was also blocked by curcumin. In addition to the above effects on gene expression, we found that curcumin directly inhibited the activity of COX-2. These data provide new insights into the anticancer properties of curcumin.

Alpha-Phellandrene CAS# 99-83-2

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Ocete, M. A., S. Risco, et al. (1989). “Pharmacological activity of the essential oil of Bupleurum.” Journal of Ethnopharmacology 25(3): 305-313.

Saxena, V. K. and R. N. Sharma (1998). “Constituents of the essential oil from Commiphora mukul gum resin.” Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). “Constituents of essential oil of Myrtus communis.” Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Alpha-Pinene CAS#s 7785-70-8, 80-56-8 (hept-2-ene 2,6,6-trimethyl 2-pinene)

Arora, D., M. Kumar, et al. (2002). "Centella asiatica - A review of its medicinal uses and pharmacological." Journal of Natural Remedies 2(2): 143-149. Centella asiatica, a medicinal herb widely distributed throughout the world is popular as a traditional medicine. In Ayurveda, it is used either alone or as an important ingredient of several formulations for the management of CNS, skin and gastrointestinal diseases. Several of its traditional uses have been scientifically validated and some of the active principles have also been reported. This review focuses on the details of its medicinal uses with emphasis on the pharmacological actions.

Banthorpe, D. V., B. M. Modawi, et al. (1978). "Redox interconversions of geraniol and nerol in higher plants." Phytochemistry 17(7): 1115-1118. The use of 14C, 3H-labelled precursors showed that for plant feedings carried out in winter, isothujone (trans-thujan-3-one) was formed in Tanacetum vulgare from nerol (3,7-dimethyl-octa-cis-2,6-dien-1-ol) without loss of hydrogen from C-1 of the precursor. In contrast, formation from geraniol (the corresponding trans-isomer) involved stereospecific loss of the pro-(1S) hydrogen. This suggests that geraniol and nerol were interconverted by a redox system. However, anomalous results were obtained from similar studies at other seasons with T. vulgare, and on the biosynthesis of alpha - and beta -pinenes (pin-3-ene and pin-2-(10)-ene) in Pinus pinaster, 1,8-cineole (1,8- oxidomethane) in Mentha piperita and Eucalyptus globulus, and of carvone (menth-6,8(9)-dien-2-one) in M. spicata.

Beuscher, N., M. Kietzmann, et al. (1998). "Interference of Myrtol standardized with inflammatory and allergic mediators." Arzneimittel Forschung 48(10): 985-989. The phytomedicine-based product, Myrtol standardized (the active principle of Gelomyrtol(R)/Gelomyrtol(R) forte), containing 1,8- cineole (eucalyptol), alpha-pinene and d-limonene, inhibited the activity of 5-lipoxygenase of human basophil and eosinophil leukocytes and the formation of leukotriene C4 as well as did 1,8- cineole. It inhibited the increase in prostaglandin (PGE2) levels in mucous membranes of teat cisterns of the isolated bovine udder after topical administration of TPA (tetradecanoylphorbol-13-acetate). After topical administration into the teat cisterns, it increased the surface temperature, comparable to the effects of menthol. In vitro and in vivo studies revealed spasmolytic and broncholytic effects.

Beuscher, N., M. Kietzmann, et al. (1998). "Interference of Myrtol standardized with inflammatory and allergic mediators." Arzneimittel Forschung 48(10): 985-989. The phytomedicine-based product, Myrtol standardized (the active principle of Gelomyrtol(R)/Gelomyrtol(R) forte), containing 1,8- cineole (eucalyptol), alpha-pinene and d-limonene, inhibited the activity of 5-lipoxygenase of human basophil and eosinophil leukocytes and the formation of leukotriene C4 as well as did 1,8- cineole. It inhibited the increase in prostaglandin (PGE2) levels in mucous membranes of teat cisterns of the isolated bovine udder after topical administration of TPA (tetradecanoylphorbol-13-acetate). After topical administration into the teat cisterns, it increased the surface temperature, comparable to the effects of menthol. In vitro and in vivo studies revealed spasmolytic and broncholytic effects.

Bingol, F. and B. Sener (1995). “A review of terrestrial plants and marine organisms having.” International Journal of Pharmacognosy 33(2): 81-97.

Ceschel, G. C., P. Maffei, et al. (2000). “In vitro permeation through porcine buccal mucosa of Salvia desoleana.” International Journal of Pharmaceutics 195(1-2): 171-177. In the light of recent studies, which have shown that the essential oil derived from some Lamiaceae species has appreciable anti-inflammatory activity, moderate anti-microbial action and the ability to inhibit induced hyperalgesia, an assessment of the diffusion and permeation of Salvia desoleana Atzei and Picci (S. desoleana) essential oil through porcine buccal mucosa was considered useful for a possible application in the stomatological field. Topical formulations (microemulsions, hydrogels and microemulsion- hydrogels) were prepared for application to the buccal mucosa. The mucosa permeation of the oil from the formulations was evaluated using Franz cells, with porcine buccal mucosa as septum between the formulations (donor compartment) and the receptor phase chambers. The study also aimed at optimising the permeability of the S. desoleana essential oil by means of an enhancer, the diethylene glycol monoethyl ether Transcutol(R). The diffusion of the oil through the membrane was determined by evaluating the amount of essential oil components present in the receiving solution, the flux and the permeation coefficient (at the steady state) in the different formulations at set intervals. Qualitative and quantitative determinations were done by gas chromatographic analysis. All the formulations allow a high permeability coefficient in comparison with the pure essential oil. In particular, the components with a terpenic structure (beta-pinene, cineole, alpha-terpineol and linalool) have the highest capacity to pass through the porcine buccal mucosa when compared to the other components (linalyl acetate and alpha-terpinil acetate). Moreover, the enhancer, diethylene glycol monoethyl ether largely increases the permeation of the essential oil components in relation to the concentration. (C) 2000 Elsevier Science B.V.

Choi, H. S., H. S. Song, et al. (2000). “Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl.” J Agric Food Chem 48(9): 4156-61. Thirty-four kinds of citrus essential oils and their components were investigated for radical-scavenging activities by the HPLC method using 1,1-diphenyl-2-picrylhydrazyl (DPPH). To examine the oils' relative radical-scavenging activities compared with that of a standard antioxidant, Trolox was employed. All of the essential oils were found to have scavenging effects on DPPH in the range of 17. 7-64.0%. The radical-scavenging activities of 31 kinds of citrus essential oils were comparable with or stronger than that of Trolox ($p < 0.05$). The oils of Ichang lemon (64.0%, 172.2 mg of Trolox equiv/mL), Tahiti lime (63.2%, 170.2 mg of Trolox equiv/mL), and Eureka lemon (61.8%, 166.2 mg of Trolox equiv/mL) were stronger radical scavengers than other citrus oils. Citrus volatile components such as geraniol (87.7%, 235.9 mg of Trolox equiv/mL), terpinolene (87.4%, 235.2 mg of Trolox equiv/mL), and gamma-terpinene (84.7%, 227.9 mg of Trolox equiv/mL) showed marked scavenging activities on DPPH ($p < 0.05$).

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Cometto-Muniz, J. E., W. S. Cain, et al. (1998). “Trigeminal and olfactory chemosensory impact of selected terpenes.” Pharmacol Biochem Behav 60(3): 765-70. In Experiment 1, four normosmics and four anosmics (three congenital, one idiopathic) provided odor and nasal pungency thresholds, respectively, for the following terpenes: delta3-carene, p-cymene, linalool, 1.8-cineole, and geraniol, plus the structurally related compound cumene. Additionally, all subjects provided nasal localization (i.e., right/left) and eye irritation thresholis. Trigeminally mediated thresholds (i.e., nasal pungency, nasal localization, and eye irritation) lay about three orders of magnitude above odor thresholds, which ranged between 0.1 and 1.7 ppm. The results implied uniform chemesthetic sensitivity across tasks and sites of impact. In Experiment 2, normosmics and anosmics provided odor and nasal pungency thresholds, respectively, for three pairs of isomeric terpenes: alpha- and gamma-terpinene, alpha- and beta-pinene, and R(+)- and S(-)-limonene. Odor thresholds ranged between 1.4 and 19 ppm, that is, about an order of magnitude higher than those of the previous terpenes, with no substantial differences between odor thresholds of members of a pair. Regarding chemesthetic impact, only alpha-terpinene evoked nasal pungency. The overall outcome suggests comparable trigeminal chemosensitivity between nose and eyes and between normosmics and anosmics, as shown before for homologous n-alcohols. It also lends support to a previously derived solvation model of the chemesthetic potency of airborne substances, and indicates the likely importance of certain molecular-size restrictions for effective trigeminal impact.

Drew, A. K., I. M. Whyte, et al. (2002). “Chinese herbal medicine toxicology database: Monograph on herba asari.” Journal of Toxicology - Clinical Toxicology 40(2): 169-172.

Gurdip Singh, I. P. S. Kapoor, et al. (2000). “Studies on essential oils, part 28: chemical composition, antifungal and insecticidal activities of rhizome volatile oil of Homalomena aromatica Schott.” Flavour and Fragrance Journal 15(4): 278-280. HPLC and GC-MS analysis of rhizome oil of Homalomena aromatica showed the presence of 39 components accounting for 96.9% of the total oil. The major component was linalool (62.1%), followed by terpinen-4-ol (17.2%), alpha-terpineol (2.4%), gamma-terpinene (1.9%), alpha- cadinol (1.5%), geraniol (1.4%), nerol (1.4%), alpha-terpinene (1.0%), spatulenol (1.0%) and T-cadinol (1.0%). However, the higher percentage of linalool (87.5%) was obtained in HPLC studies. This oil showed good antifungal activity against Curvularia

pallescens [Cochliobolus pallescens], Aspergillus niger and Fusarium graminearum [Gibberella zeae] as well as also showing insecticidal behaviour against white termite (Odontotermes obesus).

Hirasuna, T. J., L. J. Pestchanker, et al. (1996). “Taxol production in suspension cultures of Taxus baccata.” Plant Cell, Tissue and Organ Culture 44(2): 95-102. The response of Taxus baccata (PC2) to basic manipulations of culture conditions is described. Suspension cultures of PC2 were maintained at 25 deg C on a modified B5 medium with two-week transfers. Under these conditions, no taxol(R) was formed. However, if the cells were left in the same medium for 7 or more additional days, taxol was produced and released (ca. 90%) into the extracellular medium. Levels as high as 13 mg/litre extracellular taxol were achieved in shake flask cultures, and taxol was the primary taxane formed representing between 50 and 80% of total taxane in the medium. The cells were sensitive to changes in culture conditions and cultures cycled through periods of high (13 mg/litre) and low (<0.1 mg/litre) levels of taxol production during extended culture. Picloram was the most effective of the auxins tested with respect to cell growth but it suppressed taxol production. Addition of fructose to moderately- productive cultures (ca. 4 mg/litre) improved taxol production, but cultures in a high producing state did not respond. Glucose suppressed taxane production. Two isoprenoids (geraniol and pinene) had a modest effect on taxol production when added to cultures at 10 mg/litre.

Houmani, Z., S. Azzoudj, et al. (2002). “The essential oil composition of Algerian zaatar: Origanum spp. and.” Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp. gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-methylether (11.4%) and thymol (26.1%); the major compounds of T. willdenowii were p-cymene (15.2%), thymol (15.1%) and carvacrol (51.3%); finally, from the two samples of T. algeriensis analyzed, one was rich in linalool (78.8%) and the other was rich in thymol (62.7%).

Inouye, S., K. Uchida, et al. (2001). “Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact.” Journal of Essential Oil Research 13(1): 68-72. The flowers of Perovskia abrotanoides and Nepeta juncea and the leaves and flowers of Thymus linearis were collected at full bloom from different areas of Karakoram district, Pakistan, then dried

and examined for essential oil composition. Dried plant parts were placed in air-tight boxes with 3 species of fungi (Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes or Trichophyton rubrum) for one, 3 and 5 days, respectively. P. abrotanoides extracts contained high concentrations of 1,8-cineole (24-27%) and alpha-pinene (18-23%) and low concentrations of borneol and bornyl acetate. N. juncea essential oils contained nepetalactone (5.8 plus or minus 0.54 mg per g of dried flowers), and several minor constituents such as nerol, 1,8-cineole, neryl acetate and an unidentified component. Two Thymus linearis chemotypes were collected; that from Hunza and Rupal valley had thymol and carvacrol as major components, and the other chemotype from the Rakaposhi area had geranyl acetate and geraniol as major components. N. juncea and Thymus linearis essential oils inhibited fungal growth by vapour contact, while P. abrotanoides showed no activity. The 2 Trichophyton species were the most susceptible and C. albicans was the least susceptible to the toxic effects of plant aromatic compounds, while A. fumigatus showed intermediate susceptibility.

Isaac, O. (1994). “Calendula officinalis L. - Marigold.” Zeitschrift fur Phytotherapie 15(6): 356-370. Marigold (Calendula officinalis L.) in the process of rediscovering natural healing forces has gained importance. Increasing significance is contributed to calendula ointments, which have been used traditionally for a long time. The range of the calendula constituents is characterized by a high level of terpenoids e.g. saponosides in the form of oleanolglycosides and triterpene alcohols. The triterpenediol-3-monoesters consist for 85% of faradiolesters. The colour of the flowers depends on their content of carotinoids. Orange flowers contain carotins, especially lycopin, while the yellow varieties predominately contain xanthophylls. Characteristic calendula-flavonoids are the isorhamnetin glycosides. Contrary to other asteraceae marigold contains no sesquiterpene lactons. Also remarkable is the fat oil of the seeds which predominately consists of the conjugated trienoic acid calendula acid. Calendula preparations are mainly used for the treatments of wounds and for cosmetical purposes. The antiinflammatory principle can be isolated by lipophilic extraction, e.g. by extraction with hypercritical carbondioxide and used as ingredient of creams and ointments.

Janardhanan, M. and J. E. Thoppil (2002). “Chemical composition of two species of Hydrocotyle (Apiaceae).” Acta Pharmaceutica 52(1): 67-69. The essential oils of two species of Hydrocotyle (Apiaceae), Hydrocotyle javanica Thunb. and H. sibthorpioides Lam., were analysed by GLC. Monoterpenes, sesquiterpenes and phenols were detected in these herbs.

Jorge Neto, J. and B. Mancini (1992). “Dialium guianense (Aubl.) Sandw., Leguminosae: chromatographic analysis of the essential oil.” Revista de Ciencias Farmaceuticas 14: 125-132. The essential oil composition of leaves of the medicinal plant D. guianense, collected in Irece, Bahia, Brazil, was determined. About 90% of the isolated compounds were identified by spectral analyses. The major components included alpha-pinene (16.74%), beta-pinene (25.64%), citronellol (19.98%), farnesol (9.03%) and geraniol (4.45%).

Kohlert, C., I. Van Rensen, et al. (2000). “Bioavailability and pharmacokinetics of natural volatile terpenes in.” Planta Medica 66(6): 495-505. Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of in vitro effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the in vitro activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.

Kuhnt, M., A. Probstle, et al. (1995). “Biological and pharmacological activities and further constituents of.” Planta Medica 61(3): 227-232. Several extracts of Hyptis verticilla and isolated compounds were evaluated for their anti-inflammatory, antibacterial, antisecretory, and cytotoxic properties. The aerial parts yielded (R)-5-hydroxypyrrolidin-2-one and essential oil with the main components alpha-pinene, beta-pinene, and thymol. Spectroscopic methods (UV, IR, sup 1H-NMR, sup 1sup 3C-NMR, mass, CD) fully characterized (R)-5-hydroxypyrrolidin-2-one, as it was isolated by a bioassay guided fractionation. The essential oil, (R)-5-hydroxypyrrolidin-2-one, as well as the previously isolated rosmarinic acid and dehydropodophyllotoxine contributed to the antibacterial effects of H. verticillata. Furthermore, rosmarinic acid showed significant capillary stabilizing effects. Sideritoflavone inhibited prostaglandin synthase to a significant extent and had antisecretory effects comparable to those of NPPB. The cytotoxicity of the aqueous extract, as demonstrated using KB and HT 29 cell lines, may be of toxicological relevance in cases of internal application.

Kuhnt, M., A. Probstle, et al. (1995). “Biological and pharmacological activities and further constituents of Hyptis verticillata.” Planta Medica 61(3): 227-232. The aerial parts of H. verticillata are used by the Mixe Indians in Oaxaca (Mexico) to treat gastrointestinal disorders, skin infections and headaches. Extracts of the aerial parts of H. verticillata plants, grown in Freiburg (Germany), and several compounds isolated from these extracts, were screened for their antibacterial, antiinflammatory, antisecretory and cytotoxic properties (data presented). Bioassay guided fractionation and spectroscopic data led to the identification of (R)-5-hydroxypyrrolidin-2-one, a compound previously only known as a synthetic racemate. The known compounds rosmarinic acid and dehydropodophyllotoxin were also identified. Steam distillation of the essential oil yielded mainly alpha-pinene, beta-pinene and thymol. All of these compounds exhibited a potent antibacterial activity against Bacillus subtilis, Micrococcus luteus and Escherichia coli. In the HET-CAM (Hen's Egg Test Chorioallantois Membrane) assay, in which inhibition of the permeability of capillary systems and capillary stabilizing actions were measured, rosmarinic acid was slightly more effective in stabilizing the chorioallantois membrane than the reference compound indomethacin (27.5 and 24.5% delay of haemorrhage, respectively). Sideritoflavone showed a 78.3% inhibition of prostaglandin synthase (IC50 value of 30 micro g/ml). The crude extract (at 500 micro g/ml) and sideritoflavone (at 0.1 mM/litre) also significantly inhibited secretion when

applied to the serosal side of isolated rabbit distal colon mounted in a modified Ussing chamber; the antisecretory activity of sideritoflavone was similar to that of NPPB (5-nitro-2-(3-phenylpropylamino)-benzoate), a known Cl--channel blocker. The crude extract, and the ethanolic and aqueous extracts (but not the aqueous residue obtained from the lyophilized plant) exhibited cytotoxic activity against HT 29 and KB cell lines (MIC values of 0.8-4.1 micro g/ml).

Kulevanova, S., M. Ristic, et al. (1997). "Composition of essential oils of Thymus tosevii ssp. tosevii and Thymus tosevii ssp. substriatus from Macedonia." Pharmazie 52(5): 382-386. T. tosevii subsp. tosevii and T. tosevii subsp. substriatus are used in traditional medicine in Macedonia against cold, flu, pulmonary infection and abdominal throes. The essential oils of T. tosevii subsp. tosevii and T. tosevii subsp. substriatus, growing wild in Macedonia, were investigated by means of GC and GC-MS. The main components of the oils were thymol, carvacrol, linalool, geraniol, terpenyl acetate, p-cymene and gamma-terpinene. The essential oil composition varied according to the origin and the year of plant collection.

Lal, R. N., T. K. Sen, et al. (1978). "Gas chromatography of the essential oil of Ocimum sanctum L." Parfum. u Kosmetik 59(7): 230-231. Water distillation of O. sanctum cultivated in India yielded 0.7% oil. Eugenol content of the oil was 70% by weight. Other constituents identified in the oil were nerol, eugenol methylether, caryophyllene, terpinene-4-ol, decylaldehyde gamma -selinene, alpha -pinene, beta - pinene, camphor and carvacrol.

Martin, S., E. Padilla, et al. (1993). "Anti-inflammatory activity of the essential oil of Bupleurum." Planta Medica 59(6): 533-536. The essential oil of Bupleurum fruticescens was investigated qualitatively and quantitatively by GC and GC-MS analyses. The anti-inflammatory activity of the whole essential oil and its major components was also investigated in the rat hindpaw edema model induced by carrageenin or by PGEinf 1. The anti-inflammatory activity shown by the essential oil can be attributed to the two major components, alpha-pinene and beta-caryophyllene. In order to know the role of the adrenal glands in the anti-inflammatory activity exerted by the two major components of the essential oil, they were studied against the carrageenin-induced hindpaw edema in adrenolectomized rats. It is concluded that alpha-pinene needs the integrity of the adrenal glands to exert its anti-inflammatory activity, as opposed to beta-caryophyllene which was also active in adrenolectomized animals.

Martin, S., E. Padilla, et al. (1993). "Anti-inflammatory activity of the essential oil of Bupleurum fruticescens." Planta Medica 59(6): 533-536. Bupleurum species, widespread in southeastern Spain, are used in folk medicine as antiinflammatory remedies. The essential oil composition of the flowering parts of B. fruticescens, collected in the Sierra Baza mountains, Spain, were investigated qualitatively and quantitatively (GC and GC-MS). Antiinflammatory activity, investigated in the rat hindpaw oedema model induced by carrageenan or by prostaglandin E1 (PGE1), was mainly due to alpha-pinene and beta-caryophyllene. The inhibitory effects of the 2 constituents were then studied against inflammation processes in female Wistar rats with or without adrenal glands. alpha-

Pinene was active against carrageenan and PGE1 induced inflammation, but its antiinflammatory activity against carrageenan disappeared in adrenolectomized rats. beta-Caryophyllene showed antiinflammatory activity in both models, and its antiinflammatory effect did not depend on the presence of adrenal glands.

Nin, S., P. Arfaioli, et al. (1995). "Quantitative determination of some essential oil components of selected Artemisia absinthium plants." Journal of Essential Oil Research 7(3): 271-277. In traditional medicine, A. absinthium is used as an anthelmintic, insecticide, stomachic, and tonic. The essential oils, steam- distilled from leaves and flowers of plants propagated from 49 mother plants obtained from Italy (21 plants), Austria (10), Germany (5), France (4) or USA (9), were analysed by GC. More than 90 compounds were detected, most of which occurred only in trace amounts. Quantitative and qualitative differences were observed in the contents of 8 antibacterial components (alpha- and beta-thujone, terpinen-4-ol, linalool, nerol, geraniol, alpha-pinene, and 1,8-cineole [eucalyptol]). These variations were observed between individual accessions, and between plants obtained from the same geographical location. The essential oils of some genotypes were characterized by particularly high percentages of active principles.

Ocete, M. A., S. Risco, et al. (1989). "Pharmacological activity of the essential oil of Bupleurum." Journal of Ethnopharmacology 25(3): 305-313.

Padula, L. Z., A. M. Collura, et al. (1977). "Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil." Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Perry, N. S. L., P. J. Houghton, et al. (2000). "In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes." Journal of Pharmacy and Pharmacology 52(7): 895-902. The effects of S. lavandulaefolia [S. lavandulifolia] essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole [eucalyptol] (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma- terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha- pinene and were 0.03 micro g/ml, 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma- terpinene) were less potent (camphor IC50 of >10 mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro

cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant

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Perry, N. S. L., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” Journal of Pharmacy and Pharmacology 53(10): 1347-1356. S. lavandulaefolia (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in vitro and in vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, antiinflammatory and oestrogenic effects. Results of in vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg ml-1) and the monoterpenoids (0.1 M) alpha- and beta- pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible antiinflammatory activity (eicosanoid inhibition in rat leukocytes) was found in the EtOH extract (50 micro g ml-1) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible oestrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg ml-1) and the monoterpenoid geraniol (0.1- 2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit oestrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Perry, N. S., P. J. Houghton, et al. (2000). “In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes.” J Pharm Pharmacol 52(7): 895-902. Sage (Salvia spp) is reputed in European herbal encyclopaedias to enhance memory, and current memory-enhancing/anti-dementia drugs are based on enhancing cholinergic activity by inhibiting cholinesterase. In this study the effects of Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil batch used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma-terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha-pinene and were 0.03 microL [corrected] mL(-1), 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma-terpinene) were less potent (camphor IC50: >10mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. These findings suggest that if the inhibitory activity of the essential oil is primarily due to the main inhibitory terpenoid constituents identified, there is a major synergistic effect among the constituents. Since no single constituent tested was

particularly potent, it remains to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S., P. J. Houghton, et al. (2001). “In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease.” J Pharm Pharmacol 53(10): 1347-56. Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in-vitro and in-vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, anti-inflammatory and estrogenic effects. Results of in-vitro tests for these activities are reported here for S. lavandulaefolia extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg mL(-1)) and the monoterpenoids (0.1 M) alpha- and beta-pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible anti-inflammatory activity (eicosanoid inhibition in rat leucocytes) was found in the EtOH extract (50 microg mL(-1)) and was shown by the monoterpenoids alpha-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible estrogenic activity (via induction of beta-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg mL(-1)) and the monoterpenoid geraniol (0.1-2 mM). 1,8-Cineole, alpha- and beta-pinene and thujone did not exhibit estrogenic activity in this analysis. These results demonstrate that S. lavandulaefolia, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Pifferi, G. (1994). “The essential oil of turpentine and its derivatives in cosmetics and pharmaceuticals.” Rivista Italiana EPPOS(14): 37-48. The essential oil of turpentine is obtained from the resin of conifers (mainly Pinus pinaster, P. sylvestris and P. mugo). Its composition and uses in cosmetic and pharmaceutical products, and in perfumery, are discussed. The main constituents of the oil are alpha- and beta-pinene (73 and 17%, respectively). Turpentine oil is used in traditional medicine to treat problems of the respiratory tract. More recently, oxygenated derivatives have been synthesized from turpentine constituents, which have analeptic and antiinflammatory properties. These compounds appear to be specific for the respiratory tract, and exhibit low levels of toxicity.

Rojas, M. C., L. Chayet, et al. (1983). “Substrate and metal specificity in the enzymic synthesis of cyclic monoterpenes from geranyl and neryl pyrophosphate.” Arch Biochem Biophys 222(2): 389-96. A partially purified enzyme (carbocyclase) from the flavedo of Citrus limonum formed alpha-pinene, beta-pinene, limonene, and gamma-terpinene from geranyl pyrophosphate (GPP) and neryl pyrophosphate. The maximum specific activities obtained were 7.0 and 3.6 nmol/min/mg, respectively. Cross-inhibition by the two substrates were observed and the ability to utilize neryl pyrophosphate was almost completely lost with aging. Citronellyl pyrophosphate and dimethylallyl pyrophosphate

were the most effective inhibitors of carbocyclase. Isopentenyl pyrophosphate, the monophosphate esters of nerol and geraniol, as well as inorganic pyrophosphate were much less effective inhibitors. The enzyme had an absolute requirement for Mn2+. It could be replaced with about 2% effectiveness by Mg2+ and Co2+. Kinetic studies showed that the observed reaction rate correlates with the calculated concentration of the GPP (Mn2+)2 species. Previous evidence with nonenzymatic reactions and the results presented support the view that the mechanism of carbocyclase may be the intramolecular analog of prenyltransferase.

Sangalli, B. C. and W. Chiang (2000). “Toxicology of nutmeg abuse.” Journal of Toxicology - Clinical Toxicology 38(6): 671-678. Background: Unpleasant and frightening side effects associated with the abuse of nutmeg occasionally generate emergency department referrals. We report a young patient's first-time experience with nutmeg and review the mechanisms of its toxicity. Case Report: A 13-year-old female ingested 15-24 g of nutmeg over a 3-hour period and smoked and shared 2 joints of marijuana. To facilitate ingestion, the nutmeg was put into 00-000 gelatin capsules. Bizarre behavior and visual, auditory, and tactile hallucinations developed. She also experienced nausea, gagging, hot/cold sensations, and blurred vision followed by numbness, double, and 'triple' vision, headache, and drowsiness. Nystagmus, muscle weakness, and ataxia were present. Her vital signs and laboratory tests were normal. She received 50 g of activated charcoal and except for complaints of dizziness and visual changes, her 2-day admission was uneventful. The central nervous system activity of nutmeg is often postulated to result from biotransformation of its chemical components to amphetamine-like compounds, but this has not been proven. Nutmeg contains several compounds with structural similarities to substances with known central nervous system neuromodulatory activity.

Saxena, V. K. and R. N. Sharma (1998). “Constituents of the essential oil from Commiphora mukul gum resin.” Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Seidakhmetova, R. B., A. A. Beisenbaeva, et al. (2002). “Chemical composition and biological activity of the essential oil from.” Pharmaceutical Chemistry Journal 36(3): 135-138.

Soon, S. L., H. S. Kuk, et al. (2002). “Effect of the essential oil from the flowers of Magnolia sieboldii on.” Planta Medica 68(5): 459-462. The essential oil from the flowers of Magnolia sieboldii was tested for its effects on lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and prostaglandin ESUB2 (PGESUB2) by rat peritoneal macrophages. It was shown to induce the production of NO and PGESUB2 in a concentration-dependent manner (3-30 mug/ml). Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) led to the identification of sixty

compounds, of which beta-elemene (18.0%), alpha-terpinene (14.83%) and beta-myrcene (12.72%) were the major constituents. Among these three compounds, alpha-terpinene was found to be the most effective one with inhibitory activity on NO and PGESUB2 production by LPS-stimulated rat peritoneal macrophages.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). "Constituents of essential oil of Myrtus communis." Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Wheeler, C. J., C. A. Mihaliak, et al. (1990). "Uncompetitive inhibition of monoterpene cyclases by an analog of the substrate geranyl pyrophosphate and inhibition of monoterpene biosynthesis in vivo by an analog of geraniol." Arch Biochem Biophys 279(2): 203-10. Monoterpene cyclases catalyze the divalent metal ion-dependent conversion of the acyclic precursor geranyl pyrophosphate to a variety of monocyclic and bicyclic monoterpene skeletons. Examination of the kinetics of inhibition of cyclization by the pyrophosphate ester of (E)-4-[2-diazo-3-trifluoropropionyloxy]-3-methyl-2-buten-1-o l, a photolabile structural analog of the substrate, using a partially purified preparation of geranyl pyrophosphate:(+)-pinene cyclase and geranyl pyrophosphate:(+)-bornyl pyrophosphate cyclase from common sage (Salvia officinalis) evidenced (under dark conditions) strictly uncompetitive inhibition with K'i values of 3.2 and 4.7 microM, respectively. These values are close to the corresponding Km values for the substrate with these two enzymes. This novel property of the substrate analog was also examined in the presence of two other inhibitors which bind to different domains of the cyclase active site (inorganic pyrophosphate and a sulfonium ion analog of a cyclic carbocationic intermediate of the reaction sequence (dimethyl-(4-methylcyclohex-3-en-1-yl)sulfonium iodide)) in order to address the mechanistic origins of the uncompetitive inhibition of cyclization. It was not possible, however, to rule out either an induced-fit mechanism or a sequential binding mechanism since the substrate is recognized by at least two binding domains and because direct examination of the effects of binding on cyclase conformation is currently not feasible. The substrate analog, although photoactive, did not give rise to light-dependent enzyme inactivation of greater magnitude than that obtained from ultraviolet light alone. The unusual behavior of the analog was attributed to intramolecular interaction of the electron-rich carbonyl group of the diazoester with the required divalent metal ion that is chelated by the pyrophosphate group. A photostable analog of geraniol that resembled the photoactive substrate analog in bearing a carbonyl function at C6 (6-oxo-3,7-dimethyloct-2(trans)en-1-ol) was prepared. Following foliar application to rapidly growing sage plants, this analog was seemingly activated to the corresponding pyrophosphate ester in vivo and selectively inhibited the activity of several cyclases in this tissue as evidenced by diminished production of the corresponding monoterpene end products.

Ziment, I. (2002). "Herbal antitussives." Pulmonary Pharmacology and Therapeutics 15(3): 327-333. The mechanisms of actions of cough medicines are not always known.

The problem is exacerbated for herbal medicines, where the effectiveness of the plant or its phytochemicals have rarely been carefully evaluated. Moreover, the most active phytomedicinal constituent is difficult to identify, and the expense and difficulty of such studies discourages sponsors who may not be able to benefit by subsequent exclusive marketing of the herbal remedy. Most popular herbs used as cough medicines appear to be demulcents whose action is confined to the oropharynx. It is probable that the vast majority of allegedly effective herbal cough medicines act as non-specific emetic-expectorants. The proof of activity of even marketed herbal derivatives such as guaifenesin and codeine is difficult to obtain. It is therefore likely that herbal cough medications will never be shown to be more active than placebos. Nevertheless, these plant products will continue to be popular remedies for patients and their health care advisors.

Beta-Caryophyllene CAS# 87-44-5 (bicyclo undec-4-ene 8-methylene)

Arora, D., M. Kumar, et al. (2002). "Centella asiatica - A review of its medicinal uses and pharmacological." Journal of Natural Remedies 2(2): 143-149. Centella asiatica, a medicinal herb widely distributed throughout the world is popular as a traditional medicine. In Ayurveda, it is used either alone or as an important ingredient of several formulations for the management of CNS, skin and gastrointestinal diseases. Several of its traditional uses have been scientifically validated and some of the active principles have also been reported. This review focuses on the details of its medicinal uses with emphasis on the pharmacological actions.

Chinou, I. B., V. Roussis, et al. (1997). "Chemical and antibacterial studies of two Helichrysum species of Greek origin." Planta Med 63(2): 181-3. The chemical composition of the essential oils obtained from the aerial parts of Helichrysum stoechas ssp. barrelieri and H. taenari was analysed by GC and GC/MS. From the thirty-nine identified constituents representing the 73.87% and 87.41% of the two oils, respectively, beta-elemene, beta-caryophyllene, geraniol, and camphene were the major components. Furthermore, it was found that the oils exhibited significant antibacterial activity against six Gram (+/-) bacteria.

Chowdhury, A. R. and V. P. Kapoor (2000). "Essential oil from the fruit of Apium graveolens." Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Houmani, Z., S. Azzoudj, et al. (2002). "The essential oil composition of Algerian zaatar: Origanum spp. and." Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp.

gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-methylether (11.4%) and thymol (26.1%); the major compounds of T. willdenowii were p-cymene (15.2%), thymol (15.1%) and carvacrol (51.3%); finally, from the two samples of T. algeriensis analyzed, one was rich in linalool (78.8%) and the other was rich in thymol (62.7%).

Isaac, O. (1994). "Calendula officinalis L. - Marigold." Zeitschrift fur Phytotherapie 15(6): 356-370. Marigold (Calendula officinalis L.) in the process of rediscovering natural healing forces has gained importance. Increasing significance is contributed to calendula ointments, which have been used traditionally for a long time. The range of the calendula constituents is characterized by a high level of terpenoids e.g. saponosides in the form of oleanolglycosides and triterpene alcohols. The triterpenediol-3-monoesters consist for 85% of faradiolesters. The colour of the flowers depends on their content of carotinoids. Orange flowers contain carotins, especially lycopin, while the yellow varieties predominately contain xanthophylls. Characteristic calendula-flavonoids are the isorhamnetin glycosides. Contrary to other asteraceae marigold contains no sesquiterpene lactons. Also remarkable is the fat oil of the seeds which predominately consists of the conjugated trienoic acid calendula acid. Calendula preparations are mainly used for the treatments of wounds and for cosmetical purposes. The antiinflammatory principle can be isolated by lipophilic extraction, e.g. by extraction with hypercritical carbondioxide and used as ingredient of creams and ointments.

Janardhanan, M. and J. E. Thoppil (2002). "Chemical composition of two species of Hydrocotyle (Apiaceae)." Acta Pharmaceutica 52(1): 67-69. The essential oils of two species of Hydrocotyle (Apiaceae), Hydrocotyle javanica Thunb. and H. sibthorpioides Lam., were analysed by GLC. Monoterpenes, sesquiterpenes and phenols were detected in these herbs.

Kedzia, B., M. Krzyzaniak, et al. (1994). "Composition and antimicrobial characteristics of Ol. Melissae and its components." Herba Polonica 40(1-2): 5-11. Ol. Melissae [the essential oil of Melissa officinalis] was analysed, and the main constituents were citronellal (25.2%), geraniol (16.4%) and citronellol (11.0%). The essential oil inhibited the growth of Staphylococcus aureus, Streptococcus faecalis and Candida albicans (MIC values of 100, 250 and 300 micro g/ml, respectively), and inhibited the growth of Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa to a lesser extent (MIC values of 500 micro g/ml). Citronellol, beta-caryophyllene, thymol, carvacrol and citronellal were the most active oil components against the microorganisms.

Lal, R. N., T. K. Sen, et al. (1978). "Gas chromatography of the essential oil of Ocimum sanctum L." Parfum. u Kosmetik 59(7): 230-231. Water distillation of O. sanctum cultivated in India yielded 0.7% oil. Eugenol content of the oil was 70% by weight. Other constituents identified in the oil were nerol, eugenol methylether, caryophyllene, terpinene-4-ol, decylaldehyde gamma -selinene, alpha -pinene, beta - pinene, camphor and carvacrol.

Limberger, R. P., M. E. G. Sobral, et al. (2001). “Biological activities and essential oil composition of leaves of.” Pharmaceutical Biology 39(4): 308-311. Infusions obtained from dried and fresh leaves of Blepharocalyx salicifolius were assessed in antibacterial (with S. aureus and E. coli), antiinflammatory, antinociceptive, antispasmodic and intestinal transit models. All samples analyzed showed significant antibacterial activity against Gram-positive and Gram-negative bacteria. The highest activity was observed with the dried leaves against E. coli. An infusion from fresh leaves inhibited the stimulating action of acetylcholine on intestinal musculature (average inhibition 45%). Other biological assays gave no significant results with doses up to 300 and 600 mg/kg for dried and fresh material, respectively. The essential oil obtained from fresh leaves by hydrodistillation (0.9%) was analyzed by GC and GC/MS, where 42 components were identified. The main components were 1,8-cineole (25.2%), linalool (20.4%) and beta-caryophyllene (22.9%).

Martin, S., E. Padilla, et al. (1993). “Anti-inflammatory activity of the essential oil of Bupleurum.” Planta Medica 59(6): 533-536. The essential oil of Bupleurum fruticescens was investigated qualitatively and quantitatively by GC and GC-MS analyses. The anti-inflammatory activity of the whole essential oil and its major components was also investigated in the rat hindpaw edema model induced by carrageenin or by PGEinf 1. The anti-inflammatory activity shown by the essential oil can be attributed to the two major components, alpha-pinene and beta-caryophyllene. In order to know the role of the adrenal glands in the anti-inflammatory activity exerted by the two major components of the essential oil, they were studied against the carrageenin-induced hindpaw edema in adrenolectomized rats. It is concluded that alpha-pinene needs the integrity of the adrenal glands to exert its anti-inflammatory activity, as opposed to beta-caryophyllene which was also active in adrenolectomized animals.

Martin, S., E. Padilla, et al. (1993). “Anti-inflammatory activity of the essential oil of Bupleurum fruticescens.” Planta Medica 59(6): 533-536. Bupleurum species, widespread in southeastern Spain, are used in folk medicine as antiinflammatory remedies. The essential oil composition of the flowering parts of B. fruticescens, collected in the Sierra Baza mountains, Spain, were investigated qualitatively and quantitatively (GC and GC-MS). Antiinflammatory activity, investigated in the rat hindpaw oedema model induced by carrageenan or by prostaglandin E1 (PGE1), was mainly due to alpha-pinene and beta-caryophyllene. The inhibitory effects of the 2 constituents were then studied against inflammation processes in female Wistar rats with or without adrenal glands. alpha-Pinene was active against carrageenan and PGE1 induced inflammation, but its antiinflammatory activity against carrageenan disappeared in adrenolectomized rats. beta-Caryophyllene showed antiinflammatory activity in both models, and its antiinflammatory effect did not depend on the presence of adrenal glands.

Tambe, Y., H. Tsujiuchi, et al. (1996). “Gastric cytoprotection of the non-steroidal anti-inflammatory.” Planta Medica 62(5): 469-470. The gastric cytoprotective effect of beta-caryophyllene (1), an anti-inflammatory sesquiterpene, was investigated in rats. The oral administration of beta-caryophyllene to rats significantly inhibited gastric mucosal injuries induced by necrotizing agents such as absolute ethanol and 0.6 N HCl, although

it failed to prevent water immersion stress- and indomethacin-induced gastric lesions. In addition, this compound hardly affected the secretion of gastric acid and pepsin. Thus, beta-caryophyllene elicited anti-inflammatory effects without any indication of gastric mucosal damage typical of non-steroidal anti-inflammatory agents. Furthermore, this compound manifested cytoprotective effects, rendering the two-dimensional efficacious beta-caryophyllene to be a clinically safe and potentially useful agent.

Tanaka, S., Y. Sakata, et al. (2001). “Influence of natural and synthetic compounds on cell surface expression.” Planta Medica 67(2): 108-113. Various natural and synthetic compounds including alkaloids, terpenoids and phenolics were tested for inhibition of the cell surface expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both of which are crucial in the regulation of immune response and inflammation. Of 40 compounds tested, two compounds significantly downregulated the expression of VCAM-1 on murine endothelial cells (F-2) and ten compounds that of ICAM-1 on mouse myeloid leukemia cells (M1). Sanguinarine chloride (5) and isoliquiritigenin (13) were capable of lowering the levels of both ICAM-1 and VCAM-1. The structure-activity relationships study on chalcone and flavone derivatives related to 13 suggested that the inhibitory activity of the chalcone derivatives is attributable to the 4-hydroxy group as well as the possible coplanarity between the phenyl ring and the adjacent conjugated ketone.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). “Constituents of essential oil of Myrtus communis.” Planta Medica 39(2): 164-167. beta-Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Beta-Citronellol CAS # 106-22-9 (Cephrol, Rodinol, Elenol, Rhodinol, 2,6 dimethyl octen, 2,3-Dihydrogeraniol)

Aggarwal, K. K., Ateeque Ahmad, et al. (2000). "Antimicrobial activity spectra of Pelargonium graveolens L. and Cymbopogon winterianus Jowitt oil constituents and acyl derivatives." Journal of Medicinal and Aromatic Plant Sciences 22(1B): 544-548. The essential oils of citronella Java (Cymbopogon winterianus) and rose geranium (Pelargonium graveolens) were partitioned into different fractions under high vacuum in a packed fractionating column for their separation into pure constituents such as citronellal, d-citronellol [(+)-citronellol], l-citronellol [(+)- citronellol] and geraniol. The formate derivatives of geraniol, l- and d-citronellol could be prepared with optimum yield and confirmed through GC. The spectra of the antimicrobial activities of the essential oils and their constituents in relation to optical isomers and their derivatives were analysed. Differential antimicrobial activities were studied through in vitro bioassays against 12 bacterial (Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis, Streptococcus mutans, Yersinia enterocolitica, Salmonella typhi, Escherichia coli and Mycobacterium smegmatis) and 7 fungal (Microsporum gypseum, Aspergillus niger, A. flavus, Trichophyton rubrum, Sporothrix schenckii, Candida albicans AI and C. albicans) strains. The distinct activity patterns indicated structure-function relationships for the optical isomers.

Binder, G., T. v. d. Berg, et al. (1996). "Regeneration of plants and production of volatiles from callus cultures of Melissa officinalis L. 3. Effect of exogenous growth regulators on essential oil composition." Angewandte Botanik 70(5/6): 181-184. Regenerates of lemon balm (M. officinalis) were established as an in vitro-system to study the effects of exogenous growth regulators (NAA, BAP [benzyladenine] and ABA) on morphology and essential oil composition. Long term cultivation resulted only in changes of minor essential oil constituents; citronellal, citronellol, nerol and geraniol contents were elevated, whereas geranyl acetate content was reduced. The essential oil composition of plants growing in the presence of NAA exhibited similar changes in citronellal, citronellol and geranyl acetate, i.e. like plants at an advanced stage of development. Supplementation of the medium with a high BAP concentration induced plants to accumulate >10 % alloaromadendrene, a sesquiterpene hydrocarbon found only as a trace compound in control plants and not described before for the leaf essential oil of naturally grown M. officinalis. ABA had a slight effect on the production of some minor compounds, and showed a synergistic effect in combination with BAP.

Chaumont, J. P. and D. Leger (1992). "[Campaign against allergenic moulds in dwellings. Inhibitor properties of essential oil of Geranium 'Bourbon', citronellol, geraniol and citral]." Ann Pharm Fr 50(3): 156-66. Many fungal airborne spora show allergenic effects. Indoor (dwelling, work rooms, hospital chambers) can be disinfected by elimination of living particles. We have undertaken experiments in more and more spacious bulks for evaluation of the antifungal effects of vapours of essential oils and some volatiles compounds. Results show that the Mucorales and Geotrichum resist strongly. On the contrary, the Cladosporium strains, some Aspergillus and Penicillium,

Trichothecium roseum are the most sensitive, specially towards the citral vapours. Experiments in hospital can be undertaken.

Dharmendra Saikia, S. P. S. Khanuja, et al. (2001). “Comparative antifungal activity of essential oils and constituents from three distinct genotypes of Cymbopogon spp.” Current Science 80(10): 1264-1266. The antifungal activity of the essential oils of palmarosa (C. martini) cv. CIMAP/PRC-1, lemon grass (C. flexuosus) cv. Pragati and citronella (C. winterianus) cv. BIO-13, as well as some essential oil components, viz. citral, geraniol, citronellol and citronellal, were tested against 4 human pathogenic fungi (Microsporum gypseum, Candida albicans, Sporothrix schenckii, Aspergillus niger) to identify plant substances for future antifungal formulations. Among the essential oils and components tested, lemon grass oil and geraniol, respectively, recorded the highest antifungal activity. M. gypseum was highly sensitive to all the essential oils and tested components, with inhibition zones 1.5- to 2-fold larger than the other fungal pathogens and generally low minimum inhibitory dilutions (MID). Citral produced the smallest inhibition zones but demonstrated the highest activity in terms of MID and minimum fungicidal concentration (MFC) values, which were comparable to lemon grass oil. Lemon grass, palmarosa oil and geraniol recorded the highest inhibition of C. albicans, A. niger and S. schenckii, respectively. However, the highest antifungal activity in terms of MFC was recorded by lemon grass for all organisms tested.

Frosch, P. J., B. Pilz, et al. (1995). “Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes.” Contact Dermatitis 33(5): 333-42. The objective of this study was to determine the frequency of reactivity to a series of commonly used fragrances in dermatological patients. A total of 48 fragrances (FF) were chosen, based on the publication of Fenn in 1989 in which the top 25 constituents of 3 types (1. perfumes, 2. household products, 3. soaps) of 400 commercial products on the US market had been determined. In a pilot study on a total of 1069 patients in 11 centres, the appropriate test concentration and vehicle were examined. For most fragrances, 1% and 5% were chosen, and petrolatum proved to be the best vehicle in comparison to isopropyl myristate and diethyl phthalate. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8% fragrance mix (FM) and its 8 constituents. In patients with a positive reaction to any of the 48 FF, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were tested in 11 centres. The 8% FM was positive in 89 patients (8.3% of 1072 patients). Allergic reactions to the constituents were most frequent to oak moss (24), isoeugenol (20), eugenol (13), cinnamic aldehyde (10) and geraniol (8). Reactions read as allergic on day 3/4 were observed only 10X to 7 materials of the new series (Iso E Super (2), Lyral (3), Cyclacet (1), DMBCA (1), Vertofix (1), citronellol (1) and amyl salicylate (1)). The remaining 41 fragrances were negative. 28 irritant or doubtful reactions on day 3/4 were observed to a total of 19 FF materials (more than 1 reaction: 5% citronellol (2), 1% amyl salicylate (2), 1% isononyl acetate (3), 0.1% musk xylol (2), 1% citral (2), and 1% ionone beta (2)). Clinical relevance of positive reactions to any of the FF series was not proved in a single

case. This included the 4 reactions in patients who were negative to the 8% FM. In conclusion, the top 25 fragrances commonly found in various products caused few reactions in dermatological patients and these few appeared to be clinically irrelevant, with the possible exception of Lyral. However, this data should be interpreted in the light of the relatively small number of patients tested (only 100 in most centres).

Jorge Neto, J. and B. Mancini (1992). “Dialium guianense (Aubl.) Sandw., Leguminosae: chromatographic analysis of the essential oil.” Revista de Ciencias Farmaceuticas 14: 125-132. The essential oil composition of leaves of the medicinal plant D. guianense, collected in Irece, Bahia, Brazil, was determined. About 90% of the isolated compounds were identified by spectral analyses. The major components included alpha-pinene (16.74%), beta-pinene (25.64%), citronellol (19.98%), farnesol (9.03%) and geraniol (4.45%).

Kahlos, K., J. L. Kiviranta, et al. (1994). “Volatile constituents of wild and in vitro cultivated Gloeophyllum odoratum.” Phytochemistry 36(4): 917-22. The brown-rot fungus Gloeophyllum odoratum was collected from spruce stumps in southern Finland. The volatiles in the fruiting body and fungal cultures grown in malt extract and liquid medium were investigated. Chitin, chitosan and D-(+)-glucosamine at a concentration of 450 mgl-1 medium were used as elicitors. Chitosan completely inhibited growth in the solid medium. The main volatile(s) according to GC and GC-MS analysis were either linalool, citronellol, geraniol and methyl p-methoxyphenylacetate or drimenol depending on the culture type and elicitor. The composition of volatiles in the natural fungus differed slightly from that of the cultivated fungus since the major compound was methyl p-methoxyphenylacetate. The volatile oils were toxic to larvae of the brine shrimp, Artemia salina, indicating that they may possess insecticidal and cytotoxic activity.

Kedzia, B., M. Krzyzaniak, et al. (1994). “Composition and antimicrobial characteristics of Ol. Melissae and its components.” Herba Polonica 40(1-2): 5-11. Ol. Melissae [the essential oil of Melissa officinalis] was analysed, and the main constituents were citronellal (25.2%), geraniol (16.4%) and citronellol (11.0%). The essential oil inhibited the growth of Staphylococcus aureus, Streptococcus faecalis and Candida albicans (MIC values of 100, 250 and 300 micro g/ml, respectively), and inhibited the growth of Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa to a lesser extent (MIC values of 500 micro g/ml). Citronellol, beta-caryophyllene, thymol, carvacrol and citronellal were the most active oil components against the microorganisms.

Larsen, W., H. Nakayama, et al. (2002). “Fragrance contact dermatitis - a worldwide multicenter investigation (Part III).” Contact Dermatitis 46(3): 141-4. The purpose of this study was to determine the frequency of responses to selected fragrance materials in patients who were fragrance sensitive. 218 fragrance sensitive subjects were evaluated in eight centres worldwide with a fragrance mixture (FM) and 17 less well-studied fragrance materials. Reaction to the fragrance mixture (FM) occurred in 76% of the subjects. The (FM) detected all reactions to nerol and hydroxycitronellol and 93% of the reactions to clove bud oil. Ten fragrance materials were not detected by the FM and deserve further study: benzenepropanol, beta, beta, 3-trimethyl, hexyl-salicylate, dl-citronellol, synthetic

ylang ylang oil, benzyl mixture, cyclohexyl-acetate, eugenyl methyl ether, isoeugenyl methyl ether, 3-phenyl-1-propanol, and 3, 7-dimethyl-7-methoxyoctan-2-ol.

Lis-Balchin, M. and S. G. Deans (1997). “Bioactivity of selected plant essential oils against Listeria monocytogenes.” J Appl Microbiol 82(6): 759-62. Ninety-three different commercial essential oils were screened for activity against 20 Listeria monocytogenes strains in vitro and the results correlated against the actual chemical composition of each oil. There was a substantial difference in the activity between different essential oils as expected, but there was also a difference in activity between different samples of the same essential oil. Strong anti-Listeria activity was often correlated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamaldehyde, thymol, and sometimes with citronellol, limonene and geraniol. However, as there was often no correlation between the anti-Listeria activity and the main chemical components, it is possible that either there is a more complex relationship with the chemical composition (which includes the minor components) or that substantial adulteration had occurred in some essential oil samples.

Lis-Balchin, M. T. and S. L. Hart (1994). “A pharmacological appraisal of the folk medicinal usage of Pelargonium grossularioides and Erodium cicutarium.” Journal of Herbs, Spices & Medicinal Plants 2(3): 41-48. In Africa, P. grossularioides and E. cicutarium are used in traditional medicine for their abortifacient properties, and to treat fevers, dysentery, wounds and worm infestations. The pharmacological effects of extracts (hexane, methanol and water) of leaves of P. grossularioides (obtained from South Africa) and E. cicutarium (obtained from Cambridge University, UK), were studied in vitro using guinea pig ileum, rat uterus, rat phrenic nerve preparations, and rabbit hearts. Extracts from both plants increased the tone, and reduced the strength or inhibited contraction, of guinea pig ileum. Extracts stimulated contractions of the rat uterus, increased the tension of the isolated diaphragm muscle in phrenic nerve preparations, and produced a negative ionotropic action in the rabbit heart. Hexane extracts were the most active, followed by methanol extracts. The compositions of the essential oils from both species were compared. Both species contained methyl eugenol, geraniol, citronellol, isomenthone and linalool. Sesquiterpenes, which accounted for 10% of the E. cicutarium essential oil, were absent from P. grossularioides.

Lis-Balchin, M., J. Patel, et al. (1998). “Studies on the mode of action of essential oils of scented-leaf Pelargonium (Geraniaceae).” Phytotherapy Research 12(3): 215-217. Essential oils, steam-distilled from species, hybrids and cultivars of scented-leaf Pelargonium, were assessed for their mode of spasmolytic activity in vitro using an isolated smooth muscle preparation. Their mechanism of action was postsynaptic and not atropine-like. Spasmolytic action was correlated with the chemical composition of the essential oils assessed by GC-MS. The spasmolytic effect of Pelargonium essential oils with a rose-like odour was most likely mediated through cAMP, and not through cGMP; the action of all other essential oils with diverse odours was neither through cAMP, cGMP, nor via calcium channel blockade nor potassium channel activation. The mechanism of action of the main components of the rose-like pelargoniums, citronellol and geraniol, reflected that of the whole oils.

Mahmoud, A. L. (1994). “Antifungal action and antiaflatoxigenic properties of some essential oil constituents.” Lett Appl Microbiol 19(2): 110-3. The effect of 20 essential oil constituents on Aspergillus flavus growth and aflatoxin production was tested at the level of 1000 ppm. Some of the tested oils exhibited inhibitory effects on fungal growth and toxin formation. Five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth and aflatoxin synthesis. Trials for determining the minimum inhibitory concentration (MIC) of these oils revealed that geraniol, nerol and citronellol were effective at 500 ppm, while thymol and cinnamaldehyde were highly effective at doses as low as 250 and 200 ppm, respectively. It was observed that citral, citronellol and eugenol prevented fungal growth and toxin formation for up to 8 d. However, after 15 d of incubation, toxin production was greater than the controls.

Mehmood, Z., S. Ahmad, et al. (1997). “Antifungal activity of some essential oils and their major constituents.” Indian Journal of Natural Products 13(2): 10-13. Essential oils from lemongrass (Cymbopogon flexuosus), palmarosa (C. martini), cinnamon (Cinnamomum zeylanicum) and mint (Mentha arvensis) were tested for antifungal activity against Aspergillus, Fusarium and Cladosporium isolates from an ophthalmology specimen. Cinnamon oil was the most active against Aspergillus, and overall; palmarosa was the most active against Fusarium, and lemongrass against Cladosporium. The most active constituent found was eugenol, from citral and geraniol; citronellol and cinnamaldehyde were inactive.

Onisei, T., E. T. Toth, et al. (1995). “Growth and volatile oil production of two different vitroclones of Pelargonium roseum Ait.” Rivista Italiana EPPOS(16): 13-19. In vitro-cultured plants of P. roseum, derived by direct organogenesis from stem nodes taken from 2 plants of different origin (Fundulea and Chisinau), were transplanted in the field in June. At the end of July and in mid Sep., plants were assessed for growth parameters and essential oil yield (data presented). Differences in in vitro culture establishment, shoot regeneration, shoot multiplication, root induction, plant acclimatization and essential oil production are discussed in relation to the explant source. The plants retained the characteristics of the parental genotype. Fundulea plants were more productive with regard to all the parameters assessed (plant height, number of branches, number of axillary buds, and plant FW and DW). The major essential oil constituents were geraniol, linalool and citronellal; these were present in higher quantities in plants derived from Fundulea explants, compared with those of Chisinau origin. Citronellol was only detected in plants of Fundulea origin.

Pawar, P. V., R. N. Sharma, et al. (1991). “Action of some insect growth regulators on mosquito vectors: Part I--Citronellol based diethers.” J Commun Dis 23(2): 118-22. New series of compounds starting from common terpenoids (Geraniol, citronellol) have been examined for biological activity on mosquito larvae. Many of these exhibited development disruption on eggs as well larvae. Some also affected adult oviposition behaviour. Developmental disturbances were classified as JH type by inducing typical metamorphosis inhibition in Dysdercus koenigii in the standard Hemipteran JH bioassay.

Where indicated simulated field trials were also undertaken. This report describes results of bioevaluation of the citronellol based compounds. The results indicate that these show multifarious activity against mosquitoes but at relatively high doses, suggesting that exploration of further structural variety is needed before truly promising analogues can be obtained.

Rastogi, S. C., S. Heydorn, et al. (2001). "Fragrance chemicals in domestic and occupational products." Contact Dermatitis 45(4): 221-5. Epidemiological studies have described an increasing prevalence of fragrance allergy and indicated an association with hand eczema. 59 domestic and occupational products intended for hand exposure were subjected to gas chromatography-mass spectrometric (GC-MS) analyses to test the hypothesis that fragrance chemicals known to have the potential to cause contact allergy but not included in fragrance mix (FM) may be common ingredients in these products. A quantitative analysis of 19 selected fragrances was performed by GC-MS. Further analysis of GC-MS data revealed the presence of 43 other fragrance chemicals/groups of fragrance chemicals in the products investigated. Among the 19 target substances the most commonly detected were limonene in 78%, linalool in 61% and citronellol in 47% of the products investigated. The FM ingredients were present in these products with the following frequencies: oak moss (evernic acid methylester) 2%, cinnamic alcohol 2%, cinnamic aldehyde (cinnamal) 3%, isoeugenol 5%, alpha-amylcinnamic aldehyde (amyl cinnamal) 8%, hydroxycitronellal 12%, eugenol 27%, and geraniol 41%. Thus, the chemical analyses of domestic and occupational products indicates that investigation of potential contact allergy related to these products types should consider fragrance allergens additional to those in the FM, since these may occur with high frequency.

Sharma, R. N., P. V. Pawar, et al. (1993). "Action of some new insect growth regulators on mosquito vectors. Part II: Geraniol based diethers." J Commun Dis 25(1): 30-5. Biological activity of saturated diethers viz. 1-benzyloxy/phenoxy-8-alkoxy and 1-alkoxy-8-benzyloxy-3,7-dimethyl-1, 8-octanes (IIa-IIq) prepared from Geraniol, were studied on three mosquito species and the bug Dysdercus koenigii. These diethers exhibited oviposition deterrent and developmental inhibition activities of greater magnitudes than the compounds based on citronellol reported in Part I of this paper. Some of these new compounds inhibit development of mosquitoes at 0.05 ppm and deter oviposition at 0.05 per cent doses. Tests were extended to field simulated conditions in selected cases.

Viollon, C. and J. P. Chaumont (1994). "Antifungal properties of essential oils and their main components upon Cryptococcus neoformans." Mycopathologia 128(3): 151-3. Cryptococcus neoformans opportunistic fungus met in the last phasis of AIDS is inhibited in vitro by several essential oils on natural volatile compounds. The minimal inhibitory concentration may reach 100 microliters/l and minimal fungicidal concentration 200 microliters/l with Palmarosa or Cinnamon oils. Among phenolic compounds, thymol and carvacrol are most fungitoxic. Terpenoids, citral, geraniol, and citronellol show best activities.

Yan, D. W., Z. J. Zhang, et al. (1994). “Analysis of the chemical compositions of essential oils from scented leaves of Pelargonium hybrids acclimated in Yunnan Province.” Fruits (Paris) 49(1): 22. The leaves of Pelargonium hybrids, collected from plants grown at Kunming, in May, June, Aug., Sept. and Oct. [year unspecified], were analysed for their essential oil compositions, using GC. Whereas geraniol contents continously increased between May and Oct., citronellol contents slightly decreased. The geraniol concentration of plants grown in Kunming was 4-13% higher than that of plants grown in Bin Chuang (Yunnan Province, China), Reunion or Morocco.

Calixto, J. B., A. Beirith, et al. (2000). "Naturally occurring antinociceptive substances from plants." Phytotherapy Research 14(6): 401-418. Despite the progress that has occurred in recent years in the development of therapy, there is still a need for effective and potent analgesics, especially for the treatment of chronic pain. One of the most important analgesic drugs employed in clinical practice today continues to be the alkaloid morphine. In this review, emphasis will be given to the important contribution and the history of Papaver somniferum, Salix species, Capsicum species and Cannabis sativa in the development of new analgesics and their importance in the understanding of the complex pathways related to electrophysiological and molecular mechanisms associated with pain transmission. Recently discovered antinociceptive substances include alkaloids, terpenoids and flavonoid. Plant-derived substances have, and will certainly continue to have, a relevant place in the process of drug discovery, particularly in the development of new analgesic drugs. (C) 2000 John Wiley and Sons, Ltd.

Chagonda, L. S., C. Makanda, et al. (2000). "The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe." Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Houmani, Z., S. Azzoudj, et al. (2002). "The essential oil composition of Algerian zaatar: Origanum spp. and." Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor

qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp. gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-methylether (11.4%) and thymol (26.1%); the major compounds of T. willdenowii were p-cymene (15.2%), thymol (15.1%) and carvacrol (51.3%); finally, from the two samples of T. algeriensis analyzed, one was rich in linalool (78.8%) and the other was rich in thymol (62.7%). (c) 2002 by The Haworth Press, Inc. All rights reserved.

Martinetz, D. (1993). "African and Indian Bdellium." Zeitschrift fur Phytotherapie 14(1): 34-36.

Ocete, M. A., S. Risco, et al. (1989). "Pharmacological activity of the essential oil of Bupleurum." Journal of Ethnopharmacology 25(3): 305-313.

Padula, L. Z., A. M. Collura, et al. (1977). "Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil." Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Saxena, V. K. and R. N. Sharma (1998). "Constituents of the essential oil from Commiphora mukul gum resin." Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Schaneberg, B. T. and I. A. Khan (2002). "Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC." J Agric Food Chem 50(6): 1345-9. A gas chromatography flame ionization detection method for the quantification of bioactive marker compounds (neral, geranial, geraniol, limonene, citronellal, and beta-myrcene) in the essential oil of Cymbopogon citratus (lemon grass) was developed. Four procedures for the extraction of essential oils from C. citratus were compared including solvent extraction, steam distillation extraction, accelerated solvent extraction, and supercritical fluid extraction. Solvent extraction by sonication with nonpolar solvents showed comparable results to the steam distillation method. Several commercial products prepared from C. citratus and Cymbopogon flexuosus were analyzed and compared.

Sidibe, L., J. C. Chalchat, et al. (2001). "Aromatic plants of Mali (IV): chemical composition of essential oils of Cymbopogon citratus (DC) Stapf and C. giganteus (Hochst.) Chiov." Journal of Essential Oil Research 13(2): 110-112. The composition of

the essential oils of C. citratus and C. giganteus from Mali and Cote d'Ivoire, collected in 1993 and 1994, was determined by GC and GC/MS, and they were found to contain 19 and 27 constituents, respectively. C. citratus oil from Mali contained a high proportion of citral (approximately 75%) (geranial/neral ca 2/1), some myrcene (6.2-9.1%) and geraniol (3.0-5.6%). It differed from the oil of the Ivory Coast in which the contents of geranial, neral and myrcene each ranged between 18-35%. C. giganteus oil was characterized by high proportions of cis- and trans-p-mentha-1(7), 8- dien-2-ols (approx. 50%) and p-mentha-2,8-dien-1-ols (approx.25%) together with isopiperitenol-carveol (approx. 10%) and traces of carvone (<5%).

Soon, S. L., H. S. Kuk, et al. (2002). “Effect of the essential oil from the flowers of Magnolia sieboldii on.” Planta Medica 68(5): 459-462. The essential oil from the flowers of Magnolia sieboldii was tested for its effects on lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and prostaglandin ESUB2 (PGESUB2) by rat peritoneal macrophages. It was shown to induce the production of NO and PGESUB2 in a concentration-dependent manner (3-30 mug/ml). Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) led to the identification of sixty compounds, of which beta-elemene (18.0%), alpha-terpinene (14.83%) and beta-myrcene (12.72%) were the major constituents. Among these three compounds, alpha-terpinene was found to be the most effective one with inhibitory activity on NO and PGESUB2 production by LPS-stimulated rat peritoneal macrophages.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). “Constituents of essential oil of Myrtus communis.” Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Borneol CAS # 507-70-0 (Camphol, 2-Bornanol, Bornyl alcohol, 2-Camphanol, Camphane 2-hydroxy, Bicyclo heptan-2-ol trimethyl, 2-Hydroxy trimethyl norbornane)

Buchbauer, G., L. Jirovetz, et al. (1993). "Fragrance compounds and essential oils with sedative effects upon inhalation." Journal of Pharmaceutical Sciences 82(6): 660-664. In experiments with female 6- to 8-week-old Swiss mice [see also Planta Medica (1987) 53, 315-318], a total of 44 fragrance compounds and essential oils, obtained from Dragoco Company (Vienna, Austria) and known to possess sedative properties, were screened for their potential aromatherapeutic value when administered by inhalation. The motility of untreated mice was compared with that of mice exposed to a specific compound after no pretreatment or after a caffeine-induced overagitation treatment. Compared with the motility of untreated mice (100%) that of mice exposed to lavender [Lavandula sp.] oil, neroli [Citrus aurantium] oil, linalool, linalyl acetate, citronellal, benzaldehyde, 2-phenylethyl acetate, alpha-terpineol and sandalwood [Santalum album] oil was decreased by 78.4, 65.3, 73.0, 69.1, 49.8, 43.7, 45.0, 45.0 and 40.0%, respectively. In contrast, an increased motility was observed after exposure to geraniol, isoborneol, isoeugenol, orange [Citrus sp.] terpenes and thymol. The sedative effect of lavender oil, isoeugenol, linalool, maltol, carvone and linalyl acetate counteracted caffeine-induced overagitation; overagitation was amplified by anthranilic acid methyl ester, farnesol, lime (Tilia sp.) blossom oil and nerol inhalation. Serum samples, taken shortly after the inhalation treatment, were analysed by GC-MS, GC-fourier transform infrared and GC-flame inonization techniques in order to identify active constituents. A total of 21 substances were identified at concentrations of up to 0.1 ng/ml serum. Correlations of the aroma detection thresholds and sedative properties associated with these substances indicated that there might be a direct pharmacological interaction of fragrance molecules with body tissues rather than a reflective interaction caused by a pleasant feeling.

Chagonda, L. S., C. Makanda, et al. (2000). "The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe." Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Ditzel, P. (1988). “Self-medication with phytopharmaceuticals: Possibilities and.” Deutsche Apotheker Zeitung 128(40): 2094-2095.

Figueiredo, A. C., M. J. Almendra, et al. (1996). “Biotransformation of monoterpenes and sesquiterpenes by cell suspension cultures of Achillea millefolium L. ssp. millefolium.” Biotechnology Letters 18(8): 863-868. The transformation capacity of Achillea millefolium spp. millefolium (yarrow) cell suspension cultures was investigated using geraniol (50 mg/l) and borneol, menthol, thymol and farnesols (25 mg/l) as substrates. Apart from converting these substrates into several biotransformation products, the cell suspension cultures were also able to glycosylate both the substrates and the biotransformation products.

Houmani, Z., S. Azzoudj, et al. (2002). “The essential oil composition of Algerian zaatar: Origanum spp. and.” Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp. gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-methylether (11.4%) and thymol (26.1%); the major compounds of T. willdenowii were p-cymene (15.2%), thymol (15.1%) and carvacrol (51.3%); finally, from the two samples of T. algeriensis analyzed, one was rich in linalool (78.8%) and the other was rich in thymol (62.7%). (c) 2002 by The Haworth Press, Inc. All rights reserved.

Inouye, S., K. Uchida, et al. (2001). “Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact.” Journal of Essential Oil Research 13(1): 68-72. The flowers of Perovskia abrotanoides and Nepeta juncea and the leaves and flowers of Thymus linearis were collected at full bloom from different areas of Karakoram district, Pakistan, then dried and examined for essential oil composition. Dried plant parts were placed in air-tight boxes with 3 species of fungi (Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes or Trichophyton rubrum) for one, 3 and 5 days, respectively. P. abrotanoides extracts contained high concentrations of 1,8-cineole (24-27%) and alpha-pinene (18-23%) and low concentrations of borneol and bornyl acetate. N. juncea essential oils contained nepetalactone (5.8 plus or minus 0.54 mg per g of dried flowers), and several minor constituents such as nerol, 1,8-cineole, neryl acetate and an unidentified component. Two Thymus linearis chemotypes were collected; that from

Hunza and Rupal valley had thymol and carvacrol as major components, and the other chemotype from the Rakaposhi area had geranyl acetate and geraniol as major components. N. juncea and Thymus linearis essential oils inhibited fungal growth by vapour contact, while P. abrotanoides showed no activity. The 2 Trichophyton species were the most susceptible and C. albicans was the least susceptible to the toxic effects of plant aromatic compounds, while A. fumigatus showed intermediate susceptibility.

Kohlert, C., I. Van Rensen, et al. (2000). "Bioavailability and pharmacokinetics of natural volatile terpenes in." Planta Medica 66(6): 495-505. Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of in vitro effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the in vitro activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.

Mumcuoglu, K. Y., R. Galun, et al. (1996). "Repellency of essential oils and their components to the human body louse, Pediculus humanus humanus." Entomologia Experimentalis et Applicata 78(3): 309-314. Five essential oils and 9 of their components were compared with diethyltoluamide (DEET) for their repellent activity against P. humanus humanus [P. humanus]. The absolute or intrinsic repellency of the compounds was tested by applying the repellent to corduroy patches and comparing them with untreated patches. It was found that the most effective repellents were DEET and citronella, whose activity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days. The repellent activity of the oil components such as citronellal and geraniol lasted more than 15 and 8 days, respectively. DEET remained effective at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1. The comparative or standard repellency of the candidate repellents was examined with the aid of a new screening technique using hairs treated with ammonium bicarbonate which is attractive to lice. Using this technique it could be shown that the repellent activity of citronella and geraniol lasted 2 days and that of rosemary and citronellal for only 1 day. DEET was active for <1 day. Serial dilutions of these substances also revealed that citronella was the most potent repellent for lice, followed by citronellal, rosemary, geraniol and DEET. The differences however, were not significant.

Sangalli, B. C. and W. Chiang (2000). "Toxicology of nutmeg abuse." Journal of Toxicology - Clinical Toxicology 38(6): 671-678. Background: Unpleasant and frightening side effects associated with the abuse of nutmeg occasionally generate emergency department referrals. We report a young patient's first-time experience with nutmeg and review the mechanisms of its toxicity. Case Report: A 13-year-old female ingested 15-24 g of nutmeg over a 3-hour period and smoked and shared 2 joints of marijuana. To facilitate ingestion, the nutmeg was put into 00-000 gelatin capsules.

Bizarre behavior and visual, auditory, and tactile hallucinations developed. She also experienced nausea, gagging, hot/cold sensations, and blurred vision followed by numbness, double, and 'triple' vision, headache, and drowsiness. Nystagmus, muscle weakness, and ataxia were present. Her vital signs and laboratory tests were normal. She received 50 g of activated charcoal and except for complaints of dizziness and visual changes, her 2-day admission was uneventful. The central nervous system activity of nutmeg is often postulated to result from biotransformation of its chemical components to amphetamine-like compounds, but this has not been proven. Nutmeg contains several compounds with structural similarities to substances with known central nervous system neuromodulatory activity.

Sharma, J. N., K. C. Srivastava, et al. (1994). “Suppressive effects of eugenol and ginger oil on arthritic rats.” Pharmacology 49(5): 314-318. This study examined the effect of eugenol and ginger oil on severe chronic adjuvant arthritis in rats. Severe arthritis was induced in the right knee and right paw of male Sprague-Dawley rats by injecting 0.05 ml of a fine suspension of dead Mycobacterium tuberculosis bacilli in liquid paraffin (5 mg/ml). Eugenol (33 mg/kg) and ginger oil (33 mg/kg), given orally for 26 days, caused a significant suppression of both paw and joint swelling. These findings suggest that eugenol and ginger oil have potent antiinflammatory and/or antirheumatic properties.

Thoma, K. and P. Serno (1984). “Physical instability of drugs as a result of polymorphism.” Deutsche Apotheker Zeitung 124(43): 2162-2170.

Vogel, G. (1975). “Predictability of the activity of drug combinations. Yes or no?” Arzneimittel-Forschung/Drug Research 25(9): 1356-1365. The following experiments were carried out on the question of the 'predictability' of the effect of drug combinations. A methanol extract from Viscum album causes a rise in coronary perfusion in isolated and perfused (Langendorff's method) guinea pig hearts. Administration of a methanol extract from Crataegus oxyacantha results not only in a rise in coronary perfusion, but also in a positive inotropic effect. Combined application of the drugs leads to a pure addition of effects, which are therefore 'predictable'. Extracts from Rhamnus frangula, Rheum sinens., and Senna lead to a dose dependent reduction in the gastrointestinal passage time. Combining the three extracts results in a pure addition of the effects. An extract from Potentilla anserina, which when given alone lengthens the gastrointestinal passage time, antagonises the laxative effect of extracts from Rheum sinens., Rhamnus frangula and Senna. All the effects tested in the area of the gastrointestinal tract behaved in a purely additive way and were therefore 'predictable'. When given separately the ethereal oils menthol, oleum juniperi, borneol and eugenol as well as dehydrocholic acid Na salt and alpha naphthyl acetic acid Na salt cause, in rats, a dose dependent increase in bile flow. Combining two or three of these substances results in a pure addition of the effects. The effects of all the test substances were therefore 'predictable'. When given separately valepotriate, morphine, scopolamine, ajmalicine, rescinnamine, reserpinine and reserpine, as depressants of the central nervous system (CNS), lead to a dose dependent lengthening of hexobarbital induced sleeping time in mice. However, when combined, these substances lead to a lengthening of hexobarbital sleeping time which is well above what was to be expected following application of the single substances. So, it

can happen that the combination of the two single doses which, when taken separately produce no effect, leads to a lengthening of hexobarbital induced sleeping time by more than 100%. In the case of CNS depressants, it is therefore true to say that their effect, when given in combination, was 'unpredictable'.

Camphene CAS # 79-92-5 (Dimethyl 3-methylene norbornane, Dimethyl 2-methylene norcamphane)

Chagonda, L. S., C. Makanda, et al. (2000). "The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe." Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Chinou, I. B., V. Roussis, et al. (1997). "Chemical and antibacterial studies of two Helichrysum species of Greek origin." Planta Med 63(2): 181-3. The chemical composition of the essential oils obtained from the aerial parts of Helichrysum stoechas ssp. barrelieri and H. taenari was analysed by GC and GC/MS. From the thirty-nine identified constituents representing the 73.87% and 87.41% of the two oils, respectively, beta-elemene, beta-caryophyllene, geraniol, and camphene were the major components. Furthermore, it was found that the oils exhibited significant antibacterial activity against six Gram (+/-) bacteria.

Houmani, Z., S. Azzoudj, et al. (2002). "The essential oil composition of Algerian zaatar: Origanum spp. and." Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp. gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-

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Janardhanan, M. and J. E. Thoppil (2002). “Chemical composition of two species of Hydrocotyle (Apiaceae).” Acta Pharmaceutica 52(1): 67-69. The essential oils of two species of Hydrocotyle (Apiaceae), Hydrocotyle javanica Thunb. and H. sibthorpioides Lam., were analysed by GLC. Monoterpenes, sesquiterpenes and phenols were detected in these herbs.

Mumcuoglu, K. Y., R. Galun, et al. (1996). “Repellency of essential oils and their components to the human body louse, Pediculus humanus humanus.” Entomologia Experimentalis et Applicata 78(3): 309-314. Five essential oils and 9 of their components were compared with diethyltoluamide (DEET) for their repellent activity against P. humanus humanus [P. humanus]. The absolute or intrinsic repellency of the compounds was tested by applying the repellent to corduroy patches and comparing them with untreated patches. It was found that the most effective repellents were DEET and citronella, whose activity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days. The repellent activity of the oil components such as citronellal and geraniol lasted more than 15 and 8 days, respectively. DEET remained effective at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1. The comparative or standard repellency of the candidate repellents was examined with the aid of a new screening technique using hairs treated with ammonium bicarbonate which is attractive to lice. Using this technique it could be shown that the repellent activity of citronella and geraniol lasted 2 days and that of rosemary and citronellal for only 1 day. DEET was active for <1 day. Serial dilutions of these substances also revealed that citronella was the most potent repellent for lice, followed by citronellal, rosemary, geraniol and DEET. The differences however, were not significant.

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Limonene CAS#s 5989-54-8, 5989-27-5, 138-86-3, 7705-14-8 (Mentha-1,8-diene, Carvene, citrene, Dipentene, Cajeputen, Cajeputene, Cinen, Cinene, Dipenten, Eulimen, Kautschin, Limonen, Nesol, 1,8-Terpodiene, Dipanol, p-Menthane, Unitene, pentene, menthadiene dipentene, Methyl 4-isopropenyl cyclohexene, Cyclohexene 1-methyl 1-methylethenyl)

(1978). "d-Limonene." Drugs of Today 14(9): 404-406.

Aharonson, Z., M. Gana-Weisz, et al. (1998). "Stringent structural requirements for anti-Ras activity of S-prenyl analogues." Biochim Biophys Acta 1406(1): 40-50. The carboxy terminal S-farnesylcysteine of Ras oncoproteins is required for their membrane anchorage and transforming activities. We showed previously that S-farnesylthiosalicylic acid (FTS) affects the membrane anchorage of activated H-Ras in EJ cells and inhibits their growth. We report here on structural elements in S-prenyl derivatives that specifically inhibit the growth of EJ cells, but not of untransformed Rat-1 cells. Inhibition of the Ras-dependent extracellular signal-regulated protein kinase (ERK), of DNA synthesis and of EJ cell growth were apparent after treatment with FTS or its 5-fluoro, 5-chloro and 4-fluoro derivatives or with the C20 S-geranylgeranyl derivative of thiosalicylic acid. The 4-Cl-FTS analogue was a weak inhibitor of EJ cell growth. The 3-Cl-FTS analogue and the FTS carboxyl methyl ester were inactive, as were the C10 S-geranyl derivative of thiosalicylic acid, farnesoic acid, N-acetyl-S-farnesyl-L-cysteine and S-farne-sylthiopropionic acid. The structural requirements for anti-Ras activity of S-prenyl analogues thus appear to be rather stringent. With regard to chain length, the C15 farnesyl group linked to a rigid backbone seems to be necessary and sufficient. A free carboxyl group in an appropriately rigid orientation, as in thiosalicylic acid, is also required. Halogenic substitutents on the benzene ring of the thiosalicylic acid are tolerated only at position 5 or 4. This information may facilitate the design of potent Ras antagonists and deepen our understanding of the mode of association of Ras with the plasma membrane.

Baker, F. C., B. Mauchamp, et al. (1983). "Farnesol and farnesal dehydrogenase(s) in corpora allata of the tobacco hornworm moth, Manduca sexta." J Lipid Res 24(12): 1586-94. The metabolism of [3H]farnesol was studied in cell-free preparations of corpora allata from the tobacco hornworm, Manduca sexta, to assess the role of this presumed biosynthetic precursor of juvenile hormone (JH) III. A reversed-phase ion-pair liquid chromatographic (RP-IPC) procedure was devised to separate farnesol from several potential intermediates in its presumed metabolism to JH III: farnesal, farnesoic acid, 10,11-epoxyfarnesoic acid, and methyl farnesoate. Following incubation of (2E,6E)-[1,5,9-3H]farnesol with homogenates of corpora allata from fifth instar larvae or adult female M. sexta, and analysis by RP-IPC, the major radiolabeled products corresponded to farnesoic acid, farnesal, and a polar product(s) presumably derived from the tritium on C-1 of farnesol. Inclusion of NAD+ in the incubations conducted with crude homogenates resulted in enhanced [3H]farnesol metabolism, decreased accumulation of [3H]farnesal, and increased levels of [3H]farnesoic acid. Substitution of NADP+ for NAD+ was ineffective, suggesting that farnesol and/or farnesal dehydrogenase were NAD+-dependent enzymes. Pellet fractions obtained by differential centrifugation of

crude homogenates exhibited both farnesol and farnesal dehydrogenase activity but only the latter was clearly stimulated by addition of NAD+. The alcohol/aldehyde dehydrogenase(s) showed some substrate specificity for the 2E isomer; nerol and (2Z,6E)-farnesol were barely metabolized under conditions in which either geraniol or (2E,6E)-farnesol were rapidly oxidized. The identity of the [3H]farnesal zone obtained from RP-IPC was further established by normal-phase liquid chromatography and by gas-liquid chromatography-mass spectrometry.

Bansal, N., D. A. Nyquist, et al. (1992). "Protein-linked isoprenoid lipids in dexamethasone-treated human lymphoid lines in culture." Biochem Cell Biol 70(6): 489-95. Accumulation of isoprenoids was studied in two cell lines derived from acute T-cell leukemia: CEM-C7 cells, whose growth is inhibited by the glucocorticoid dexamethasone, and CEM-C1 cells, which are resistant to this steroid. Isoprenoids were measured by growing the cells in serum-free medium in the presence of lovastatin, which blocks synthesis of mevalonate, and then labeling with exogenous [3H]mevalonolactone. In both cell lines, isoprenoids associated with proteins were detected in cytoplasm, nucleus, and chromatin, and in the chromatin residue that remains after extraction of histone and nonhistone proteins. Differences in labeling were detected after treatment with dexamethasone in the CEM-C7 line, showing a decrease in the cytoplasmic fraction with a corresponding increase in both the nuclear and chromatin fractions as compared with untreated cells. No change was seen in the CEM-C1 line. In both cell lines, 25-30% of the incorporated label was released by treatment with acid or alkali. However, the majority of the label required treatment with methyl iodide for the release of organic-soluble tritiated products. After extraction with chloroform, the lipid fractions contained farnesol, geraniol, dolichols, and possibly nerolidol.

Barnes, J. (1998). "Complementary medicine: Aromatherapy." Pharmaceutical Journal 260(6998): 862-867.

Beaupre, D. M. and R. Kurzrock (1999). "RAS inhibitors in hematologic cancers: Biologic considerations and." Investigational New Drugs 17(2): 137-143. As the molecular mechanisms responsible for the development and propagation of cancer are becoming elucidated, the nascent field of gene-directed therapy is emerging. Recently, several investigators have described inhibitors of the Ras protein. This molecule has been targeted because RAS is one of the most commonly mutated oncogenes in human neoplasia. In this review, we will discuss the role of Ras in the pathogenesis of hematologic neoplasms, and the biology behind the development of novel compounds which specifically suppress Ras function.

Beuscher, N., M. Kietzmann, et al. (1998). "Interference of Myrtol standardized with inflammatory and allergic mediators." Arzneimittel Forschung 48(10): 985-989. The phytomedicine-based product, Myrtol standardized (the active principle of Gelomyrtol(R)/Gelomyrtol(R) forte), containing 1,8- cineole (eucalyptol), alpha-pinene and d-limonene, inhibited the activity of 5-lipoxygenase of human basophil and eosinophil leukocytes and the formation of leukotriene C4 as well as did 1,8- cineole. It inhibited the increase in prostaglandin (PGE2) levels in mucous membranes of teat

cisterns of the isolated bovine udder after topical administration of TPA (tetradecanoylphorbol-13-acetate). After topical administration into the teat cisterns, it increased the surface temperature, comparable to the effects of menthol. In vitro and in vivo studies revealed spasmolytic and broncholytic effects.

Beuscher, N., M. Kietzmann, et al. (1998). “Interference of Myrtol standardized with inflammatory and allergic mediators.” Arzneimittel Forschung 48(10): 985-989. The phytomedicine-based product, Myrtol standardized (the active principle of Gelomyrtol(R)/Gelomyrtol(R) forte), containing 1,8- cineole (eucalyptol), alpha-pinene and d-limonene, inhibited the activity of 5-lipoxygenase of human basophil and eosinophil leukocytes and the formation of leukotriene C4 as well as did 1,8- cineole. It inhibited the increase in prostaglandin (PGE2) levels in mucous membranes of teat cisterns of the isolated bovine udder after topical administration of TPA (tetradecanoylphorbol-13-acetate). After topical administration into the teat cisterns, it increased the surface temperature, comparable to the effects of menthol. In vitro and in vivo studies revealed spasmolytic and broncholytic effects.

Blazer-Yost, B. L., C. L. Hughes, et al. (1997). “Protein prenylation is required for aldosterone-stimulated Na+ transport.” Am J Physiol 272(6 Pt 1): C1928-35. Aldosterone stimulation of transcellular Na+ flux in polarized epithelial cells is dependent on at least one transmethylation reaction, but the substrate of this signaling step is unknown. Because it is clear that the majority of cellular protein methylation occurs in conjunction with protein prenylation, we examined the importance of prenylation to aldosterone-stimulated Na+ transport in the A6 cell line. Lovastatin, an inhibitor of the first committed step of the mevalonate pathway, inhibits the natriferic effect of aldosterone but does not inhibit insulin-stimulated Na+ flux. The addition of a farnesyl group does not appear to be involved in aldosterone's action. Neither alpha-hydroxyfarnesylphosphonic acid, an inhibitor of farnesyl:protein transferase, nor N-acetyl-S-farnesyl-L-cysteine, an inhibitor of farnesylated protein methylation, inhibits the hormone-induced increase in Na+ transport. In contrast, N-acetyl-S-geranyl-geranyl-L-cysteine, an inhibitor of geranylgeranyl protein methylation, completely abolishes the aldosterone-induced increase in Na+ flux with no effect on insulin-mediated Na+ transport or cellular protein content. These data indicate that methylation of a geranylgeranylated protein is involved in aldosterone's natriferic action.

Boone, C. W. (1994). “Current strategies of cancer chemoprevention: 13th Sapporo Cancer.” Cancer Research 54(12): 3315-3318.

Boone, C. W., J. W. Bacus, et al. (1997). “Properties of intraepithelial neoplasia relevant to cancer.” Proceedings of the Society for Experimental Biology and Medicine 216(2): 151-165. Cancer chemoprevention is defined as the prevention of cancer by the administration of diet supplements or drugs. A drug discovery effort should therefore focus on finding agents that will avert the process of intraepithelial neoplasia which precedes invasive cancer. Over 30 agents developed by the chemoprevention program at the National Cancer Institute are being tested against intraepithelial neoplasia of many organ sites in more than 80 clinical trials. Two basic mechanisms underlie the onset and

development of intraepithelial neoplasia. First is the development of the two precursor lesions of chronic diffuse epithelial hyperplasia and genomic instability, the latter being produced by 'mutator' mutations in genes responsible for genomic stability, by gene copy amplification or loss from DNA breakage-fusion-anaphase-bridge cycles, by unequal sister chromatid exchange, and by accumulation of double minutes. Second is the development of multicentric intraepithelial neoplastic lesions which independently progress through each of the following processes at a continuously accelerating rate: clonal evolution, hyperproliferation, production of genomic structural variants, and apoptosis. Recommended chemoprevention strategies based on these mechanisms are (i) the development of better technology for early diagnosis, (ii) the development of multiple agents that block intralesional proliferation at steps along the signal pathway of mitotic signal transduction and along the signal pathway of synthesis of daughter cell components, (iii) the development of nontoxic anti- inflammatory agents, antioxidants, antimutagens, and proapoptotics, (iv) the avoidance of 'clonal escape' through use of drug combinations, and (v) the use of computer-assisted quantitative image analysis to assay modulation of surrogate end points in chemoprevention clinical trials.

Buchbauer, G., L. Jirovetz, et al. (1993). “Fragrance compounds and essential oils with sedative effects upon inhalation.” Journal of Pharmaceutical Sciences 82(6): 660-664. In experiments with female 6- to 8-week-old Swiss mice [see also Planta Medica (1987) 53, 315-318], a total of 44 fragrance compounds and essential oils, obtained from Dragoco Company (Vienna, Austria) and known to possess sedative properties, were screened for their potential aromatherapeutic value when administered by inhalation. The motility of untreated mice was compared with that of mice exposed to a specific compound after no pretreatment or after a caffeine-induced overagitation treatment. Compared with the motility of untreated mice (100%) that of mice exposed to lavender [Lavandula sp.] oil, neroli [Citrus aurantium] oil, linalool, linalyl acetate, citronellal, benzaldehyde, 2-phenylethyl acetate, alpha-terpineol and sandalwood [Santalum album] oil was decreased by 78.4, 65.3, 73.0, 69.1, 49.8, 43.7, 45.0, 45.0 and 40.0%, respectively. In contrast, an increased motility was observed after exposure to geraniol, isoborneol, isoeugenol, orange [Citrus sp.] terpenes and thymol. The sedative effect of lavender oil, isoeugenol, linalool, maltol, carvone and linalyl acetate counteracted caffeine-induced overagitation; overagitation was amplified by anthranilic acid methyl ester, farnesol, lime (Tilia sp.) blossom oil and nerol inhalation. Serum samples, taken shortly after the inhalation treatment, were analysed by GC-MS, GC-fourier transform infrared and GC-flame inonization techniques in order to identify active constituents. A total of 21 substances were identified at concentrations of up to 0.1 ng/ml serum. Correlations of the aroma detection thresholds and sedative properties associated with these substances indicated that there might be a direct pharmacological interaction of fragrance molecules with body tissues rather than a reflective interaction caused by a pleasant feeling.

Buffatti, G. (1965). “[Geranyl farnesyl acetate in the treatment of gastroenteritis in the infant].” Clin Pediatr (Bologna) 47(9): 710-28.

Burke, Y. D., M. J. Stark, et al. (1997). “Inhibition of pancreatic cancer growth by the dietary isoprenoids farnesol and geraniol.” Lipids 32(2): 151-6. Fruits and vegetables

have protective effects against many human cancers, including pancreatic cancer. Isoprenoids are one class of phytochemicals which have antitumor activity, but little is known about their effects on cancer of the pancreas. We tested the hypothesis that isoprenoids would inhibit the growth of pancreatic tumor cells. Significant (60-90%) inhibition of the anchorage-independent growth of human MIA PaCa2 pancreatic tumor cells was attained with 25 microM farnesol, 25 microM geranylgeraniol, 100 microM perillyl amine, 100 microM geraniol, or 300 microM perillyl alcohol. We then tested the relative in vivo antitumor activities of dietary farnesol, geraniol, and perillyl alcohol against transplanted PC-1 hamster pancreatic adenocarcinomas. Syrian Golden hamsters fed geraniol or farnesol at 20 g/kg diet exhibited complete inhibition of PC-1 pancreatic tumor growth. Both farnesol and geraniol were more potent than perillyl alcohol, which inhibited tumor growth by 50% at 40 g/kg diet. Neither body weights nor plasma cholesterol levels of animals consuming isoprenoid diets were significantly different from those of pair-fed controls. Thus, farnesol, geraniol, and perillyl alcohol suppress pancreatic tumor growth without significantly affecting blood cholesterol levels. These dietary isoprenoids warrant further investigation for pancreatic cancer prevention and treatment.

Cardullo, A. C., A. M. Ruszkowski, et al. (1989). “Allergic contact dermatitis resulting from sensitivity to citrus peel, geraniol, and citral.” J Am Acad Dermatol 21(2 Pt 2): 395-7. A bartender with hand dermatitis had allergic contact sensitivity to the skin of lemon, lime, and orange but not to their juices. Although most reported cases of citrus peel allergy are due to d-limonene, for our patient, reactions to patch tests for geraniol and citral, two minor components of citrus peel oil, were positive, whereas those for d-limonene were negative. Contact allergy to citrus peel oil should be considered in patients with hand dermatitis who are occupationally exposed to citrus fruits.

Cesco, G. (1967). “[Geranyl farnesylacetate as a preventive and therapeutic agent in disorders of the digestive system caused by corticoids].” Minerva Gastroenterol 13(4): 152-7.

Chernin, T. (2001). “An update on cancer chemoprevention.” Oncology Spectrums 2(4): 276-282.

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Colli, S., S. Eligini, et al. (1997). “Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis.” Arterioscler Thromb Vasc Biol 17(2): 265-72. We examined the effect of fluvastatin, the first entirely synthetic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor that is structurally different from other vastatins, on tissue factor (TF) expression in human macrophages spontaneously differentiated in culture from blood monocytes. Fluvastatin decreased TF activity in a dose-dependent manner (1 to 5 mumol/L) in both unstimulated and lipopolysaccharide-stimulated macrophages, and this reduction paralleled the decrease in immunologically recognized TF protein. The same results were obtained with another lipophilic vastatin, simvastatin, but not with hydrophilic pravastatin. The reduction in TF expression was also observed in macrophages enriched in cholesterol after exposure to 50 micrograms/mL acetylated low density lipoprotein. The inhibitory effect of fluvastatin on TF activity and antigen was fully reversible by coincubation with 100 mumol/L mevalonate or 10 mumol/L all-trans-geranylgeraniol but not with dolichol, farnesol, or geraniol. Suppression of TF antigen and activity was accompanied by a diminution in TF mRNA levels, which was completely prevented by mevalonate. Furthermore, fluvastatin impaired bacterial lipopolysaccharide-induced binding of c-Rel/p65 heterodimers to a kappa B site in the TF promoter, indicating that this drug influences induction of the TF gene. We conclude that lipophilic vastatins inhibit TF expression in macrophages, and because this effect is prevented by mevalonate and geranylgeraniol, a geranylgeranylated protein plays a crucial role in the regulation of TF biosynthesis. The suppression of TF in macrophages by vastatins indicates a potential mechanism by which these drugs interfere with the formation and progression of atherosclerotic plaque as well as thrombotic events in hyperlipidemic patients.

Cometto-Muniz, J. E., W. S. Cain, et al. (1998). “Trigeminal and olfactory chemosensory impact of selected terpenes.” Pharmacol Biochem Behav 60(3): 765-70. In Experiment 1, four normosmics and four anosmics (three congenital, one idiopathic) provided odor and nasal pungency thresholds, respectively, for the following terpenes: delta3-carene, p-cymene, linalool, 1.8-cineole, and geraniol, plus the structurally related compound cumene. Additionally, all subjects provided nasal localization (i.e., right/left) and eye irritation thresholis. Trigeminally mediated thresholds (i.e., nasal pungency, nasal localization, and eye irritation) lay about three orders of magnitude above odor thresholds, which ranged between 0.1 and 1.7 ppm. The results implied uniform chemesthetic sensitivity across tasks and sites of impact. In Experiment 2, normosmics and anosmics provided odor and nasal pungency thresholds, respectively, for three pairs of isomeric terpenes: alpha- and gamma-terpinene, alpha- and beta-pinene, and R(+)- and S(-)-limonene. Odor thresholds ranged between 1.4 and 19 ppm, that is, about an order of magnitude higher than those of the previous terpenes, with no substantial differences between odor thresholds of members of a pair. Regarding chemesthetic impact, only alpha-terpinene evoked nasal pungency. The overall outcome suggests comparable trigeminal chemosensitivity between nose and eyes and between normosmics and anosmics, as shown before for homologous n-alcohols. It also lends support to a previously derived solvation model of the chemesthetic potency of airborne substances, and indicates the likely importance of certain molecular-size restrictions for effective trigeminal impact.

Corey, E. J. and S. E. Lazerwith (1998). “A direct and efficient stereocontrolled synthetic route to the.” Journal of the American Chemical Society 120(49): 12777-12782. Described herein is a new synthetic route to pseudopterosin aglycone (3), a key intermediate for the synthesis of a group of antiinflammatory natural products including pseudopterosin A (1) and E (2). The pathway of synthesis starts with the abundant and inexpensive (S)-(-)-limonene and its long-known cyclic hydroboration product (4) and leads to the chiral hydroxy ketone 6. Conversion of 6 to 10 followed by a novel aromatic annulation produced 15 which underwent a highly diastereoselective cyclization to afford the protected pseudopterosin aglycone 16. The naturally occurring pseudopterosins such as 1 and 2 are readily available from this key intermediate.

Correll, C. C., L. Ng, et al. (1994). “Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase.” J Biol Chem 269(26): 17390-3. The degradation of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is highly regulated and is dependent on both a sterol and non-sterol derivative of mevalonic acid (MVA). We recently proposed that the non-sterol component is derived from farnesyl diphosphate (FPP), presqualene pyrophosphate, or squalene (Correll, C. C. and Edwards, P. A. (1994) J. Biol. Chem. 269, 633-638). In the current study, we have used digitonin-permeabilized cells to further define this MVA-derived non-sterol component required for the regulated degradation of HMG-CoA reductase. The addition of either FPP or farnesol to digitonin-permeabilized cells resulted in a rapid and dose-dependent degradation of HMG-CoA reductase. The effect of FPP, but not farnesol, was blocked by the phosphatase inhibitor sodium fluoride. The enhanced degradation of HMG-CoA reductase in permeabilized cells specifically required farnesol, since the addition of any of the structurally related isoprenoids geraniol, geranyl diphosphate, geranylgeranyl diphosphate, nerolidol, or all-cis-farnesol, or of the non-sterol squalene to the permeabilized cells did not stimulate enzyme degradation. The present studies demonstrate for the first time that the accelerated degradation of HMG-CoA reductase can be initiated in vitro. Further, since farnesol is shown to be specifically required for the enhanced degradation of the enzyme in vitro, we propose that this isoprenoid alcohol is important in this process in intact cells.

Corsini, A., M. Mazzotti, et al. (1993). “Relationship between mevalonate pathway and arterial myocyte proliferation: in vitro studies with inhibitors of HMG-CoA reductase.” Atherosclerosis 101(1): 117-25. The role of mevalonate and its products (isoprenoids) in the control of cellular proliferation was examined by investigating the effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (vastatins) on growth and on cholesterol biosynthesis of cultured arterial myocytes (SMC). Simvastatin (S) and fluvastatin (F), but not pravastatin (P), decreased the rate of growth of rat vascular SMC. The inhibition, evaluated as cell number, was dose-dependent with IC50 values of 2.8 and 2.2 microM for S and F, respectively; P (1-500 microM) was inactive. The inhibition of cell growth induced by 3.5 microM S (70% decrease) was prevented completely by the addition of 100 microM mevalonate, partially (70-85%) by the addition of 10 microM geraniol, 10 microM farnesol and 5 microM geranylgeraniol, but

not by the addition of squalene, confirming the specific role of isoprenoid metabolites in regulating cell proliferation. All the tested vastatins inhibited the incorporation of [14C]acetate into cholesterol but P had 800 times lower potency than S and F. Similar results were obtained in SMC from human femoral artery. At least 80% inhibition of cholesterol synthesis was necessary to induce a decrease in SMC proliferation. To further investigate the relationship between cholesterol synthesis and cell growth, two enantiomers of F were investigated. The enantiomer more active on HMG-CoA reductase was 70- and 1.6-fold more potent on arterial myocyte proliferation than its antipode and the racemic mixture, respectively.

Crowell, P. L. (1999). "Prevention and therapy of cancer by dietary monoterpenes." J Nutr 129(3): 775S-778S. Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity. For example, d-limonene, which comprises >90% of orange peel oil, has chemopreventive activity against rodent mammary, skin, liver, lung and forestomach cancers. Similarly, other dietary monoterpenes have chemopreventive activity against rat mammary, lung and forestomach cancers when fed during the initiation phase. In addition, perillyl alcohol has promotion phase chemopreventive activity against rat liver cancer, and geraniol has in vivo antitumor activity against murine leukemia cells. Perillyl alcohol and d-limonene also have chemotherapeutic activity against rodent mammary and pancreatic tumors. As a result, their cancer chemotherapeutic activities are under evaluation in Phase I clinical trials. Several mechanisms of action may account for the antitumor activities of monoterpenes. The blocking chemopreventive effects of limonene and other monoterpenes during the initiation phase of mammary carcinogenesis are likely due to the induction of Phase II carcinogen-metabolizing enzymes, resulting in carcinogen detoxification. The post-initiation phase, tumor suppressive chemopreventive activity of monoterpenes may be due to the induction of apoptosis and/or to inhibition of the post-translational isoprenylation of cell growth-regulating proteins. Chemotherapy of chemically induced mammary tumors with monoterpenes results in tumor redifferentiation concomitant with increased expression of the mannose-6-phosphate/insulin-like growth factor II receptor and transforming growth factor beta1. Thus, monoterpenes would appear to act through multiple mechanisms in the chemoprevention and chemotherapy of cancer.

Cruz, T., M. M. Cabo, et al. (1993). "Chemical composition and antimicrobial activity of the essential oils of different samples of Thymus baeticus Boiss." Phytotherapy Research 7(1): 92-94. The aerial parts of female and hermaphrodite plants were collected during the flowering period from 2 sites in Granada, Lanjaron and Ugijar. The components of the essential oils were determined by GC (data tabulated). No phenolic components were detected in any of the samples. There were marked quantitative differences in oil composition between the 2 geographic sources. The main constituent of the essential oil of hermaphrodite plants from Ugijar was 1,8-cineole (21.4%), and these plants also contained limonene (4.5%), camphor (0.5%), and bornyl acetate (0.7%) which were not detected in the oil from female plants from this area. The main constituent of the essential oil obtained from female plants from Ugijar was terpinen-4- ol (22.8%). The main constituents of the essential oil of hermaphrodite plants from Lanjaron were geraniol

(20.7%) and 1,8- cineole (15.8); the main constituent of the essential oil of female plants from Lanjaron was 1,8-cineole (20.9%). In assays against 8 species of human pathogenic bacteria, and 1 yeast (Candida albicans), all the oils showed considerable activity against most of the strains. Only Salmonella typhimurium and Staphylococcus aureus were unaffected by any oil at the highest concentration tested, 175 mug/ml. Antimicrobial activity was greatest in oils with a high geraniol content.

Da Grada, C. T., O. Greco, et al. (1967). "[Endoscopic study of clinical trials of geranyl farnesylacetate]." Minerva Gastroenterol 13(4): 157-64.

de Montellano, P. R., J. S. Wei, et al. (1977). "Inhibition of squalene synthetase by farnesyl pyrophosphate analogues." J Med Chem 20(2): 243-9. The pyrophosphates of the following farnesol analogues have been synthesized: 2-methylfarnesol; 7,11-dimethyl-3-ethyl-2,6,10-dodecatrien-1-ol; 3-demethylfarnesol; 4-methylthiofarnesol; 7,11-dimethyl-3-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyl02-iodo-2,6,10-dodecatrien-1-ol; 7,11-dimethyldodeca-6,10-dien-2-yn-1-ol; phytol; 3,7,11-trimethyl-2-dodecen-1-ol; 3,7,11-trimethyldodecan-1-ol; and geraniol. The double bonds in all the above compounds were in the E configuration, except phytol, which was a 7:3 mixture of 2E and 2Z isomers. Each of the pyrophosphates inhibits the incorporation of labeled farnesyl pyrophosphate into squalene by a yeast enzyme preparation. Free alcohols and monophosphates are inactive. The analogues, listed in order of decreasing inhibitory strength, are, by kinetic analysis, competitive or mixed inhibitors. Irreversible inhibition is not observed. The results suggest that binding to the enzyme is primarily mediated by the pyrophosphate moiety assisted by relatively nonspecific lipophilic interactions. Decreasing the chain length and saturating double bonds severely reduces binding, while substitution at the 2,3, and 4 positions, and lengthening of the chain, is well tolerated.

de Ropp, J. S. and F. A. Troy (1984). "Chemical synthesis and 2H NMR investigations of polyisoprenols: dynamics in model membranes." Biochemistry 23(12): 2691-5. Polyisoprenols (PIs) such as dolichol and undecaprenol have been shown to play an important role as enzymatic cofactors in the synthesis of glycoconjugates of both prokaryotic and eukaryotic cells. Presented here is a synthetic route used for obtaining specifically labeled [omega,omega-(C2H3)2]PIs that initiates with the selective oxidation of the omega-terminal double bond of the PI with N-bromosuccinimide. Continuation of the reaction sequence produces an omega-terminal aldehyde three carbons shorter than the original PI. A Wittig reaction with an appropriate deuterium-labeled phosphonium salt is then used to form an omega-terminal-deuterated PI identical with the starting material except for replacement of 1H with 2H at the two omega-terminal methyls of the PI. Deuterium NMR spectra of [omega, omega-(C2H3)2]geraniol and -farnesol incorporated into phospholipid multilamellar vesicles show powder patterns. The quadrupole splitting of the 2H NMR signals was interpretable in terms of the degree of orderedness of the 2H-labeled site. The pure trans isomer geraniol gave rise to a single set of splittings for each C2H3 group while farnesol, a mixture of isomers, showed multiple quadrupole splittings. The quadrupole splittings of the PIs increased with increasing concentration of label and with lowering of temperature. Deuterium NMR T1 measurements, revealing rates of motion of the 2H-labeled site, showed fast motion for

[omega,omega-(C2H3)3]geraniol relative to [omega,omega-(C2H3)2]cholesterol under similar conditions. A correlation time of 5 X 10(-10) s was estimated for [omega,omega-(C2H3)2]geraniol, which was 1 order of magnitude faster than for [26,27-(C2H3)2]cholesterol.

Dorsey, J. K. and J. W. Porter (1968). "The inhibition of mevalonic kinase by geranyl and farnesyl pyrophosphates." J Biol Chem 243(18): 4667-70.

El-Bayoumy, K. (1994). "Evaluation of chemopreventive agents against breast cancer and proposed." Carcinogenesis 15(11): 2395-2420. This review is based on literature data pertaining to the use of single agents or complex mixtures that have been shown to offer protection against mammary cancer in rodents. The species and strains of animals, dose, route, frequency and duration of carcinogen administration, as well as types, levels, route of administration and duration of chemopreventive treatments are described in detail. A brief description regarding the mechanism(s) responsible for the chemopreventive action of each class of agent is provided. The reader is encouraged to use additional sources for detailed information. The epidemiological observations that implicate certain agents or complex mixtures as playing a role in reducing human breast cancer are also reported and a comparison is made with results from assays in laboratory animals. Possible intermediate biomarkers that indicate risk for breast cancer are discussed. The purpose of this review is to provide the reader with a fairly concise knowledge of the subject. The information in this review is gathered together to stimulate studies toward appropriate strategies for future clinical chemoprevention trials.

Elson, C. E. and S. G. Yu (1994). "The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables." J Nutr 124(5): 607-14. Anutritive isoprenoid constituents of fruits, vegetables, cereal grains and essential oils exhibit a spectrum of anticarcinogenic activities. The induction of hepatic Phase II detoxifying activities by dietary isoprenoids appears to underlie their blocking action. The second anticarcinogenic action of the dietary isoprenoids, suppression of the growth of chemically initiated and transplanted tumors is, we suggest, secondary to the inhibition of mevalonate pathway activities. Mevinolin, a competitive inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase activity, depletes cells of the intermediate products of the pathway that are required for the posttranslational modification of proteins, a process giving the proteins lipophilic anchors that bind to membranes. As a consequence, nuclear lamins and ras oncoproteins remain in nascent states, and cells do not proliferate. gamma-Tocotrienol, perillyl alcohol, geraniol and d-limonene suppress hepatic HMG-CoA reductase activity, a rate-limiting step in cholesterol synthesis, and modestly lower serum-cholesterol levels of animals. These isoprenoids also suppress tumor growth. The HMG-CoA reductase of neoplastic tissues differs from that of sterologenic tissues in being markedly resistant to sterol feedback inhibition. Our review suggests that the mevalonate pathway of tumor tissues is uniquely sensitive to the inhibitory actions of the dietary isoprenoids.

Everitt, Z. M. and G. B. Lockwood (1992). "Biotransformation of geraniol by agitating and immobilised cultures of Anethum graveolens." Fitoterapia 63(6): 534-536. Free and

immobilized suspensions, and callus, derived from seeds of A. graveolens (dill) were grown in supplemented Gamborg's BS medium. Terpenoid (limonene and carvone) accumulation ceased after the 7th generation of callus. Feeding geraniol at 20 or 30 p.p.m. resulted in the production of nerol by suspensions over 24 h; thereafter no geraniol or nerol was detected in the cells or medium of suspension cultures. Higher concentrations (50 and 100 p.p.m.) of geraniol inhibited conversion to nerol.

Figueiredo, A. C., M. J. Almendra, et al. (1996). "Biotransformation of monoterpenes and sesquiterpenes by cell suspension cultures of Achillea millefolium L. ssp. millefolium." Biotechnology Letters 18(8): 863-868. The transformation capacity of Achillea millefolium spp. millefolium (yarrow) cell suspension cultures was investigated using geraniol (50 mg/l) and borneol, menthol, thymol and farnesols (25 mg/l) as substrates. Apart from converting these substrates into several biotransformation products, the cell suspension cultures were also able to glycosylate both the substrates and the biotransformation products.

Godwin, D. A. and B. B. Michniak (1999). "Influence of drug lipophilicity on terpenes as transdermal penetration enhancers." Drug Dev Ind Pharm 25(8): 905-15. Percutaneous absorption-enhancing effects on the skin of hairless mice of 11 monoterpenes [1, (+)-limonene; 2, (-)-menthone; 3, (+)-terpinen-4-ol; 4, alpha-terpineol; 5, 1,8-cineole; 6, (+)-carvone; 7, (-)-verbenone; 8, (-)-fenchone; 9, p-cymene; 10, (+)-neomenthol; and 11, geraniol] were investigated using three different model drugs (caffeine, hydrocortisone, triamcinolone acetonide [TA]) with varying lipophilicities. Terpenes were applied at 0.4 M in propylene glycol (PG) to mouse skin. The model drugs were applied as suspensions in PG 1 hr following enhancer pretreatment. The combination of terpenes in PG provided significant enhancement of the permeation of caffeine through mouse skin. The most active compounds 10 and 11 increased permeation by between 13-fold and 16-fold. The terpenes also enhanced the delivery of hydrocortisone, but not to as great an extent. The most active compounds 3 and 4 increased permeation between 3.9-fold and 5-fold. The compounds examined did not significantly increase the delivery of TA. The most active compound 4 only increased delivery 2.5-fold, while the next most active compound 6 only increased delivery 1.7-fold. Overall, these results indicate that the combination of terpenes with PG can significantly increase the transdermal penetration of the hydrophilic drug caffeine and the polar steroid hydrocortisone.

Greenwald, P., G. J. Kelloff, et al. (1995). "Genetic and cellular changes in colorectal cancer: Proposed targets of." Cancer Epidemiology Biomarkers and Prevention 4(7): 691-702. Progress in development of a genetic model for colorectal tumorigenesis and human chemoprevention research may allow the mechanism-based identification of targets and chemopreventive agents that will protect against colorectal cancer. For example, numerous mutagenic events can occur throughout colorectal carcinogenesis, including loss of heterozygosity in tumor suppressor genes such as APC, MCC, DCC, and p53, as well as in oncogenes such as K-ras. Chemopreventive agents that inhibit mutagenic activity such as N-acetyl-l-cysteine, oltipraz, and nonsteroidal anti-inflammatory drugs may protect against these mutations. Also, agents such as perillyl alcohol and lovastatin that interfere with protein isoprenylation and, hence, inhibit

oncogene activation may protect against aberrant K-ras expression. Hyperproliferation in normal mucosa, leading to early adenomas, and cellular proliferation, leading to growth and progression of neoplasia, are also aspects of colorectal carcinogenesis that can be controlled by chemopreventive agents. Calcium is a chemopreventive agent for which there is both clinical and experimental evidence of inhibition of cell proliferation in colon mucosa. Other examples of antiproliferative agents with potential chemopreventive efficacy in colon are 2-difluoromethylornithine, dehydroepiandrosterone, and selenium. Differentiating agents such as retinoids and deltanoids also may slow proliferation and progression. Antioxidants have potential for interfering with both mutagenicity and proliferation (e.g., by preventing oxidative activation of carcinogens and scavenging activated oxygen species generated during inflammation). The same mechanistic principles apply to identification of dietary chemopreventive intervention for colorectal carcinogenesis. For example, lowering dietary fat and increasing dietary fiber lead to lower colorectal mucosal proliferation, and cruciferous vegetables contain agents such as indoles and dithiolthiones that have shown antimutagenic activity.

Guin, J. D., B. N. Meyer, et al. (1984). “The effect of quenching agents on contact urticaria caused by cinnamic.” Journal of the American Academy of Dermatology 10(1): 45-51. Inhibition (quenching) of contact sensitization by cinnamic aldehyde (CA) reportedly occurs from eugenol (E) and d-limonene (d-L). Experimentally the former inhibited nonimmunologic contact urticaria (NICU) from CA in seven of eleven test subjects, which prompted a search for possible mechanisms, including chemical interaction, altered absorption, anti-inflammatory activity, and competitive inhibition. Mixtures of CA and E and CA and d-L showed no chemical changes or intermolecular bonding. Absorption was not increased by cellophane tape stripping, and neither E nor d-L inhibited urticaria formation following stimulation of skin of five subjects with dermographism. Competitive inhibition at the receptor level may best explain the quenching phenomenon observed. Additional factors are presented that complicate the already numerous caveats in interpreting test results in NICU.

Havel, C., E. R. Rector, 2nd, et al. (1986). “Isopentenoid synthesis in isolated embryonic Drosophila cells. Possible regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by shunted mevalonate carbon.” J Biol Chem 261(22): 10150-6. Our previous studies (Watson, J. A., Havel, C. M., Lobos, D. V., Baker, F. C., and Morrow, C. J. (1985) J. Biol. Chem. 260, 14083-14091) suggested that a matabolite, distal to isopentenyl 1-pyrophospate (IPP), served as a regulatory signal for sterol-independent modulation of Kc cell 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. This report summarizes efforts to localize the potential source of the post-IPP regulatory signal molecule. We found no direct correlation between mevalonate-mediated suppression of Kc cell HMG-CoA reductase activity and the rates of [1-14C]-, [3-14C]-, [5-14C]-, or [5-3H]mevalonate incorporation into either carbon dioxide, neutral lipids, water, or water-soluble isopentenoid pyrophosphate esters. [1-14C]Mevalonate's rate of conversion to 14CO2 (a measure of total isopentenyl 1-pyrophosphate synthesis) was minimally 5-fold greater than that for neutral isopentenoid lipid synthesis (measured with either [5-3H]-, [3-14C]-, or [5-14C]mevalonate). However, [5-3H]mevalonate's rate of conversion into [3H]H2O (measure of shunted mevalonate carbon) was equivalent or

greater than that measured for neutral isopentenoid lipid synthesis. [5-14C]Mevalonate radioactivity was incorporated into macromolecules and n-fatty acids. Kc cell extracts (100,000 X g supernatant fluid) readily oxidized alcohols with the following activity sequence: geraniol = nerol greater than farnesol = dimethylallyl alcohol greater than geranylgeraniol, isopentenyl alcohol, and allyl alcohol. Oxidation required NAD, and ethanol was not a substrate. We conclude that (a) Kc cells shunted a significant fraction (greater than or equal to 40%) of their post-IPP carbon to prenols for oxidative catabolism and (b) that shunted mevalonate carbon may play a significant role in the mevalonate-mediated regulation of Kc cell HMG-CoA reductase activity.

He, L., H. Mo, et al. (1997). “Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo.” J Nutr 127(5): 668-74. Sundry mevalonate-derived constituents (isoprenoids) of fruits, vegetables and cereal grains suppress the growth of tumors. This study estimated the concentrations of structurally diverse isoprenoids required to inhibit the increase in a population of murine B16(F10) melanoma cells during a 48-h incubation by 50% (IC50 value). The IC50 values for d-limonene and perillyl alcohol, the monoterpenes in Phase I trials, were 450 and 250 micromol/L, respectively; related cyclic monoterpenes (perillaldehyde, carvacrol and thymol), an acyclic monoterpene (geraniol) and the end ring analog of beta-carotene (beta-ionone) had IC50 values in the range of 120-150 micromol/L. The IC50 value estimated for farnesol, the side-chain analog of the tocotrienols (50 micromol/L) fell midway between that of alpha-tocotrienol (110 micromol/L) and those estimated for gamma- (20 micromol/L) and delta- (10 micromol/L) tocotrienol. A novel tocotrienol lacking methyl groups on the tocol ring proved to be extremely potent (IC50, 0.9 micromol/L). In the first of two diet studies, experimental diets were fed to weanling C57BL female mice for 10 d prior to and 28 d following the implantation of the aggressively growing and highly metastatic B16(F10) melanoma. The isomolar (116 micromol/kg diet) and the Vitamin E-equivalent (928 micromol/kg diet) substitution of d-gamma-tocotrienol for dl-alpha-tocopherol in the AIN-76A diet produced 36 and 50% retardations, respectively, in tumor growth ($P < 0.05$). In the second study, melanomas were established before mice were fed experimental diets formulated with 2 mmol/kg d-gamma-tocotrienol, beta-ionone individually and in combination. Each treatment increased ($P < 0.03$) the duration of host survival. Our finding that the effects of individual isoprenoids were additive suggests the possibility that one component of the anticarcinogenic action of plant-based diets is the tumor growth-suppressive action of the diverse isoprenoid constituents of fruits, vegetables and cereal grains.

Hegerhorst, D. F., R. B. Bhat, et al. (1988). “Seasonal changes of selected secondary plant products in Chrysothamnus nauseosus ssp. turbinatus.” Great Basin Naturalist 48(1): 1-8. Previous physiological studies of latex and resin production during the growing season indicated a negative correlation between latex and resin content. The resin was highest in the spring and lowest in the summer, whereas latex was highest in the summer and lowest in the spring. In the present study, using the same plants, individual compounds were followed during the growing season to see if they correlated with the latex or resin trend. The total compounds in the cyclohexane fraction followed the resin pattern. Individual compounds varied in their changes during the growing

season. Limonene, for example, was negatively correlated with latex production, whereas beta-cubebene was positively correlated. The possible metabolic pathways between resin and latex are discussed.

Hegerhorst, D. F., R. B. Bhat, et al. (1988). “Seasonal changes of selected secondary plant products in Chrysothamnus nauseosus ssp. turbinatus.” Great Basin Naturalist 48(1): 1-8. Previous physiological studies of latex and resin production during the growing season indicated a negative correlation between latex and resin content. The resin was highest in the spring and lowest in the summer, whereas latex was highest in the summer and lowest in the spring. In the present study, using the same plants, individual compounds were followed during the growing season to see if they correlated with the latex or resin trend. The total compounds in the cyclohexane fraction followed the resin pattern. Individual compounds varied in their changes during the growing season. Limonene, for example, was negatively correlated with latex production, whereas beta-cubebene was positively correlated. The possible metabolic pathways between resin and latex are discussed.

Hinson, D. D., K. L. Chambliss, et al. (1997). “Post-translational regulation of mevalonate kinase by intermediates of the cholesterol and nonsterol isoprene biosynthetic pathways.” J Lipid Res 38(11): 2216-23. To assess the potential for feedback inhibition by isoprene intermediates in the cholesterol and nonsterol isoprene biosynthetic pathway, we expressed human cDNAs encoding mevalonate kinase (MKase), phosphomevalonate kinase (PMKase), and mevalonate diphosphate decarboxylase (MDDase) as fusion proteins in Escherichia coli DH5alpha, and purified these proteins by affinity chromatography. Several phosphorylated and non-phosphorylated isoprenes were analyzed as inhibitors of the enzymes using a standard spectrophotometric assay. Of the three proteins, only MKase was inhibited through competitive interaction at the ATP-binding site. The intermediates studied (and their relative inhibitory capacity) were: geranylgeranyl-diphosphate (GGPP, C20) > farnesyl-diphosphate (FPP, C15) > geranyl-diphosphate (GPP, C10) > isopentenyl-diphosphate (IPP, C5) > or = 3,3-dimethylallyl-diphosphate (DMAPP, C5) > farnesol (C15) > dolichol-phosphate (DP, C(80-100)). Mevalonate-diphosphate, geraniol, and dolichol were not inhibitors. Our data further define the spectrum of physiologic inhibitors of MKase, and provide the first evidence for feedback inhibition of MKase by a nonsterol isoprene produced by the branched pathway, dolichol-phosphate. These results provide additional evidence that MKase may occupy a central regulatory role in the control of cholesterol and nonsterol isoprene biosynthesis.

Holloway, P. W. and G. Popjak (1967). “The purification of 3,3-dimethylallyl- and geranyl-transferase and of isopentenyl pyrophosphate isomerase from pig liver.” Biochem J 104(1): 57-70.

Hornby, J. M., E. C. Jensen, et al. (2001). “Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol.” Appl Environ Microbiol 67(7): 2982-92. The inoculum size effect in the dimorphic fungus Candida albicans results from production of an extracellular quorum-sensing molecule (QSM). This molecule prevents mycelial

development in both a growth morphology assay and a differentiation assay using three chemically distinct triggers for germ tube formation (GTF): L-proline, N-acetylglucosamine, and serum (either pig or fetal bovine). In all cases, the presence of QSM prevents the yeast-to-mycelium conversion, resulting in actively budding yeasts without influencing cellular growth rates. QSM exhibits general cross-reactivity within C. albicans in that supernatants from strain A72 are active on five other strains of C. albicans and vice versa. The QSM excreted by C. albicans is farnesol (C(15)H(26)O; molecular weight, 222.37). QSM is extracellular, and is produced continuously during growth and over a temperature range from 23 to 43 degrees C, in amounts roughly proportional to the CFU/milliliter. Production is not dependent on the type of carbon source nor nitrogen source or on the chemical nature of the growth medium. Both commercial mixed isomer and (E,E)-farnesol exhibited QSM activity (the ability to prevent GTF) at a level sufficient to account for all the QSM activity present in C. albicans supernatants, i.e., 50% GTF at ca. 30 to 35 microM. Nerolidol was ca. two times less active than farnesol. Neither geraniol (C(10)), geranylgeraniol (C(20)), nor farnesyl pyrophosphate had any QSM activity.

Hursting, S. D., T. J. Slaga, et al. (1999). “Mechanism-based cancer prevention approaches: Targets, examples, and.” Journal of the National Cancer Institute 91(3): 215-225. Humans are exposed to a wide variety of carcinogenic insults, including endogenous and man-made chemicals, radiation, physical agents, and viruses. The ultimate goal of carcin genesis research is to elucidate the processes involved in the induction of human cancer so that interventions may be developed to prevent the disease, either in the general population or in susceptible subpopulations. Progress to date in the carcinogenesis field, particularly regarding the mechanisms of chemically induced cancer, has revealed several points along the carcinogenesis pathway that may be amenable to mechanism-based prevention strategies. The purpose of this review is to examine the basic mechanisms and stages of chemical carcinogenesis, with an emphasis on ways in which preventive interventions can modify those processes. Possible ways of interfering with tumor initiation events include the following: i) modifying carcinogen activation by inhibiting enzymes responsible for that activation or by direct scavenging of DNA-reactive electrophiles and free radicals; ii) enhancing carcinogen detoxification processes by altering the activity of the detoxifying enzymes; and iii) modulating certain DNA repair processes. Possible ways of blocking the processes involved in the promotion and progression stages of carcinogenesis include the following: i) scavenging of reactive oxygen species; ii) altering the expression of genes involved in cell signaling, particularly those regulating cell proliferation, apoptosis, and differentiation; and iii) decreasing inflammation. In addition, the utility for mechanism-based cancer prevention research of new animal models that are based on the overexpression or inactivation of specific cancer-related genes is examined.

Ishihara, M., F. Takayama, et al. (2000). “Cytotoxic activity of polyprenylalcohols and vitamin K2 derivatives.” Anticancer Res 20(6B): 4307-13. Cytotoxic activity of 9 polyprenylalcohols and 6 vitamin K2 derivatives (MK-1 to MK-6) with various lengths of prenyl units was investigated. Among these compounds, geranylgeraniol with 4 prenyl units, and MK-2 with 2 prenyl units, showed the highest cytotoxic activity against human

oral tumor cell lines (HSC-2, HSG), without induction of internucleosomal DNA fragmentation. Higher molecular weight compounds showed selective cytotoxicity against tumor cell lines than normal human gingival fibroblasts HGF. ESR spectroscopy showed that all polyprenylalcohols did not produce radical, nor scavenged O2- generated by hypoxanthine and xanthine oxidase reaction, and only slightly enhanced the radical intensity of sodium ascorbate. Vitamin K2 derivatives scavenged O2- more efficiently, but did not produce radical (except MK-3) and only slightly modified the ascorbate radical intensity. Cytotoxic activity of these compounds might be affected by the molecular weight, hydrophobicity, van der Waals area and stabilization of hydration of the molecule.

Jorge Neto, J. and B. Mancini (1992). “Dialium guianense (Aubl.) Sandw., Leguminosae: chromatographic analysis of the essential oil.” Revista de Ciencias Farmaceuticas 14: 125-132. The essential oil composition of leaves of the medicinal plant D. guianense, collected in Irece, Bahia, Brazil, was determined. About 90% of the isolated compounds were identified by spectral analyses. The major components included alpha-pinene (16.74%), beta-pinene (25.64%), citronellol (19.98%), farnesol (9.03%) and geraniol (4.45%).

Kohlert, C., I. Van Rensen, et al. (2000). “Bioavailability and pharmacokinetics of natural volatile terpenes in.” Planta Medica 66(6): 495-505. Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of in vitro effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the in vitro activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.

Kurtz, D. B., T. L. White, et al. (2001). “Odorant confusion matrix: The influence of patient history on patterns.” Physiology and Behavior 72(4): 595-602. The odorant confusion matrix (OCM) is an odorant identification test in which the number of correct odorant identifications quantifies the level of olfactory function. As with other confusion matrices, the OCM reflects distortions of sensory perception as errors in identification. Previous work with the OCM suggests that, within an individual, hyposmia is associated with a stable shift in odorant perception. The current study examined whether consistent shifts in odorant perception are also characteristic of the various pathologies that lead to an olfactory loss. In a retrospective study, OCM response patterns for 135 hyposmic patients were fit into a five-dimensional space in which the distances between subjects reflected the dissimilarities between their OCM response patterns. Multivariate regression was performed relating position in the five-dimensional space to each of 11 factors representing 33 demographic and medical history variables. One factor, named congestion (gathering the variables of past polyposis, current polyposis, and current nasal obstruction due to swelling), was significantly indicative of patterns of responses on the

OCM, independent of the level of hyposmia. These data suggest that conductive olfactory loss may be associated with alterations in odorant perception, which are reflected in consistent odorant confusions. Such alterations in perception may eventually serve as a basis for a clinical test to provide differential diagnoses as to the sources of olfactory losses. (c) 2001 Elsevier Science Inc.

Langman, J. M. (1995). "d-Limonene: Is it a safe, effective alternative to xylene?" Journal of Histotechnology 18(2): 131-138. d-Limonene (Cinf 1inf 0Hinf 1inf 6) is a hydrocarbon of the monoterpene sub-group and is the major constituent of citrus peel oils (90-95%) and other ethereal oils. Used in the food and cosmetic industry for many years, it has been generally regarded as safe. In recent years, d-limonene-based products have been used in some pathology laboratories as a replacement for xylene, a known toxic substance. d-Limonene is readily absorbed into the body, metabolized, and cleared from the body. It appears to perform adequately as a wax solvent and clearing agent, and it has a reduced fire risk compared with xylene. Although product safety data sheets mention several adverse effects of exposure to d-limonene, exposure to 76 ppm for 2 hr had no irritative or central nervous system effects in volunteers. Headaches and nausea have been reported from some laboratories that have used d-limonene, and proper ventilation is required. Some users have reported skin irritations as well. It does induce renal pathology in some strains of adult male rats but has no proven genetic effects. In comparison, xylene has toxic effects on many organ systems, although it, too, does not appear to be mutagenic or carcinogenic. d-Limonene appears to be a safe and effective replacement for xylene, although more extensive use and further experimental studies may require revision of this conclusion.

Lis-Balchin, M. and S. G. Deans (1997). "Bioactivity of selected plant essential oils against Listeria monocytogenes." J Appl Microbiol 82(6): 759-62. Ninety-three different commercial essential oils were screened for activity against 20 Listeria monocytogenes strains in vitro and the results correlated against the actual chemical composition of each oil. There was a substantial difference in the activity between different essential oils as expected, but there was also a difference in activity between different samples of the same essential oil. Strong anti-Listeria activity was often correlated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamaldehyde, thymol, and sometimes with citronellol, limonene and geraniol. However, as there was often no correlation between the anti-Listeria activity and the main chemical components, it is possible that either there is a more complex relationship with the chemical composition (which includes the minor components) or that substantial adulteration had occurred in some essential oil samples.

Machida, K., T. Tanaka, et al. (1998). "Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast Saccharomyces cerevisiae." J Bacteriol 180(17): 4460-5. The mechanism of farnesol (FOH)-induced growth inhibition of Saccharomyces cerevisiae was studied in terms of its promotive effect on generation of reactive oxygen species (ROS). The level of ROS generation in FOH-treated cells increased five- to eightfold upon the initial 30-min incubation, while cells treated with other isoprenoid compounds, like geraniol,

geranylgeraniol, and squalene, showed no ROS-generating response. The dependence of FOH-induced growth inhibition on such an oxidative stress was confirmed by the protection against such growth inhibition in the presence of an antioxidant such as alpha-tocopherol, probucol, or N-acetylcysteine. FOH could accelerate ROS generation only in cells of the wild-type grande strain, not in those of the respiration-deficient petite mutant ([rho0]), which illustrates the role of the mitochondrial electron transport chain as its origin. Among the respiratory chain inhibitors, ROS generation could be effectively eliminated with myxothiazol, which inhibits oxidation of ubiquinol to the ubisemiquinone radical by the Rieske iron-sulfur center of complex III, but not with antimycin A, an inhibitor of electron transport that is functional in further oxidation of the ubisemiquinone radical to ubiquinone in the Q cycle of complex III. Cellular oxygen consumption was inhibited immediately upon extracellular addition of FOH, whereas FOH and its possible metabolites failed to directly inhibit any oxidase activities detected with the isolated mitochondrial preparation. A protein kinase C (PKC)-dependent mechanism was suggested to exist in the inhibition of mitochondrial electron transport since FOH-induced ROS generation could be effectively eliminated with a membrane-permeable diacylglycerol analog which can activate PKC. The present study supports the idea that FOH inhibits the ability of the electron transport chain to accelerate ROS production via interference with a phosphatidylinositol type of signal.

Marcos Becerro, J. F. and F. J. Rodriguez Gorostiza (1968). "[The effect of farnesyl geranyl acetate on duodenal ulcer]." Rev Esp Enferm Apar Dig 27(4): 633-50.

Martinez-Gonzalez, J., B. Raposo, et al. (2001). "3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition prevents endothelial NO synthase downregulation by atherogenic levels of native LDLs: balance between transcriptional and posttranscriptional regulation." Arterioscler Thromb Vasc Biol 21(5): 804-9. Atherogenic levels of native low density lipoproteins (nLDLs) decrease the bioavailability of endothelium-derived NO and downregulate endothelial NO synthase (eNOS) expression in cultured human endothelial cells. Here, we show that simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, within the therapeutic range (0.01 to 1 &mgr;mol/L) prevented the downregulation of eNOS mRNA and protein promoted by nLDL (180 mg cholesterol/dL, 48 hours) in human umbilical vein endothelial cells. This effect of simvastatin was completely reversed by mevalonate, the product of the reaction, and to a lesser extent by farnesol and geranyl geraniol. Simvastatin significantly stabilized eNOS mRNA in cells treated with nLDL during 48 hours (eNOS mRNA half-life approximately 11 hours in controls versus >24 hours in nLDL per 0.1 micromol/L simvastatin-treated cells). The downregulation of eNOS by nLDL was abrogated by cycloheximide, an inhibitor of protein synthesis, and by N-acetyl-leucyl-leucyl-norleucinal, a protease inhibitor that reduces the catabolism of sterol regulatory element binding proteins. Sterol deprivation increased the downregulation produced by nLDL on eNOS and sterol regulatory element binding protein-2 expression levels. However, no differential modulation of the retardation bands corresponding to the putative sterol-responsive element present in the eNOS promoter was detected by electrophoretic mobility shift assay. Our results suggest that nLDL promote eNOS downregulation operating at a

transcriptional level, whereas simvastatin prevents such an effect through a posttranscriptional mechanism.

McCarty, M. F. (2001). “Current prospects for controlling cancer growth with non-cytotoxic.” Medical Hypotheses 56(2): 137-154. In animal or cell culture studies, the growth and spread of cancer can be slowed by many nutrients, food factors, herbal extracts, and well-tolerated, available drugs that are still rarely used in the clinical management of cancer, in part because they seem unlikely to constitute definitive therapies in themselves. However, it is reasonable to expect that mechanistically complementary combinations of these measures could have a worthwhile impact on survival times and, when used as adjuvants, could improve the cure rates achievable with standard therapies. The therapeutic options available in this regard include measures that: down-regulate serum free IGF-I; suppress the synthesis of mevalonic acid and/or certain derivatives thereof; modulate arachidonate metabolism by inhibiting 5-lipoxygenase, 12-lipoxygenase, or COX-2; antagonize the activation of AP-1 transcription factors; promote the activation of PPAR-gamma transcription factors; and that suppress angiogenesis by additional mechanisms. Many of these measures appear suitable for use in cancer prevention. (c) 2001 Harcourt Publishers Ltd.

Melnykovych, G., J. S. Haug, et al. (1992). “Growth inhibition of leukemia cell line CEM-C1 by farnesol: effects of phosphatidylcholine and diacylglycerol.” Biochem Biophys Res Commun 186(1): 543-8. Acute leukemia cells of the established line CEM-C1 were treated during growth in serum-free medium with various concentrations of trans-trans farnesol. At concentrations ranging from 9.0 to 31.5 microM, farnesol inhibited growth of these cells without causing cell lysis. This effect was preceded by very rapid inhibition of choline incorporation in cellular lipid fraction. The growth inhibitory effect was prevented to a large extent by incubation with phosphatidylcholine or diacylglycerol.

Mincis, M. (1968). “[Clinical study of the effect of geranyl of farnesylacetate combined with nafiver in peptic ulcer patients].” Rev Bras Med 25(5): 324-7.

Miquel, K., A. Pradines, et al. (1996). “Farnesol and geranylgeraniol induce actin cytoskeleton disorganization and apoptosis in A549 lung adenocarcinoma cells.” Biochem Biophys Res Commun 225(3): 869-76. The effects of exogenous isoprenoids were investigated on A549 human lung adenocarcinoma cells. Among the tested isoprenoids, only farnesol and geranylgeraniol induce actin cytoskeleton disorganization, growth inhibition, and apoptosis. In contrast, desmosterol leads only to growth inhibition. We show that all tested isoprenoids are potent inhibitors of HMG CoA reductase activity, the sterols being the most powerful while they induce neither F-actin disorganization nor apoptosis. Thus the molecular mechanisms induced by farnesol and geranylgeraniol appear independent of reductase regulation. Our results point out the specific role of farnesol and geranylgeraniol on actin cytoskeleton organization and apoptosis in adenocarcinoma cells.

Mo, H., D. Tatman, et al. (2000). “Farnesyl anthranilate suppresses the growth, in vitro and in vivo, of murine B16 melanomas.” Cancer Lett 157(2): 145-53. The numbers of isoprene residues and unsaturated bonds, cis/trans configuration, and head group polarity influence the tumor-suppressive potency of acyclic isoprenoid hydrocarbons and alcohols; within the series tested, trans, trans farnesol had the greatest potency. Geraniol esters had increased potency relative to that of the free alcohol. Farnesyl anthranilate induced a concentration-dependent decrease in the B16 melanoma cell population, in part due to an increased proportion of cells in the G1 phase of the cell cycle and in part by the increased the proportion of apoptotic cells. Farnesyl anthranilate (1.5 mmol/kg diet) significantly suppressed the growth of implanted B16 melanomas and lowered the plasma cholesterol levels of tumor-free mice.

Mukhtar, H. and N. Ahmad (1999). “Cancer chemoprevention: Future holds in multiple agents.” Toxicology and Applied Pharmacology 158(3): 207-210.

Nery, A. L. and R. Nascif (1966). “[Analysis of the value of the product geranyl farnesyl acetate (Gefarnate) in the treatment of peptic ulcer].” Hospital (Rio J) 69(4): 669-89.

Nishio, E., K. Tomiyama, et al. (1996). “3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor impairs cell p4fferentiation in cultured adipogenic cells (3T3-L1).” Eur J Pharmacol 301(1-3): 203-6. Lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, inhibits the synthesis of mevalonic acid. We examined the effect of lovastatin on the differentiation of the fibroblast/adipocyte cell line (3T3-L1). Lovastatin inhibits the differentiation of 3T3-L1 cells in a dose-dependent fashion. The inhibitory effect of lovastatin was partially reversed by adding exogenous mevalonic acid to the 3T3-L1 cells. Exogenous cholesterol (15 micrograms/ml) did not prevent lovastatin inhibition of adipocyte conversion. The isoprenoids, farnesol and geraniol, partially prevented lovastatin inhibition of adipocyte conversion but squalene did not prevent lovastatin inhibition of adipocyte conversion. We conclude that the inhibitory effect of lovastatin was partially due to the blockade of the pathway leading to synthesis of isoprenoids, which are downstream products of mevalonic acid.

Nishioka, K., I. Nakatsuka, et al. (1988). “An improved method for 14C-labelling of farnesylacetic acid and its geranyl ester.” Radioisotopes 37(3): 133-9. Farnesylacetic acid was efficiently labelled with 14C at the 5-position and gefarnate, a potent ulcer inhibitor, was prepared from it in radioactive form for use in metabolic studies. Condensation of [carbonyl-14C]acetyl chloride (5) with t-butyl 2-ethoxymagnesiomalonate (6) followed by acid-catalyzed deprotection and decarboxylation gave ethyl 3-oxo[3-14C]butanoate (8). Alkylation of the keto ester (8) with geranyl bromide (9) afforded the unsaturated keto ester (10), which was hydrolyzed and decarboxylated to give geranyl[2-14C]acetone (11). Grignard reaction of 11 with cyclopropylmagnesium bromide followed by treatment with hydrobromic acid yielded [4-14C]homofarnesyl bromide (13). Cyanation of 13 with potassium cyanide and subsequent hydrolysis gave [5-14C]farnesylacetic acid (1) in 6.1% yield from barium [14C]carbonate (3). Chlorination of 1 followed by esterification with geraniol afforded [5-14C]gefarnate (2) in 88% yield.

Ocete, M. A., S. Risco, et al. (1989). “Pharmacological activity of the essential oil of Bupleurum.” Journal of Ethnopharmacology 25(3): 305-313.

Ohnuma, S., M. Watanabe, et al. (1996). “Identification and characterization of geranylgeraniol kinase and geranylgeranyl phosphate kinase from the Archaebacterium Sulfolobus acidocaldarius.” J Biochem (Tokyo) 119(3): 541-7. Geranylgeranyl diphosphate is an important precursor of archaebacterial ether-linked lipids, and it has been thought that all of this compound is "de novo" synthesized by geranylgeranyl diphosphate synthase. We studied the phosphorylation of geranylgeraniol, which seems to be related to the salvage pathway of biosynthesis of archaebacterial ether-linked lipids, in the Archaebacterium Sulfolobus acidocaldarius. Activities of geranylgeraniol kinase and geranylgeranyl phosphate kinase were detected in a cell lysate of S. acidocaldarius. The two enzymes were easily separated by ultracentrifugation. The membrane fraction and the cytosolic fraction contained geranylgeraniol kinase activity and geranylgeranyl phosphate kinase activity, respectively. Geranylgeraniol kinase, which requires divalent cation such as Mg2+, Co2+, and Mn2+ and NTP (ATP, GTP, CTP, UTP), catalyzes monophosphorylation of (all-E)-geranylgeraniol to produce geranylgeranyl phosphate. (all-E)-Farnesol, (all-E)-hexaprenol, and (all-E)-octaprenol were also active substrates, though they were less effective than (all-E)-geranylgeraniol. However, neither geraniol nor (22E,6E,10Z,14Z,18Z,22Z,26Z,++ +30Z,34Z,38Z)-undecaprenol was active. This enzyme is extremely thermostable and its pH optimal is between 6.5 and 8.5. The Michaelis constants for (all-E)-geranylgeraniol and ATP are 27 nM and 650 microM, respectively.

Padayatty, S. J., M. Marcelli, et al. (1997). “Lovastatin-induced apoptosis in prostate stromal cells.” J Clin Endocrinol Metab 82(5): 1434-9. Benign prostatic hyperplasia (BPH) is a common disease of aging men. Current medical treatment for this condition is only partially effective, therefore many patients must undergo surgery for symptomatic relief. BPH is caused by an increase in prostate epithelial and stromal cells, especially the latter. Since BPH stromal cells have a long life span and are not very responsive to androgen withdrawal, cultured BPH stromal cells were used to explore the feasibility of pharmacologically inducing apoptosis in these cells. We obtained BPH tissue during surgery, and stromal cells were isolated and maintained in culture. After cells achieved confluence, we induced apoptosis with the HMGCoA reductase inhibitor, lovastatin (30 micromol/L). The effects of testosterone (100 micromol/L), dihydrotestosterone (DHT; 100 micromol/L) and finasteride (100 micromol/L) on lovastatin-induced apoptosis were studied on cells grown in media containing charcoal stripped serum. Similarly, we examined the effect of the cholesterol pathway metabolites, mevalonic acid (30 micromol/L), geranyl geraniol (30 micromol/L), farnesol (10 micromol/L), squalene (30 micromol/L) and 7-ketocholesterol (3 micromol/L) on lovastatin-induced apoptosis. We demonstrated apoptosis by DNA laddering in agarose gels, by fluorescence microscopy following acridine orange staining, and by flow cytometry after end-labeling of DNA strand breaks with biotin-16-dUTP using deoxynucleotidyl exotransferase (TdT). Lovastatin at 30 micromol/L, but not at lower concentrations, induced apoptosis in BPH prostate stromal cells. This was seen (by flow cytometry) in 16.6 +/- 7.3% (mean +/- SD) of BPH cells treated with lovastatin at 72 h vs. 2.5 +/- 1.2% of cells treated with ethanol.

Lovastatin-induced apoptosis was not increased in stripped serum or by the addition finasteride, and was not inhibited by testosterone or DHT. Only mevalonate and geranyl geraniol, prevented lovastatin-induced apoptosis whereas farnesol, squalene, or 7-ketocholesterol did not. We conclude that lovastatin can induce apoptosis in BPH stromal cells in vitro, and this is not affected by androgen withdrawal or stimulation. It is unlikely that lovastatin, per se, will be an effective treatment for BPH in vivo, but it does provide a means for inducing apoptosis in vitro. Understanding the apoptotic process in BPH stromal cells ultimately may lead to new therapeutic strategies for BPH.

Padula, L. Z., A. M. Collura, et al. (1977). "Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil." Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Parker, T. S., G. Popjak, et al. (1978). "Inhibition of liver prenyltransferase by alkyl phosphonates and phosphonophosphates." Biochim Biophys Acta 530(1): 24-34. n-Pentyl and n-decyl phosphonate and the corresponding phosphonophosphates were found to inhibit cholesterol synthesis from mevalonate in the 10000 X g supernatants of liver homogenates and the synthesis of farnesyl pyrophosphate from geranyl and isopentenyl pyrophosphate by purified liver prenyltransferase. Kinetic analysis of the inhibition of prenyltransferase showed that the phosphonates and the phosphonophosphates interacted with two forms, or two sites, of the enzyme. The order of increasing potency was C5-phosphonate less than C10-phosphonate less than C5-phosphonophosphate less than C10-phosphonophosphate. The phosphonophosphates were at least ten times stronger inhibitors than the phosphonates.

Parra, J. L., L. Coderch, et al. (1997). "Incorporation of non-steroidal anti-inflammatory drugs into specific." Colloids and Surfaces A: Physicochemical and Engineering Aspects 123-124(-): 115-123. The presence of monophasic domains in systems made up of free unsaturated fatty acids, potassium oleate, d-limonene and transcutol was investigated to determine the possibilities of the incorporation of two anti- inflammatory drugs (piroxicam or diclofenac). The role of the unsaturation of fatty acids in obtaining suitable monophasic compositions with the additional presence of two percutaneous absorption enhancers (d-limonene and transcutol) can be noted from the results obtained. The monophasic realms visualized for the different systems studied were mainly dependent on the free fatty acid potassium oleate combination (variable weight ratio). The final pH value of the monophasic compositions obtained was also different. Alternatively, some other interesting compounds present in the stratum corneum (i.e. glycerine or squalene) can be also used. A percutaneous absorption assay of piroxicam incorporated into two monophasic compositions was carried out as a preliminary experiment, with promising results. These systems may possibly constitute suitable drug delivery strategies for the topical application of anti-inflammatory compounds.

Paubert-Braquet, M., H. Cousse, et al. (1998). “Effect of the lipidosterolic extract of Serenoa repens (Permixon) and its major components on basic fibroblast growth factor-induced proliferation of cultures of human prostate biopsies.” Eur Urol 33(3): 340-7. OBJECTIVE: To assess the effect of the lipidosterolic extract of Serenoa repens (LSESr) on in vitro cell proliferation in biopsies of human prostate MATERIAL AND METHODS: Cell proliferation was assessed by incorporation of [3H]thymidine followed by historadiography. RESULTS: Basic fibroblast growth factor (b-FGF) induced a considerable increase in human prostate cell proliferation (from +100 to +250%); the glandular epithelium was mainly affected, minimal labeling being recorded in the other regions of the prostate. Similar results were observed with epidermal growth factor (EGF), although the increase in cell proliferation was not recorded in some cases. Lovastatin, an inhibitor of hydroxymethylglutaryl coenzyme A, antagonized both the basal proliferation and the growth factor-stimulated proliferation of human prostate epithelium (EGF, mean inhibition approximately 80-95%; b-FGF, mean inhibition approximately 40-90%). Geraniol, a precursor of both farnesyl pyrophosphate and geranylgeranyl pyrophosphate, and farnesol, the precursor of farnesyl pyrophosphate, increased cell proliferation only in some prostate specimens, this effect being antagonized by lovastatin. LSESr did not affect basal prostate cell proliferation, with the exception of two prostate specimens in which a significant inhibition of basal proliferation was observed with the highest concentration of LSESr (30 micrograms/ ml). In contrast, LSESr inhibited b-FGF-induced proliferation of human prostate cell cultures; this effect was significant for the highest concentration of LSESr (30 micrograms/ml). In some prostate samples, a similar inhibition was also noted with lower concentrations. Unsaturated fatty acids (UFA), in the range 1-30 ng/ml), did not affect the basal prostate cell proliferation, only a slight increase in cell proliferation was noted in 1 prostate specimen. UFA (1, 10 or 30 micrograms/ml) markedly inhibited the b-FGF-induced cell proliferation down to the basal value. Lupenone, hexacosanol and the unsaponified fraction of LSESr markedly inhibited the b-FGF-induced cell proliferation, whereas a minimal effect on basal cell proliferation was noted. CONCLUSIONS: Despite the large variability in the response of the prostate samples to b-FGF, these results indicate that LSESr and its components affect the proliferative response of prostate cells to b-FGF more than their basal proliferation.

Perry, N. S. L., P. J. Houghton, et al. (2000). “In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes.” Journal of Pharmacy and Pharmacology 52(7): 895-902. The effects of S. lavandulaefolia [S. lavandulifolia] essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole [eucalyptol] (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma- terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha- pinene and were 0.03 micro g/ml, 0.67 mM and 0.63 mM, respectively.

Camphor and other compounds tested (geraniol, linalool and gamma- terpinene) were less potent (camphor IC50 of >10 mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S., P. J. Houghton, et al. (2000). “In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes.” J Pharm Pharmacol 52(7): 895-902. Sage (Salvia spp) is reputed in European herbal encyclopaedias to enhance memory, and current memory-enhancing/anti-dementia drugs are based on enhancing cholinergic activity by inhibiting cholinesterase. In this study the effects of Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil batch used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma-terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha-pinene and were 0.03 microL [corrected] mL(-1), 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma-terpinene) were less potent (camphor IC50: >10mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. These findings suggest that if the inhibitory activity of the essential oil is primarily due to the main inhibitory terpenoid constituents identified, there is a major synergistic effect among the constituents. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Priborsky, J., K. Takayama, et al. (1992). “Influence of limonene and lauracapram on percutaneous absorption of.” Arzneimittel-Forschung/Drug Research 42(2): 116-119. The promoting effect of cyclic monoterpenes, 1% limonene (CAS 5989-27-5) and 1% cineole (CAS 470-82-6), on percutaneous absorption of nonsteroidal anti-inflammatory drugs was investigated in the rats. Compared with 1% laurocapram, drug absorption from the gel ointments was significantly more enhanced by addition of 1% limonene, while without any enhancer only ibuprofen penetrated across the skin in the limited amount. When using formulation with propylene glycol or 50% propylene glycolethanol solution, instead of carboxyvinyl polymer gel, percutaneous absorption significantly decreased and neither limonene nor cineole or laurocapram were capable of promoting percutaneous absorption of flufenamic acid to sufficient serum level. Cineole and limonene were also evaluated in permeation experiments in vitro. Enhancement ability of limonene in the gel ointment was approximately 5 times higher comparing with enhancement ratio of cineole, while in 100% propylene glycol enhancement ability of both cyclic monoterpenes was equal. Good correlation was observed between in vivo and in vitro experiments.

Evaluation of solubility proved that in the gel ointment simulated as water-ethanol solution were relatively best condition for percutaneous absorption of flufenamic acid when comparing with propylene glycol or 50% propylene glycol-ethanol solution.

Rajab, M. S., C. L. Cantrell, et al. (1998). "Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study." Planta Med 64(1): 2-4. The crude methanol extract of the Kenyan shrub Leucas volkensii Gurke (Labiatae) displayed in a radiorespirometric bioassay antimycobacterial activity against Mycobacterium tuberculosis. Bioassay-guided fractionation of the crude extract led to the identification of (E)-phytol as the principal active component with a minimum inhibitory concentration (MIC) of 2 micrograms/ml, a value also observed for (3R,S,7R,11R)-phytanol, (Z)-phytol, and a commercially available 2:1 mixture of (E)- and (Z)-phytol. The derivatives (E)-phytol acetate, a mixture of the (2S,3S)- and (2R,3R)-isomers of (E)-phytol epoxide and (3R,S,7R,11R)-phytanic acid displayed lower activities with MICs of 8, 16, and > 128 micrograms/ml, respectively. Geraniol and farnesol, displayed MICs of 64 and 8 micrograms/ml, respectively. The activities of (E)-phytol, (Z)-phytol and (3R,S,7R,11R)-phytanol were found to be in the same range as ethambutol, a clinically useful drug with an MIC in the range 0.95-3.8 micrograms/ml.

Rastogi, S. C., S. Heydorn, et al. (2001). "Fragrance chemicals in domestic and occupational products." Contact Dermatitis 45(4): 221-5. Epidemiological studies have described an increasing prevalence of fragrance allergy and indicated an association with hand eczema. 59 domestic and occupational products intended for hand exposure were subjected to gas chromatography-mass spectrometric (GC-MS) analyses to test the hypothesis that fragrance chemicals known to have the potential to cause contact allergy but not included in fragrance mix (FM) may be common ingredients in these products. A quantitative analysis of 19 selected fragrances was performed by GC-MS. Further analysis of GC-MS data revealed the presence of 43 other fragrance chemicals/groups of fragrance chemicals in the products investigated. Among the 19 target substances the most commonly detected were limonene in 78%, linalool in 61% and citronellol in 47% of the products investigated. The FM ingredients were present in these products with the following frequencies: oak moss (evernic acid methylester) 2%, cinnamic alcohol 2%, cinnamic aldehyde (cinnamal) 3%, isoeugenol 5%, alpha-amylcinnamic aldehyde (amyl cinnamal) 8%, hydroxycitronellal 12%, eugenol 27%, and geraniol 41%. Thus, the chemical analyses of domestic and occupational products indicates that investigation of potential contact allergy related to these products types should consider fragrance allergens additional to those in the FM, since these may occur with high frequency.

Rojas, M. C., L. Chayet, et al. (1983). "Substrate and metal specificity in the enzymic synthesis of cyclic monoterpenes from geranyl and neryl pyrophosphate." Arch Biochem Biophys 222(2): 389-96. A partially purified enzyme (carbocyclase) from the flavedo of Citrus limonum formed alpha-pinene, beta-pinene, limonene, and gamma-terpinene from geranyl pyrophosphate (GPP) and neryl pyrophosphate. The maximum specific activities obtained were 7.0 and 3.6 nmol/min/mg, respectively. Cross-inhibition by the two substrates were observed and the ability to utilize neryl pyrophosphate was almost completely lost with aging. Citronellyl pyrophosphate and dimethylallyl pyrophosphate

were the most effective inhibitors of carbocyclase. Isopentenyl pyrophosphate, the monophosphate esters of nerol and geraniol, as well as inorganic pyrophosphate were much less effective inhibitors. The enzyme had an absolute requirement for Mn2+. It could be replaced with about 2% effectiveness by Mg2+ and Co2+. Kinetic studies showed that the observed reaction rate correlates with the calculated concentration of the GPP (Mn2+)2 species. Previous evidence with nonenzymatic reactions and the results presented support the view that the mechanism of carbocyclase may be the intramolecular analog of prenyltransferase.

Rosado, J. A. and S. O. Sage (2000). "Farnesylcysteine analogues inhibit store-regulated Ca2+ entry in human platelets: evidence for involvement of small GTP-binding proteins and actin cytoskeleton." Biochem J 347 Pt 1: 183-92. We have investigated the mechanism of Ca(2+) entry into fura-2-loaded human platelets by preventing the prenylation of proteins such as small GTP-binding proteins. The farnesylcysteine analogues farnesylthioacetic acid (FTA) and N-acetyl-S-geranylgeranyl-L-cysteine (AGGC), which are inhibitors of the methylation of prenylated and geranylgeranylated proteins respectively, significantly decreased thrombin-evoked increases in intracellular free Ca(2+) concentration ([Ca(2+)](i)) in the presence, but not in the absence, of external Ca(2+), suggesting a relatively selective inhibition of Ca(2+) entry over internal release. Both these compounds and N-acetyl-S-farnesyl-L-cysteine, which had similar effects to those of FTA, also decreased Ca(2+) entry evoked by the depletion of intracellular Ca(2+) stores with thapsigargin. The inactive control N-acetyl-S-geranyl-L-cysteine was without effect. Patulin, an inhibitor of prenylation that is inert with respect to methyltransferases, also decreased store-regulated Ca(2+) entry. Cytochalasin D, an inhibitor of actin polymerization, significantly decreased store-regulated Ca(2+) entry in a time-dependent manner. Both cytochalasin D and the farnesylcysteine analogues FTA and AGGC inhibited actin polymerization; however, when evoking the same extent of decrease in actin filament formation, FTA and AGGC showed greater inhibitory effects on Ca(2+) entry, indicating a cytoskeleton-independent component in the regulation of Ca(2+) entry by small GTP-binding-protein. These findings suggest that prenylated proteins such as small GTP-binding proteins are involved in store-regulated Ca(2+) entry through actin cytoskeleton-dependent and cytoskeleton-independent mechanisms in human platelets.

Roullet, J. B., H. Xue, et al. (1996). "Farnesyl analogues inhibit vasoconstriction in animal and human arteries." J Clin Invest 97(10): 2384-90. Recent studies have suggested that nonsterol, mevalonate-derived metabolites are implicated in the control of vascular tone and blood pressure. Because of the metabolic importance of farnesyl pyrophosphate, a 15-carbon (C15) intermediate of the cholesterol pathway, the vasoactive properties of the farnesyl motif were investigated. Two farnesyl analogues were used: farnesol, the natural dephosphorylated form of farnesyl pyrophosphate, and N-acetyl-S-trans,trans-farnesyl-L-cysteine (AFC), a synthetic mimic of the carboxyl terminus of farnesylated proteins. Both compounds inhibited NE-induced vasoconstriction in rat aortic rings at micromolar concentration. Their action was rapid, dose dependent, and reversible. Shorter (C10) and longer (C20) isoprenols as well as N-acetyl-S-geranyl-L-cysteine (C10) did not inhibit the response to NE. In contrast, N-acetyl-S-geranylgeranyl-L-

cysteine (C20), exhibited vasoactive properties similar to AFC. It was further demonstrated that AFC and farnesol inhibited KCl and NaF-induced contractions, suggesting a complex action on Ca2+ channels and G protein-dependent pathways. Finally, the effect of farnesol and AFC on the NE response was reproduced in human resistance arteries. In conclusion, mevalonate-derived farnesyl analogues are potent inhibitors of vasoconstriction. The study suggests that farnesyl cellular availability is an important determinant of vascular tone in animals and humans, and provides a basis for exploring farnesyl metabolism in humans with compromised vascular function as well as for using farnesyl analogues as regulators of arterial tone in vivo.

Roullet, J. B., U. C. Luft, et al. (1997). "Farnesol inhibits L-type Ca2+ channels in vascular smooth muscle cells." J Biol Chem 272(51): 32240-6. Earlier experiments with animal and human arteries have shown that farnesol, a natural 15-carbon (C15) isoprenoid, is an inhibitor of vasoconstriction (Roullet, J.-B., Xue, H., Chapman, J., McDougal, P., Roullet, C. M., and McCarron, D. A. (1996) J. Clin. Invest. 97, 2384-2390). We report here that farnesol reduced KCl- and norepinephrine-dependent cytosolic Ca2+ transients in fura-2-loaded intact arteries. An effect on Ca2+ signaling was also observed in cultured aortic smooth muscle cells (A10 cells). In these cells, farnesol reduced KCl-induced [Ca2+]i transients and mimicked the inhibitory effect of Ca2+-free medium on the [Ca2+]i response to both 12,13-phorbol myristate acetate, a protein kinase C activator, and thapsigargin, a specific endoplasmic reticulum ATPase inhibitor. Perforated patch-clamp experiments further showed in two vascular smooth muscle cell lines (A10 and A7r5), a reversible, dose-dependent inhibitory effect of farnesol on L-type Ca2+ currents (IC50 = 2.2 microM). Shorter (C10, geraniol) and longer (C20, geranylgeraniol) isoprenols were inactive. L-type Ca2+ channel blockade also occurred under tight (gigaohm) seal configuration using cell-attached, single-channel analysis, thus suggesting a possible action of farnesol from within the intracellular space. We finally demonstrated that farnesol did not affect Ca2+-sensitive pathways implicated in smooth muscle contraction, as tested with alpha-toxin permeabilized arteries. Altogether, our results indicate that farnesol is an inhibitor of vascular smooth muscle Ca2+ signaling with plasma membrane Ca2+ channel blocker properties. The data have implications for the endogenous and pharmacological regulation of vascular tone by farnesol or farnesol analogues.

Saxena, V. K. and R. N. Sharma (1998). "Constituents of the essential oil from Commiphora mukul gum resin." Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Schaneberg, B. T. and I. A. Khan (2002). "Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC." J Agric Food Chem 50(6): 1345-9. A gas chromatography flame ionization detection method for the quantification of bioactive marker compounds (neral, geranial, geraniol, limonene, citronellal, and beta-

myrcene) in the essential oil of Cymbopogon citratus (lemon grass) was developed. Four procedures for the extraction of essential oils from C. citratus were compared including solvent extraction, steam distillation extraction, accelerated solvent extraction, and supercritical fluid extraction. Solvent extraction by sonication with nonpolar solvents showed comparable results to the steam distillation method. Several commercial products prepared from C. citratus and Cymbopogon flexuosus were analyzed and compared.

Shamon, L. A., C. Chen, et al. (1994). "A correlative approach for the identification of antimutagens that." Anticancer Research 14(5 A): 1775-1778. Seventy natural and synthetic compounds were tested for potential to inhibit mutation induced by 7,12-dimethylbenz(a)anthracene (DMBA) in Salmonella typhimurium strain TM677. Results were compared with their ability to inhibit DMBA-induced preneoplastic lesions in a mouse mammary gland organ culture system. The response mediated by fifty-five of the test compounds was either positive or negative in both test systems, indicating that the combined use of these assays should aid in the discovery of antimutagenic agents that have cancer chemopreventive potential.

Sharma, J. N., K. C. Srivastava, et al. (1994). "Suppressive effects of eugenol and ginger oil on arthritic rats." Pharmacology 49(5): 314-318. This study examined the effect of eugenol and ginger oil on severe chronic adjuvant arthritis in rats. Severe arthritis was induced in the right knee and right paw of male Sprague-Dawley rats by injecting 0.05 ml of a fine suspension of dead Mycobacterium tuberculosis bacilli in liquid paraffin (5 mg/ml). Eugenol (33 mg/kg) and ginger oil (33 mg/kg), given orally for 26 days, caused a significant suppression of both paw and joint swelling. These findings suggest that eugenol and ginger oil have potent antiinflammatory and/or antirheumatic properties.

Siani, A. C., M. F. S. Ramos, et al. (1999). "Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of Protium." Journal of Ethnopharmacology 66(1): 57-69. The resins and leaves of Protium species are commonly used in folk medicine. The pharmacological effects of essential oils obtained by steam distillation (leaves and resin) from 5 Protium species (collected in Brazil) were studied. Analysis by GC-MS and retention indices calculations demonstrated that the resin oil is constituted mainly of monoterpenes and phenylpropanoids: alpha-terpinolene (22%), p-cymene (11%), p-cimen-8-ol (11%), limonene (5%), and dillapiol (16%), whereas sesquiterpenes predominate as the volatile constituents of the leaves. The resin of P. heptaphyllum (PHP) and leaves of P. strumosum (PS), P. grandifolium (PG), P. lewellyni [P. llewelyni] (PL) and P. hebetatum (PHT) were screened for: antiinflammatory activity using the mouse pleurisy model induced by zymosan (500 micro g/cavity) and lipopolysaccharide (LPS; 250 ng/cavity); antinociceptive effect (writhing test in mice); effects on NO production from stimulated macrophages; and effects on proliferation of neoplastic cell lines, Neuro-2a (mouse neuroblastoma), SP2/0 (mouse plasmacytoma) and J774 (mouse monocytic cell line). In the pleurisy model, the oils from PHP, PS and PL inhibited protein extravasation but no sample inhibited total or differential leukocyte counts after administration (100 mg/kg, p.o.) 1 h before stimulation with zymosan. The oils from PG, PL and PHT inhibited neutrophil accumulation, whereas PHP and especially PL inhibited LPS-induced eosinophil accumulation in the

mouse pleural cavity. PHT also inhibited mononuclear cell accumulation. No antinociceptive effect was observed when animals received oral administration of the essential oils (100 mg/kg). In vitro treatment with essential oils (100 micro g/well) affected NO production from stimulated mouse macrophages. PHP and PS inhibited (74% and 46%, respectively) LPS-induced NO production. In contrast, PL increased (49%) NO production. Cell proliferation was also affected by the oils assayed, rate of effectiveness was in the range of 60-100% for Neuro- 2a, 65-95% for SP2/0 and 70-90% for J774.

Siani, A. C., M. F. S. Ramos, et al. (1999). “Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of Protium.” Journal of Ethnopharmacology 66(1): 57-69. The resins and leaves of Protium species are commonly used in folk medicine. The pharmacological effects of essential oils obtained by steam distillation (leaves and resin) from 5 Protium species (collected in Brazil) were studied. Analysis by GC-MS and retention indices calculations demonstrated that the resin oil is constituted mainly of monoterpenes and phenylpropanoids: alpha-terpinolene (22%), p-cymene (11%), p-cimen-8-ol (11%), limonene (5%), and dillapiol (16%), whereas sesquiterpenes predominate as the volatile constituents of the leaves. The resin of P. heptaphyllum (PHP) and leaves of P. strumosum (PS), P. grandifolium (PG), P. lewellyni [P. llewelyni] (PL) and P. hebetatum (PHT) were screened for: antiinflammatory activity using the mouse pleurisy model induced by zymosan (500 micro g/cavity) and lipopolysaccharide (LPS; 250 ng/cavity); antinociceptive effect (writhing test in mice); effects on NO production from stimulated macrophages; and effects on proliferation of neoplastic cell lines, Neuro-2a (mouse neuroblastoma), SP2/0 (mouse plasmacytoma) and J774 (mouse monocytic cell line). In the pleurisy model, the oils from PHP, PS and PL inhibited protein extravasation but no sample inhibited total or differential leukocyte counts after administration (100 mg/kg, p.o.) 1 h before stimulation with zymosan. The oils from PG, PL and PHT inhibited neutrophil accumulation, whereas PHP and especially PL inhibited LPS-induced eosinophil accumulation in the mouse pleural cavity. PHT also inhibited mononuclear cell accumulation. No antinociceptive effect was observed when animals received oral administration of the essential oils (100 mg/kg). In vitro treatment with essential oils (100 micro g/well) affected NO production from stimulated mouse macrophages. PHP and PS inhibited (74% and 46%, respectively) LPS-induced NO production. In contrast, PL increased (49%) NO production. Cell proliferation was also affected by the oils assayed, rate of effectiveness was in the range of 60-100% for Neuro- 2a, 65-95% for SP2/0 and 70-90% for J774.

Smith, P. M., G. E. Sladen, et al. (1974). “Proceedings: A double blind trial of carbenoxolone and geranyl farnesylacetate in gastric ulcer.” Gut 15(10): 833.

Steele, V. E., R. C. Moon, et al. (1994). “Preclinical efficacy evaluation of potential chemopreventive agents in.” Journal of Cellular Biochemistry 56(SUPPL. 20): 32-54. In the NCI, Chemoprevention Branch drug development program, potential chemopreventive agents are evaluated for efficacy against chemical carcinogen-induced tumors in ainmal models. This paper summarizes the results of 144 agents in 352 tests

using various animal efficacy models. Of these results, 146 were positive, representing 85 different agents. The target organs selected for the animals model are representative of high-incidence human cancers. The assays include inhibition of tumors induced by MNU in hamster trachea, DEN in hamster lung, AOM in rat colon (including inhibition of AOM-induced aberrant crypts), MAM in mouse colon, DMBA and MNU in rat mammary glands, DMBA promoted by TPA in mouse skin, and OH-BBN in mouse bladder. The agents tested may be classified into various pharmacological and chemical structural categories that are relevant to their chemopreventive potential. These categories include antiestrogens, antiinflammatories (e.g., NSAIDs), antioxidants, arachidonic acid metabolism inhibitors, GST and GSH enhancers, ODC inhibitors, protein kinase C inhibitors, retinoids and carotenoids, organosulfur compounds, calcium compounds, vitamin Dinf 3 and analogs, and phenolic compounds (e.g., flavonoids). The various categories of compounds have different spectra of efficacy in animal models. In hamster lung, GSH-enhancing agents and antioxidants appear to have high potential for inhibiting carcinogenesis. In the colon, NSAIDs and other antiinflammatory agents appear particularly promising. Likewise, NSAIDs are very active in mouse bladder. In rat mammary glands, retinoids and antiestrogens (as would be expected) are efficacious. Several of the chemicals evaluated also appear to be promising chemopreventive agents based on their activity in several of the animal models. Particularly, the ODC inhibitor DFMO was active in the colon, mammary glands, and bladder models, while the dithiolthione, oltipraz, was efficacious in all the models listed above (i.e., lung, colon, mammary glands, skin, and bladder).

Stohs, S. J. (1995). “The role of free radicals in toxicity and disease.” Journal of Basic and Clinical Physiology and Pharmacology 6(3-4): 205-228. Free radicals are defined as atoms or molecules that contain one or more unpaired electrons. The toxicity of many xenobiotics is associated with the metabolic activation of foreign compounds to form free radicals or with the production of reactive oxygen species as superoxide anion, hydroxyl radicals or hydrogen peroxide which are responsible for the tissue damaging effects as lipid peroxidation, and DNA and protein damage. Oxidative stress associated with production of reactive oxygen species is believed to be involved not only in the toxicity of xenobiotics but also in the pathophysiology of aging, and various age-related diseases, including cataracts, atherosclerosis, neoplastic diseases, diabetes, diabetic retinopathy, chronic inflammatory diseases of the gastrointestinal tract, aging of skin, diseases associated with cartilage, Alzheimer's disease, and other neurologic disorders. The cellular sources of free radicals and reactive oxygen species, the biological targets of free radicals, and clinical conditions which are associated with free radical production and tissue damage are reviewed. In addition, potential therapeutic approaches to the prevention of free radical damage are considered. Free radical-induced injury can explain many clinical conditions.

Tachibana, A., T. Tanaka, et al. (1996). “Evidence for farnesol-mediated isoprenoid synthesis regulation in a halophilic archaeon, Haloferax volcanii.” FEBS Lett 379(1): 43-6. Farnesol strongly inhibited growth of a halophilic archaeon, Haloferax volcanii, with an IC50 value of only 2 microM (0.4 microgram/ml) in rich medium and 50 nM (0.01 microgram/ml) in minimal medium without lysis. Other isoprenoid alcohols such as

isopentenol, dimethylallyl alcohol, geraniol, and geranylgeraniol at 500 microM did not affect its growth. Mevalonate, which is the precursor of all isoprenoid membrane lipids in archaea, led to recovery of the growth inhibition of H. volcanii, but acetate had no such effect. Farnesol inhibited incorporation of acetate, but not mevalonate, into the lipid fraction. These results suggest that farnesol inhibited the biosynthetic pathway from acetate (acetyl-CoA) to mevalonate. Farnesol is known to be derived from the important intermediate of isoprenoids, farnesyl diphosphate (FPP), and found in neutral lipid fraction from this archaeon. Moreover, the cell-free extracts from H. volcanii could phosphorylate farnesol with ATP to generate farnesyl monophosphate and FPP. We conclude that farnesol-mediated isoprenoid synthesis regulation system by controlling farnesol concentration is present in H. volcanii.

Takahashi, I. and K. Ogura (1981). "Farnesyl pyrophosphate synthetase from Bacillus subtilis." J Biochem (Tokyo) 89(5): 1581-7. Farnesyl pyrophosphate synthetase was detected in extracts of Bacillus subtilis and partially purified by Sephadex G-100, hydroxylapatite, and DEAE-Sephadex chromatography. The enzyme catalyzed the exclusive formation of all-trans farnesyl pyrophosphate from isopentenyl pyrophosphate and either dimethylallyl or geranyl pyrophosphate. Mg2+ was essential for the catalytic activity and Mn2+ was less effective. The enzyme was slightly activated by sulfhydryl reagents. This enzyme was markedly stimulated by K+, NH4+, or detergents such as Triton X-100 and Tween 80, unlike the known farnesyl pyrophosphate synthetases from eucaryotes. The molecular weight of the enzyme was estimated by gel filtration to be 67,000. The Michaelis constants for dimethylallyl and geranyl pyrophosphate were 50 microM and 18 microM, respectively.

Tanaka, T. (1997). "Chemoprevention of human cancer: Biology and therapy." Critical Reviews in Oncology/Hematology 25(3): 139-174.

Toouli, J. (1991). "Symptomatic gallstones: Management options for the 1990s." HPB Surgery 4(4): 255-260.

Tse, G., D. Blankschtein, et al. (1999). "Thermodynamic prediction of active ingredient loading in polymeric microparticles." J Control Release 60(1): 77-100. The growing use of microparticles as a controlled-delivery system for pharmaceutical and non-pharmaceutical active ingredients (AIs) has prompted a costly trial-and-error development of new and effective microparticle systems. In order to facilitate a more rational design and optimization of AI loadings in microparticles, we have developed a molecular-thermodynamic theory to predict the loading of liquid AIs in polymeric microparticles that are manufactured by a solvent evaporation process. This process involves the emulsification of a liquid polymer solution (consisting of polymer and AI dissolved in a volatile solvent) in an aqueous surfactant solution. The theory describes the equilibrium distribution of the AI between the aqueous phase and the dispersed polymeric droplets. The universal functional activity coefficient (UNIFAC) and UNIFAC-Free Volume (FV) group-contribution methods are utilized to model the nonidealities in the water and polymeric droplet phases, respectively. The inputs to the theory are: (i) the chemical structures, densities and total masses of the manufacturing ingredients, (ii) the

manufacturing temperature and (iii) the glass transition temperature of the polymer. Since surfactant concentrations exceeding the critical micellar concentration (CMC) are often required in order to stabilize the dispersed polymeric droplets during the emulsion manufacturing process, the theory also accounts for AI solubilization in surfactant micelles present in the manufacturing solution. To test the AI loading predictions, we compare theoretical predictions of AI loadings in poly(lactic acid), poly(methyl methacrylate) and polystyrene microparticles to experimentally measured ones for five model AIs with varying degrees of hydrophobicity (benzyl alcohol, n-octanol, geraniol, farnesol and galaxolide). We also demonstrate how the developed theory can be utilized to screen polymers with respect to their abilities to load a given AI, as well as to provide guidelines for manufacturing microparticles having the desired AI loading.

Tsuji, M., Y. Fujisaki, et al. (1975). "Studies on d limonene as a gallstone solubilizer. (VI) The." Pharmacometrics 10(2): 187-197. The pharmacologic effects of d limonene as cholesterol gallstone solubilizer, on the biliary and gastrointestinal system were studied, and the following results were obtained. d Limonene showed constrictive action on the gallbladder in dogs when injected directly into the gallbladder or intravenously. The same effects were also observed on the isolated guinea pig gallbladder. d Limonene elevated the perfusion pressure of the sphincter of Oddi in dogs when injected directly into the common bile duct or intravenously. d Limonene exhibited constrictive action on the stomach in most dogs when injected into the stomach or intravenously, on the contrary, relaxed action in some dogs. It also had constrictive action on the fundus strip of the stomach in rats. d Limonene showed constrictive action on the small intestine in dogs when injected directly into the small intestine or intravenously. After being perfused continuously for 6 hr, d limonene showed irritant action on the gallbladder, particularly the mucous membrane, and the common bile duct in rabbits.

Uekama, K. (1981). "Pharmaceutical applications of cyclodextrin complexations." Yakugaku Zasshi 101(10): 857-873.

Van Dessel, G., H. Van Meirvenne, et al. (1992). "Uptake of dolichol by Vero cells." Biochem Cell Biol 70(6): 475-80. Characterization and kinetics of dolichol uptake by a Vero cell line are reported. Vero cells incorporate dolichol in a time- and dose-dependent manner. Optimal uptake is found at 37 degrees C and at a pH of 7.4. In contrast to cholesterol, an inhibitory effect on the dolichol incorporation is found for farnesol, geraniol, and retinol. Long chain polyprenols were slightly stimulatory. The translocation seems not to be highly energy dependent. The lack of substantial inhibition by chloroquine does not plead for a receptor-mediated endocytosis. Incorporated dolichol was distributed over both membranes and supernatant fractions, paralleling the distribution of the lysosomal marker beta-N-acetylhexosaminidase. The incorporated dolichol is subject to a fast efflux process, which is potentiated by the presence of lipid acceptors in the extracellular medium.

Van Hoogdalem, E. J. and L. G. J. De Leede (1993). "Second Jerusalem conference on pharmaceutical sciences and clinical." Pharmaceutisch Weekblad 128(2): 48-51.

Van Lieshout, E. M. M., G. H. Posner, et al. (1998). “Effects of the sulforaphane analog compound 30, indole-3-carbinol.” Biochimica et Biophysica Acta - General Subjects 1379(3): 325-336. Several dietary compounds have been demonstrated to reduce gastrointestinal cancer rates in both humans and animals. We showed that high human gastrointestinal tissue levels of glutathione S-transferase (GST), a family of detoxification enzymes consisting of class Alpha, Mu, Pi and Theta isoforms, were inversely correlated with cancer risk. We now investigated whether the sulforaphane analog compound 30, indole-3-carbinol, D-limonene or relafen, supplemented in the diet for two weeks at 1450, 250, 10,000, and 200 ppm, respectively, influenced (i) GST activity, (ii) GST isoenzyme levels, (iii) GSH levels, or (iv) glutathione peroxidase (GPx) activity in the gastrointestinal tract of male Wistar rats. Sulforaphane analog compound 30 enhanced GST activity in all organs studied (1.2-2.4 X). It induced GST Alpha levels in small intestine and liver, GST Mu levels in stomach and small intestine, GST Pi levels in stomach and small and large intestine, and GSH levels in stomach and proximal and middle small intestine. Indole-3-carbinol induced gastric GST Mu and hepatic GST Alpha levels. D-limonene induced hepatic GST Alpha, colonic GST Pi levels and proximal small intestinal GST enzyme activity and GST Pi levels. Relafen induced hepatic GST Alpha levels, distal small intestinal and gastric GST Pi levels, and oesophageal and proximal small intestinal GSH levels. GPx activity was enhanced by relafen in oesophagus, and in distal small intestine by sulforaphane analog compound 30. Enhancement of GSTs and to a lesser extent GPx and GSH, resulting in a more efficient detoxification. may explain at least in part the anticarcinogenic properties of sulforaphane analog compound 30, and to a much lesser extent of indole-3-carbinol and D-limonene.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). “Constituents of essential oil of Myrtus communis.” Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Vigushin, D. M., G. K. Poon, et al. (1998). “Phase I and pharmacokinetic study of D-limonene in patients with.” Cancer Chemotherapy and Pharmacology 42(2): 111-117. Purpose: D-Limonene is a natural monoterpene with pronounced chemotherapeutic activity and minimal toxicity in preclinical studies. A phase I clinical trial to assess toxicity, the maximum tolerated dose (MTD) and pharmacokinetics in patients with advanced cancer was followed by a limited phase II evaluation in breast cancer. Methods: A group of 32 patients with refractory solid tumors completed 99 courses of D-limonene 0.5 to 12 g/msup 2 per day administered orally in 21-day cycles. Pharmacokinetics were analyzed by liquid chromatography-mass spectrometry. Ten additional breast cancer patients received 15 cycles of D-limonene at 8 g/msup 2 per day. Intratumoral monoterpene levels were measured in two patients. Results: The MTD was 8 g/msup 2 per day: nausea, vomiting and diarrhea were dose limiting. One partial response in a breast cancer patient on 8 g/msup 2 per day was maintained for 11 months; three patients with colorectal carcinoma had prolonged stable disease. There were no responses in the phase II study. Peak plasma concentration (C(max)) for D-limonene ranged from 10.8 +/-

6.7 to 20.5 +/- 11.2 muM. Predominant circulating metabolites were perillic acid (C(max) 20.7 +/- 13.2 to 71 +/- 29.3 muM), dihydroperillic acid (C(max) 16.6 +/- 7.9 to 28.1 +/- 3.1 muM), limonene-1,2-diol (C(max) 10.1 +/- 8 to 20.7 +/- 8.6 muM), uroterpenol (C(max) 14.3 +/- 1.5 to 45.1 +/- 1.8 muM), and an isomer of perillic acid. Both isomers of perillic acid, and cis and trans isomers of dihydroperillic acid were in urine hydrolysates. Intratumoral levels of D- limonene and uroterpenol exceeded the corresponding plasma levels. Other metabolites were trace constituents in tissue. Conclusions: D-Limonene is well tolerated in cancer patients at doses which may have clinical activity. The favorable toxicity profile supports further clinical evaluation.

Vilela, M. P. and M. Mincis (1967). "[Use of geranyl farnesylacetate in patients with non-specific ulcerative rectocolitis]." Hospital (Rio J) 71(3): 691-6.

Wainwright, G., S. G. Webster, et al. (1998). "Neuropeptide regulation of biosynthesis of the juvenoid, methyl farnesoate, in the edible crab, Cancer pagurus." Biochem J 334(Pt 3)): 651-7. The neuropeptide mandibular organ (MO)-inhibiting hormone (MO-IH), synthesized and secreted from the X-organ-sinus-gland complex of the eyestalk, regulates the biosynthesis of the putative crustacean juvenile hormone, methyl farnesoate (MF). Using radiolabelled acetate as a precursor for isoprenoid biosynthesis, farnesoic acid (FA), farnesol, farnesal, MF and geranyl geraniol were detected in MOs cultured for 24 h. Treatment of MOs with extract of sinus gland inhibited the final step of biosynthesis of MF, catalysed by FA O-methyltransferase. Additionally, treatment of MOs with purified MO-IH exhibited a dose-dependent inhibition of this final step of MF synthesis. The extent of this inhibition was dependent on the ovary stage of the MO-donor animal, being maximal in MOs from animals in the early stages of ovarian development. Assay of FA O-methyltransferase activity, using [3H]FA in the presence of S-adenosyl-l-methionine, demonstrated that the enzyme was located in the cytosolic fraction of MOs and was inhibited by incubation of MOs with MO-IH prior to preparation of subcellular fractions. For cytosolic preparations taken from vitellogenic animals, both Vmax and Km were appreciably lower than for those taken from non-vitellogenic animals. Conversely, eyestalk ablation of early-vitellogenic animals, which removes the source of MO-IH in vivo, resulted in enhancement of the cytosolic FA O-methyltransferase activity. Although both Vmax and Km show an appreciable increase upon eyestalk ablation, the increased enzyme activity is probably reflected by the fact that Vmax/Km (an approximate indication of kcat) has increased 5-fold. The combined evidence demonstrates that MO-IH inhibits FA O-methyltransferase, the enzyme which catalyses the final step of MF biosynthesis in MOs.

Wargovich, M. J. (2001). "Herbals and cancer." Advances in Experimental Medicine and Biology 492(-): 195-202.

Williams, S. N., M. L. Anthony, et al. (1998). "Induction of apoptosis in two mammalian cell lines results in increased levels of fructose-1,6-bisphosphate and CDP-choline as determined by 31P MRS." Magn Reson Med 40(3): 411-20. Programmed cell death or apoptosis was induced in human promyelocytic leukemia (HL-60) and Chinese hamster ovary (CHO-K1) cells using several cytotoxic drugs that have different modes of action,

including camptothecin, ceramide, chelerythrine, etoposide, farnesol, geranyl geraniol, and hexadecylphosphocholine. The consequent changes in cellular metabolism were monitored using 31P MRS measurements on intact cells and cell extracts. Cells undergoing programmed cell death exhibited characteristic changes in the levels of glycolytic and phospholipid metabolites. The most significant changes were increases in the concentration of the glycolytic intermediate, fructose-1,6-bisphosphate and in the concentration of CDP-choline, which is an intermediate in phosphatidylcholine biosynthesis. In HL-60 cells, the increase in fructose-1,6-bisphosphate levels could be explained by depletion of cellular NAD(H) levels. All of the agents used to induce apoptosis caused the accumulation of CDP-choline. Since the resonances of this compound occur in a relatively well resolved region of tissue spectra, it could provide a marker for apoptosis that would allow the noninvasive detection of the process in vivo using 31P MRS measurements.

Gamma Cadinene CAS# 29350-73-0 (Naphthalene decahydro 1,6-dimethyl 1-methylethyl)

Isaac, O. (1994). “Calendula officinalis L. - Marigold.” Zeitschrift fur Phytotherapie 15(6): 356-370. Marigold (Calendula officinalis L.) in the process of rediscovering natural healing forces has gained importance. Increasing significance is contributed to calendula ointments, which have been used traditionally for a long time. The range of the calendula constituents is characterized by a high level of terpenoids e.g. saponosides in the form of oleanolglycosides and triterpene alcohols. The triterpenediol-3-monoesters consist for 85% of faradiolesters. The colour of the flowers depends on their content of carotinoids. Orange flowers contain carotins, especially lycopin, while the yellow varieties predominately contain xanthophylls. Characteristic calendula-flavonoids are the isorhamnetin glycosides. Contrary to other asteraceae marigold contains no sesquiterpene lactons. Also remarkable is the fat oil of the seeds which predominately consists of the conjugated trienoic acid calendula acid. Calendula preparations are mainly used for the treatments of wounds and for cosmetical purposes. The antiinflammatory principle can be isolated by lipophilic extraction, e.g. by extraction with hypercritical carbondioxide and used as ingredient of creams and ointments.

Saxena, V. K. and R. N. Sharma (1998). “Constituents of the essential oil from Commiphora mukul gum resin.” Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Beta-Citral CAS# 5392-40-5, 141-27-5 (3,7-dimethyl 2,6-octadienal, geranial, neral, geranialdehyde, Geranaldehyde)

Barnes, J. (1998). "Complementary medicine: Aromatherapy." Pharmaceutical Journal 260(6998): 862-867.

Boyd, E. M. and E. P. Sheppard (1970). "The effect of inhalation of citral and geraniol on the output and composition of respiratory tract fluid." Arch Int Pharmacodyn Ther 188(1): 5-13.

Cain, W. S., F. T. Schiet, et al. (1995). "Comparison of models of odor interaction." Chem Senses 20(6): 625-37. Subjects rated the overall perceived intensity of concentrations of the odorants cineole, geraniol, hexyl salicylate, and linalyl acetate smelled alone and in binary mixtures. The subjects also rated intensity of specified constituents (e.g. amount of cineole in cineole, and in mixtures of cineole and linalyl acetate). The intensity of the stronger component alone offered a close description of perceived intensity. In addition to the Stronger Component model, two other psychological models (Vector and U model) and two psychophysical models (UPL2 and Equiratio Mixture model) offered descriptions ranging from fair to very good. Psychological models gave better fits, but lack explanatory power. Some results indicated that weaker odors add more potently than stronger odors, an outcome incompatible with these models. The psychophysical models, based on the additivity of single components, generally overestimated perceived intensity. Judgments of individual qualities gave only slight encouragement to any expectation of differences in masking or maskability among odorants. The results highlight the need to test particular critical hypotheses regarding how people perceive mixtures.

Cardullo, A. C., A. M. Ruszkowski, et al. (1989). "Allergic contact dermatitis resulting from sensitivity to citrus peel, geraniol, and citral." J Am Acad Dermatol 21(2 Pt 2): 395-7. A bartender with hand dermatitis had allergic contact sensitivity to the skin of lemon, lime, and orange but not to their juices. Although most reported cases of citrus peel allergy are due to d-limonene, for our patient, reactions to patch tests for geraniol and citral, two minor components of citrus peel oil, were positive, whereas those for d-limonene were negative. Contact allergy to citrus peel oil should be considered in patients with hand dermatitis who are occupationally exposed to citrus fruits.

Carriere, F., G. Gil, et al. (1989). "Biotransformation of geraniol by photoautotrophic, photomixotrophic and heterotrophic plant cell suspensions." Phytochemistry 28(4): 1087-1090. Cell suspension cultures of 4 plant species (Euphorbia characias, Nicotiana tabacum, Catharanthus roseus and Glycine max) were maintained under different carbon and energy supply regimes, i.e. photoautotrophy, photomixotrophy and heterotrophy, and were assayed for their capacity to biotransform geraniol. The reactions of interconversion of geraniol into nerol, or oxidation of alcohols into their respective aldehydes, were mainly determined by the species, regardless of the modes of culture. A rapid metabolism of monoterpenic alcohols to unidentified compounds was observed. In one cell

suspension (G. max), a high biotransformation activity (40-60%) into neral and geranial was detected.

Chagonda, L. S., C. Makanda, et al. (2000). “The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe.” Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Chan, M. M. Y., C. T. Ho, et al. (1995). “Effects of three dietary phytochemicals from tea, rosemary and turmeric.” Cancer Letters 96(1): 23-29. In chronic inflammation, cytokines induce the production of nitric oxide (NO(.)) that is converted to DNA damaging and carcinogenic peroxynitrite and nitrite. The compounds epigallocatechin gallate (EGCG), carnosol, and curcumin are non-vitamin phytochemicals contained in commonly consumed dietary plants. They are known to be anti-inflammatory and cancer preventive. Therefore, we studied their effect on the generation of peroxynitrite radicals and nitrite. They inhibited lipopolysaccharide (LPS) and interferon-gamma (IFNgamma) induced nitrite production by mouse peritoneal cells by more than 50% at 2.5-10 muM. Cell viability assays verified that the inhibition was not due to general cellular toxicity.

Chaumont, J. P. and D. Leger (1992). “[Campaign against allergenic moulds in dwellings. Inhibitor properties of essential oil of Geranium 'Bourbon', citronellol, geraniol and citral].” Ann Pharm Fr 50(3): 156-66. Many fungal airborne spora show allergenic effects. Indoor (dwelling, work rooms, hospital chambers) can be disinfected by elimination of living particles. We have undertaken experiments in more and more spacious bulks for evaluation of the antifungal effects of vapours of essential oils and some volatiles compounds. Results show that the Mucorales and Geotrichum resist strongly. On the contrary, the Cladosporium strains, some Aspergillus and Penicillium, Trichothecium roseum are the most sensitive, specially towards the citral vapours. Experiments in hospital can be undertaken.

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium

graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Dharmendra Saikia, S. P. S. Khanuja, et al. (2001). “Comparative antifungal activity of essential oils and constituents from three distinct genotypes of Cymbopogon spp.” Current Science 80(10): 1264-1266. The antifungal activity of the essential oils of palmarosa (C. martini) cv. CIMAP/PRC-1, lemon grass (C. flexuosus) cv. Pragati and citronella (C. winterianus) cv. BIO-13, as well as some essential oil components, viz. citral, geraniol, citronellol and citronellal, were tested against 4 human pathogenic fungi (Microsporum gypseum, Candida albicans, Sporothrix schenckii, Aspergillus niger) to identify plant substances for future antifungal formulations. Among the essential oils and components tested, lemon grass oil and geraniol, respectively, recorded the highest antifungal activity. M. gypseum was highly sensitive to all the essential oils and tested components, with inhibition zones 1.5- to 2-fold larger than the other fungal pathogens and generally low minimum inhibitory dilutions (MID). Citral produced the smallest inhibition zones but demonstrated the highest activity in terms of MID and minimum fungicidal concentration (MFC) values, which were comparable to lemon grass oil. Lemon grass, palmarosa oil and geraniol recorded the highest inhibition of C. albicans, A. niger and S. schenckii, respectively. However, the highest antifungal activity in terms of MFC was recorded by lemon grass for all organisms tested.

Elson, C. E., G. L. Underbakke, et al. (1989). “Impact of lemongrass oil, an essential oil, on serum cholesterol.” Lipids 24(8): 677-9. To test the hypothesis that non-sterol mevalonate pathway end products lower serum cholesterol levels, we asked 22 hypercholesterolemic subjects (315 +/- 9 mg cholesterol/dl) to take a daily capsule containing 140 mg of lemongrass oil, an essential oil rich in geraniol and citral. The paired difference in serum cholesterol levels of subjects completing the 90-day study approached significance (P less than 0.06, 2-tailed t-test). The subjects segregated into two groups, one consisting of 14 subjects resistant to the protocol and the other consisting of 8 subjects who responded. Paired differences in cholesterol level at 30, 60 and 90 d for resistant subjects were +2 +/- 6, +2 +/- 7 and -1 +/- 6 mg/dl; paired differences for the responding subjects were -25 +/- 10 (p less than 0.05), -33 +/- 8 (p less than 0.01) and -38 +/- 10 (p less than 0.025), respectively. The paired difference (+8 +/- 4) in the cholesterol levels of six responders 90 days after the discontinuation of lemongrass oil was not significant.

Epinat, J. C. and T. D. Gilmore (1999). “Diverse agents act at multiple levels to inhibit the Rel/NF-kappaB.” Oncogene 18(49): 6896-6909. Rel/NF-kappaB transcription factors regulate several important physiological processes, including developmental processes,

inflammation and immune responses, cell growth, cancer, apoptosis, and the expression of certain viral genes. Therefore, they have also been sought-after molecular targets for pharmacological intervention. As details of the Rel/NF-kappaB signal transduction pathway are revealed, it is clear that modulators of this pathway can act at several levels. Inhibitors of the Rel/NF-kappaB pathway include a variety of natural and designed molecules, including anti-oxidants, proteasome inhibitors, peptides, small molecules, and dominant-negative or constitutively active polypeptides in the pathway. Several of these molecules act as general inhibitors of Rel/NF-kappaB induction, whereas others inhibit specific pathways of induction, Inhibitors of Rel/NF-kappaB are likely to gain stature as treatments for certain cancers and neurodegenerative and inflammatory diseases.

Frosch, P. J., B. Pilz, et al. (1995). “Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes.” Contact Dermatitis 33(5): 333-42. The objective of this study was to determine the frequency of reactivity to a series of commonly used fragrances in dermatological patients. A total of 48 fragrances (FF) were chosen, based on the publication of Fenn in 1989 in which the top 25 constituents of 3 types (1. perfumes, 2. household products, 3. soaps) of 400 commercial products on the US market had been determined. In a pilot study on a total of 1069 patients in 11 centres, the appropriate test concentration and vehicle were examined. For most fragrances, 1% and 5% were chosen, and petrolatum proved to be the best vehicle in comparison to isopropyl myristate and diethyl phthalate. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8% fragrance mix (FM) and its 8 constituents. In patients with a positive reaction to any of the 48 FF, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were tested in 11 centres. The 8% FM was positive in 89 patients (8.3% of 1072 patients). Allergic reactions to the constituents were most frequent to oak moss (24), isoeugenol (20), eugenol (13), cinnamic aldehyde (10) and geraniol (8). Reactions read as allergic on day 3/4 were observed only 10X to 7 materials of the new series (Iso E Super (2), Lyral (3), Cyclacet (1), DMBCA (1), Vertofix (1), citronellol (1) and amyl salicylate (1)). The remaining 41 fragrances were negative. 28 irritant or doubtful reactions on day 3/4 were observed to a total of 19 FF materials (more than 1 reaction: 5% citronellol (2), 1% amyl salicylate (2), 1% isononyl acetate (3), 0.1% musk xylol (2), 1% citral (2), and 1% ionone beta (2)). Clinical relevance of positive reactions to any of the FF series was not proved in a single case. This included the 4 reactions in patients who were negative to the 8% FM. In conclusion, the top 25 fragrances commonly found in various products caused few reactions in dermatological patients and these few appeared to be clinically irrelevant, with the possible exception of Lyral. However, this data should be interpreted in the light of the relatively small number of patients tested (only 100 in most centres).

Gbile, Z. O. and S. K. Adesina (1987). “Nigerian flora and its pharmaceutical potential.” Journal of Ethnopharmacology 19(1): 1-16.

Gee, J. M. and I. T. Johnson (2001). “Polyphenolic compounds: Interactions with the gut and implications for.” Current Medicinal Chemistry 8(11): 1245-1255. Polyphenolic compounds are abundant throughout the plant kingdom and are found in a wide variety of human foods. The flavonoids, which are the best defined group of polyphenols in the human diet, themselves comprise a large and complex group, all of which contain a three-ring structure with two aromatic centres and a central oxygenated heterocycle. Recent evidence suggests that significant quantities of quercetin and possibly myricetin and kaempferol are absorbed in the gut. A larger fraction probably remains in the lumen, and thus a substantial proportion of the gastrointestinal mucosa is exposed to biologically significant concentrations of these compounds. A substantial body of experimental work has established that flavonoids can suppress carcinogenesis in animal models and there is considerable interest in the biological effects of these compounds at the cellular level. Flavonoids interact with cellular signal pathways controlling the cell cycle, differentiation and apoptosis. Their potentially antineoplastic effects include antioxidant activity, induction of Phase II enzyme activity, inhibition of protein kinases and interactions with Type II estrogen binding sites. Naturally occuring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential for pharmaceutical uses as chemopreventive agents against neoplastic changes in the alimentary tract. Future research should therefore focus on the biological effects of flavonoids in the human body, using biomarkers to define their effects at each stage in the onset of neoplasia.

Geldof, A. A., C. Engel, et al. (1992). “Estrogenic action of commonly used fragrant agent citral induces prostatic hyperplasia.” Urol Res 20(2): 139-44. A rat model for benign prostatic hyperplasia in man (BPH) was investigated. Citral treatment of male Copenhagen rats for 4 months via the transdermal route resulted in a marked hyperplasia of glandular epithelium and interglandular stroma in the ventral prostate. Despite the cellular hyperplasia there was not a significant increase in prostate weight. Investigations of the mechanism of action of citral showed that application of citral directly to the vagina in female, ovariectomized rats resulted in an increased proliferation of vaginal epithelium and a significant increase in the BrdUrd incorporation in vaginal epithelial cells, in short a similar effect to that of estrogen application. In an in vitro assay citral proved to inhibit estrogen binding to estrogen receptors, while no such inhibition was observed with testosterone for androgen receptors. These observations together with the estrogen implication in the BPH and the reported incidence of gynecomastia following exposure to geraniol, a precursor of citral, strongly suggest that the prostatic hyperplasia-inducing capacity of citral may be due to its estrogenic action.

Hornby, J. M., E. C. Jensen, et al. (2001). “Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol.” Appl Environ Microbiol 67(7): 2982-92. The inoculum size effect in the dimorphic fungus Candida albicans results from production of an extracellular quorum-sensing molecule (QSM). This molecule prevents mycelial development in both a growth morphology assay and a differentiation assay using three chemically distinct triggers for germ tube formation (GTF): L-proline, N-acetylglucosamine, and serum (either pig or fetal bovine). In all cases, the presence of QSM prevents the yeast-to-mycelium conversion, resulting in actively budding yeasts

without influencing cellular growth rates. QSM exhibits general cross-reactivity within C. albicans in that supernatants from strain A72 are active on five other strains of C. albicans and vice versa. The QSM excreted by C. albicans is farnesol (C(15)H(26)O; molecular weight, 222.37). QSM is extracellular, and is produced continuously during growth and over a temperature range from 23 to 43 degrees C, in amounts roughly proportional to the CFU/milliliter. Production is not dependent on the type of carbon source nor nitrogen source or on the chemical nature of the growth medium. Both commercial mixed isomer and (E,E)-farnesol exhibited QSM activity (the ability to prevent GTF) at a level sufficient to account for all the QSM activity present in C. albicans supernatants, i.e., 50% GTF at ca. 30 to 35 microM. Nerolidol was ca. two times less active than farnesol. Neither geraniol (C(10)), geranylgeraniol (C(20)), nor farnesyl pyrophosphate had any QSM activity.

Hursting, S. D., T. J. Slaga, et al. (1999). “Mechanism-based cancer prevention approaches: Targets, examples, and.” Journal of the National Cancer Institute 91(3): 215-225. Humans are exposed to a wide variety of carcinogenic insults, including endogenous and man-made chemicals, radiation, physical agents, and viruses. The ultimate goal of carcin genesis research is to elucidate the processes involved in the induction of human cancer so that interventions may be developed to prevent the disease, either in the general population or in susceptible subpopulations. Progress to date in the carcinogenesis field, particularly regarding the mechanisms of chemically induced cancer, has revealed several points along the carcinogenesis pathway that may be amenable to mechanism-based prevention strategies. The purpose of this review is to examine the basic mechanisms and stages of chemical carcinogenesis, with an emphasis on ways in which preventive interventions can modify those processes. Possible ways of interfering with tumor initiation events include the following: i) modifying carcinogen activation by inhibiting enzymes responsible for that activation or by direct scavenging of DNA-reactive electrophiles and free radicals; ii) enhancing carcinogen detoxification processes by altering the activity of the detoxifying enzymes; and iii) modulating certain DNA repair processes. Possible ways of blocking the processes involved in the promotion and progression stages of carcinogenesis include the following: i) scavenging of reactive oxygen species; ii) altering the expression of genes involved in cell signaling, particularly those regulating cell proliferation, apoptosis, and differentiation; and iii) decreasing inflammation. In addition, the utility for mechanism-based cancer prevention research of new animal models that are based on the overexpression or inactivation of specific cancer-related genes is examined.

Kessler, O. J., Y. Keisari, et al. (1998). “Role of chronic inflammation in the promotion of prostatic hyperplasia.” Journal of Urology 159(3): 1049-1053. Purpose: Chronic prostatitis is a common histopathological finding in prostatectomized patients. A possible interrelationship between the presence of leukocyte exudate and the extent of the hyperplastic lesions has been suggested. The aim of the present study was to analyze the effect of the immunomodulator compounds, Complete Freund Adjuvant (CFA) and cyclosporin A (CsA), administered alone or together with citral on the induction and extent of rat prostatic hyperplasia. Materials and Methods: Adolescent Wistar rats (42 days old) were given citral alone or combined with CFA or CsA for one month.

Semiquantitative analysis of the extent of the hyperplastic lesions was made with the histoscore protocol. Results: CsA did not induce hyperplastic changes or abolish the ability of citral to promote hyperplastic changes or to affect the extent of the lymphocytic exudate in the stroma. CFA itself, however, had a proliferative action on the prostatic epithelium, and it augmented the hyperplastic changes induced by citral and even induced atypical transformations of the acinar epithelium. Conclusions: Immunoinflammatory stimulators might play a role in the prostatic epithelial cell growth and proliferation processes, most probably by modulation of the cytokine system.

Kohlert, C., I. Van Rensen, et al. (2000). "Bioavailability and pharmacokinetics of natural volatile terpenes in." Planta Medica 66(6): 495-505. Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of in vitro effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the in vitro activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.

Krishnan, K. and D. E. Brenner (1997). "Nonsteroidal anti-inflammatory drugs (NSAIDS) in colorectal cancer." Cancer Journal 10(1): 10-16. Colorectal carcinoma is an important, feasible and attractive target for chemoprevention because a) it is a major cause of mortality in the United States and in other developed countries worldwide, b) there is a high mortality associated with advanced disease, c) there is a well described molecular carcinogenesis pathway and d) recent advances in molecular genetics will improve the ability to identify high-risk subjects. Epidemiological data, colonoscopic screening and advances in molecular genetics has made possible the identification and selection of subjects at increased risk of developing colorectal cancer. Due to this new information it may be possible to impede malignant cellular transformation with drugs. Such intervention with relatively simple maneuvers, such as a low daily dose of aspirin, can potentially reduce mortality from colorectal cancer. Prospective trials need to confirm experimental and epidemiological data supporting the efficacy of aspirin and other NSAID as chemopreventive agents before they can be used in the general population at risk. To use cancer chemopreventives effectively and safely in an asymptomatic population, the risks should be minimized and the benefits maximized by determination of optimal dose, schedule and chemopreventive mechanism of the NSAID. By linking the putative mechanism of drug action to effect endpoints, we expect to know whether the chemopreventive intervention is likely to be effective in a given individual.

Kulevanova, S., A. Kaftandzieva, et al. (2000). "Investigation of antimicrobial activity of essential oils of several Macedonian Thymus L. species (Lamiaceae)." Boll Chim Farm 139(6): 276-80. Antimicrobial activity of twenty specimens of essential oils of eleven Thymus species, naturally occurring in the Macedonian flora, was investigated by agar diffusion and broth dilution methods. Inhibition of growth and microbicidal action was

examined on three Gram positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumoniae). In spite of wide variability in essential oil composition, ranging from traces of thymol to the amount of about 50% thymol in oils, all examined samples of Thymus essential oil possessed strong antibacterial activity. Zones of inhibition of growth (for 25% dilution of oils) was from 10-54 mm in diameters. MICs ranging from 0.012-0.1% while MMCs were from 0.025-0.4% for essential oils that contained large amounts of phenols and 0.2-1.6% for those which contained traces of phenols and large amounts of geraniol, linalool and (Z + E)-citral.

Langman, J. M. (1995). "d-Limonene: Is it a safe, effective alternative to xylene?" Journal of Histotechnology 18(2): 131-138. d-Limonene (Cinf 1inf 0Hinf 1inf 6) is a hydrocarbon of the monoterpene sub-group and is the major constituent of citrus peel oils (90-95%) and other ethereal oils. Used in the food and cosmetic industry for many years, it has been generally regarded as safe. In recent years, d-limonene-based products have been used in some pathology laboratories as a replacement for xylene, a known toxic substance. d-Limonene is readily absorbed into the body, metabolized, and cleared from the body. It appears to perform adequately as a wax solvent and clearing agent, and it has a reduced fire risk compared with xylene. Although product safety data sheets mention several adverse effects of exposure to d-limonene, exposure to 76 ppm for 2 hr had no irritative or central nervous system effects in volunteers. Headaches and nausea have been reported from some laboratories that have used d-limonene, and proper ventilation is required. Some users have reported skin irritations as well. It does induce renal pathology in some strains of adult male rats but has no proven genetic effects. In comparison, xylene has toxic effects on many organ systems, although it, too, does not appear to be mutagenic or carcinogenic. d-Limonene appears to be a safe and effective replacement for xylene, although more extensive use and further experimental studies may require revision of this conclusion.

Mahmoud, A. L. (1994). "Antifungal action and antiaflatoxigenic properties of some essential oil constituents." Lett Appl Microbiol 19(2): 110-3. The effect of 20 essential oil constituents on Aspergillus flavus growth and aflatoxin production was tested at the level of 1000 ppm. Some of the tested oils exhibited inhibitory effects on fungal growth and toxin formation. Five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth and aflatoxin synthesis. Trials for determining the minimum inhibitory concentration (MIC) of these oils revealed that geraniol, nerol and citronellol were effective at 500 ppm, while thymol and cinnamaldehyde were highly effective at doses as low as 250 and 200 ppm, respectively. It was observed that citral, citronellol and eugenol prevented fungal growth and toxin formation for up to 8 d. However, after 15 d of incubation, toxin production was greater than the controls.

Manson, M. M., A. Gescher, et al. (2000). "Blocking and suppressing mechanisms of chemoprevention by dietary." Toxicology Letters 112-113(-): 499-505. Many dietary constituents are chemopreventive in animal models, and experiments with cultured cells are revealing various potential mechanisms of action. Compounds classified as blocking agents can prevent, or greatly reduce, initiation of carcinogenesis, while suppressing

agents affect later stages of the process by reducing cell proliferation. Many compounds have both types of activity. Blocking mechanisms include alteration of drug metabolising activities and scavenging of reactive oxygen species. Mechanisms which suppress tumorigenesis often involve modulation of signal transduction pathways, leading to altered gene expression, cell cycle arrest or apoptosis. As our knowledge of how these dietary components affect cell biochemistry improves, so the likelihood of success in chemoprevention trials and in provision of dietary advice to the general population to optimise the chances of preventing disease is increased. Copyright (C) 2000 Elsevier Science Ireland Ltd.

Mashanov, V. I. and I. E. Logvinenko (1979). “Artemisia balchanorum under cultivation.” Doklady Vsesoyuznoi Ordena Lenina Akademii Sel'skokhozyaistvennykh Nauk Imeni V.I. Lenina(1): 23-24. In 1972, the high-yielding varieties Krymchanka [Crimean], Balkhanka, Yuzhanka [Southerner], Evrika [Eurika] and Slavyanka were selected from a population in the Ukraine for high content of citral, linalool and geraniol. During 1975-77, they were studied in various zones of the southern Ukraine. The best for yield of essential oil in all zones were Evrika and Balkhanka.

Mehmood, Z., S. Ahmad, et al. (1997). “Antifungal activity of some essential oils and their major constituents.” Indian Journal of Natural Products 13(2): 10-13. Essential oils from lemongrass (Cymbopogon flexuosus), palmarosa (C. martini), cinnamon (Cinnamomum zeylanicum) and mint (Mentha arvensis) were tested for antifungal activity against Aspergillus, Fusarium and Cladosporium isolates from an ophthalmology specimen. Cinnamon oil was the most active against Aspergillus, and overall; palmarosa was the most active against Fusarium, and lemongrass against Cladosporium. The most active constituent found was eugenol, from citral and geraniol; citronellol and cinnamaldehyde were inactive.

Milone, C., M. L. Tropeano, et al. (2002). “Selective liquid phase hydrogenation of citral on Au/Fe2O3 catalysts.” Chem Commun (Camb)(8): 868-9. Gold supported on iron oxide hydrogenates citral (an alpha,beta-unsaturated aldehyde) to the corresponding alpha,beta-unsaturated alcohols (geraniol and nerol) with a selectivity higher than 95%.

Moleyar, V. and P. Narasimham (1992). “Antibacterial activity of essential oil components.” Int J Food Microbiol 16(4): 337-42. Antibacterial activity of fifteen essential oil components towards food borne Staphylococcus sp., Micrococcus sp., Bacillus sp. and Enterobacter sp. was studied by an agar plate technique. Cinnamic aldehyde was the most active compound followed by citral, geraniol, eugenol and menthol. At 500 micrograms/ml, cinnamic aldehyde completely inhibited the bacterial growth for more than 30 days at 30 degrees C that was comparable to 200 micrograms/ml of butylated hydroxy anisole (BHA). At lower temperatures, 25 and 20 degrees C, antibacterial activity of the five essential oil components increased. Addition of sodium chloride at 4% level (w/v) in the medium had no effect on the inhibitory activity of cinnamic aldehyde. In mixtures of cinnamic aldehyde and eugenol or BHA an additive effect was observed.

Okutani, F., J. J. Zhang, et al. (2002). “Non-specific olfactory aversion induced by intrabulbar infusion of the GABA(A) receptor antagonist bicuculline in young rats.” Neuroscience 112(4): 901-6. On postnatal day 12, young rats show an aversion to an odor to which they had been exposed along with presentations of foot shock on postnatal day 11. The acquisition of this aversive learning involves and requires disinhibition of the mitral/tufted cells induced by centrifugal noradrenergic activation during somatosensory stimulation. This olfactory learning is established only for the odor to which the rat has been exposed during conditioning. Infusion of the GABA(A) receptor antagonist bicuculline at a high dose (2.0 nmol/each olfactory bulb) into the olfactory bulb in the presence of an odor is capable of developing olfactory aversive responses without somatosensory stimulation in young rats. The purpose of this study is to characterize the properties of bicuculline-induced aversive responses. In contrast to the odor specificity of aversive learning produced by odor-shock conditioning, bicuculline-induced aversive responses lack odor specificity. Namely, bicuculline infusion in the presence of a citral odor results, in a dose-dependent manner, in subsequent aversive responses to strange odors (benzaldehyde and vanillin) that have never been presented. Moreover, bicuculline infusion alone is sufficient to produce dose-dependent aversive responses to strange odors (citral, benzaldehyde and geraniol).From these results we suggest that disinhibition of mitral/tufted cells from granule cells by bicuculline infusion makes young rats aversive to strange odors non-specifically, as if the rats had learned the odor aversion as a result of odor exposure paired with foot shock. Different mechanisms of disinhibition of the mitral/tufted cells may underlie both the pharmacological manipulation and noradrenergic activation by somatosensory stimulation.

Padula, L. Z., A. M. Collura, et al. (1977). “Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil.” Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Pattnaik, S., V. R. Subramanyam, et al. (1997). “Antibacterial and antifungal activity of aromatic constituents of essential oils.” Microbios 89(358): 39-46. Five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) were tested for antimicrobial activity against 18 bacteria (including Gram positive cocci and rods, and Gram negative rods) and 12 fungi (Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, A. oryzae, Alternaria citrii, Fusarium oxysporum, F. solani, Helminthosporium compactum, Macrophoma phaseolina, Sclerotium rolfsii, Sporothrix schenckii and Trichophyton mentagrophytes). In terms of antibacterial activity linalool was the most effective and inhibited 17 bacteria, followed by cineole, geraniol (each of which inhibited 16 bacteria), menthol and citral aromatic compounds, which inhibited 15 and 14 bacteria, respectively. Citral and geraniol oils were the most effective against fungi (inhibiting all 12 fungi), followed by linalool (which inhibited 10 fungi), cineole and menthol (each of which inhibited 7 fungi) compounds.

Rastogi, S. C., J. D. Johansen, et al. (1996). “Natural ingredients based cosmetics. Content of selected fragrance sensitizers.” Contact Dermatitis 34(6): 423-6. In the present study, we have investigated 42 cosmetic products based on natural ingredients for content of 11 fragrance substances: geraniol, hydroxycitronellal, eugenol, isoeugenol, cinnamic aldehyde, cinnamic alcohol, alpha-amylcinnamic aldehyde, citral, coumarin, dihydrocoumarin and alpha-hexylcinnamic aldehyde. The study revealed that the 91% (20/22) of the natural ingredients based perfumes contained 0.027%-7.706% of 1 to 7 of the target fragrances. Between 1 and 5 of the chemically defined synthetic constituents of fragrance mix were found in 82% (18/22) of the perfumes. 35% (7/20) of the other cosmetic products (shampoos, creams, tonics, etc) were found to contain 0.0003-0.0820% of 1 to 3 of the target fragrances. Relatively high concentrations of hydroxycitronellal, coumarin, cinnamic alcohol and alpha-amyl cinnamic aldehyde were found in some of the investigated products. The detection of hydroxycitronellal and alpha-hexylcinnamic aldehyde in some of the products demonstrates that artificial fragrances, i.e., compounds not yet regarded as natural substances, may be present in products claimed to be based on natural ingredients.

Rastogi, S. C., J. D. Johansen, et al. (1998). “Deodorants on the European market: quantitative chemical analysis of 21 fragrances.” Contact Dermatitis 38(1): 29-35. Deodorants are one of the most frequently used types of cosmetics and side-effects from them are common. Recent studies relate perfume allergy to this type of product. 73 deodorants were analyzed by gas chromatography--mass spectrometry for the determination of the contents of 7 wellknown fragrance allergens from the fragrance mix and 14 other commonly used fragrance materials. The deodorants were purchased at retail outlets in 5 European countries. It was found that in general, fragrance mix ingredients were more frequently present in vapo- and aerosol sprays than in roll-on products. The levels of the fragrance mix substances ranged from 0.0001-0.2355%. The products investigated contained cinnamic aldehyde and isoeugenol less frequently (17% and 29% respectively), and eugenol and geraniol most frequently (57% and 76% respectively). The 14 other fragrance materials were found in 40-97% of the deodorants, with hedione and benzyl acetate the most frequently found substances. The concentration of these 14 substances ranged from 0.0001-2.7%. It is concluded that the levels of cinnamic aldehyde and isoeugenol found in the deodorants could prove to be relevant for elicitation of contact dermatitis. No conclusions could be drawn about the other fragrance mix constituents, as threshold levels in sensitized individuals have not been investigated. Furthermore, all of the fragrance materials investigated were frequently found in deodorants and, apart from the fragrance mix ingredients, the extent of problems with sensitization to these fragrance materials is largely unknown.

Sargenti, S. R. and F. M. Lancas (1997). “Supercritical fluid extraction of Cymbopogon citratus (DC.) Stapf.” Chromatographia 46(5/6): 285-290. Steam extraction of the leaves of C. citratus (known as lemon grass) produces a volatile oil used for medicinal purposes. Supercritical fluid extraction of C. citratus in sequential and dynamic extraction modes is described. Different modifiers for supercritical carbon dioxide were used for the extractions. Principal compounds in the essential oils were separated by GC and GC-MS

and identified as neral, geraniol, geranial, nerolic acid and geranic acid. Different chromatographic profiles were obtained when the type and proportion of modifier were changed. When the modifier was 10% hexane, the extract yield was similar to that for steam extraction but with additional compounds; with 30% hexane the extract was similar to that from Soxhlet extraction with hexane; 10 or 20% acetone liberated compounds not extracted by other agents; 10% methanol was not selective.

Schaneberg, B. T. and I. A. Khan (2002). "Comparison of extraction methods for marker compounds in the essential oil of lemon grass by GC." J Agric Food Chem 50(6): 1345-9. A gas chromatography flame ionization detection method for the quantification of bioactive marker compounds (neral, geranial, geraniol, limonene, citronellal, and beta-myrcene) in the essential oil of Cymbopogon citratus (lemon grass) was developed. Four procedures for the extraction of essential oils from C. citratus were compared including solvent extraction, steam distillation extraction, accelerated solvent extraction, and supercritical fluid extraction. Solvent extraction by sonication with nonpolar solvents showed comparable results to the steam distillation method. Several commercial products prepared from C. citratus and Cymbopogon flexuosus were analyzed and compared.

Schilcher, H. (1986). "Pharmacology and toxicology of volatile oils." Therapiewoche 36(11): 1100-1112. Essential oils are not only very complex, they are also chemically very heterogeneous. Therefore it is not surprising that many different effects are known. Based on pharmacological and clinical tests and from the experiences of traditional medicine, essential oils have the following effects when applied externally: hyperaemic, antiinflammatory, antiseptic, granulation-promoting, deodorant, insecticide and repellent actions. When orally applied, the following effects are observed: expectorant, appetite-stimulating (digestants), choleretic, cholekinetic, carminative, spasmolytic, antiinflammatory, antiseptic, diuretic, calming (sedatives) and CNS-stimulating. Many other effects are reported in traditional medicine. The main side-effects of essential oils are allergic reactions; some have phototoxic effects. Only a few essential oils show necrotic, narcotic, abortive, nephrotoxic, hepatotoxic and cancer-producing reactions. In many cases the side-effects are pure toxic effects when misuse or abuse are the real reasons.

Scolnik, M., M. Konichezky, et al. (1994). "Immediate vasoactive effect of citral on the adolescent rat ventral." Prostate 25(1): 1-9. The vasoactive effect of a fragrance compound, citral, on rat ventral prostate is presented. A combined method of Indian-ink perfusion and postfixation transparency was used. One single dose of citral applied on the back skin of the rat induced an immediate triple response-like effect on the prostatic vascular bed. A slight vasodilatation appeared already after 21/2 min, followed by a marked vasoconstriction process after 5-min postcitral administration. Toward the period of 10 min, a second vasodilatation process was noted which persisted for the following 24 hr of observation. This study has demonstrated that changes in the microvascularization of the rat prostate can be estimated by counting the number of carbon-stained blood vessels. The results obtained by this technique are closely related with the findings in the vascular network profile of the macroangiographic observations. The mechanism of action of citral on the microvascularization of the rat ventral prostate

is yet unknown. Based on the present findings, we suggest that the neoplastic capacity of citral upon the prostatic epithelia is activated via a nonspecific inflammatory reaction modulated either by local release of neurotransmitters or throughout a direct effect of citral on the endothelial cells.

Sekiya, K., S. Kadota, et al. (1997). “Study on baths with crude drug. III. The effect of Ligustici Chuanxiong.” Biological and Pharmaceutical Bulletin 20(9): 983-987. To investigate the permeability of natural compounds through hairless mouse skin, compounds having a range of lipophilicity, i.e., ginsenoside-Re (1), baicalin (2), glycyrrhizin (3), baicalein (4), wogonin (5), honokiol (6), magnolol (7) bergapten (8), shikonin (9) and sinomenine (10) were used. These compounds permeated through the skin a little, however, they were generally accumulated trite the skin. The uptake amount into the skin of each compound related to their lipophilicities in the in vitro experiment. Furthermore, Ligustici Chuanxiong Rhizoma (Senkyu) ether extract (SEE) enhanced their permeability into the skin; especially, it exhibited an effect on the skin permeability of moderately lipophilic compounds such as 4, 8. The effect of SEE in vivo was similar to that obtained in the in vitro experiment. From these results, it was clarified that natural compounds having high lipophilicity sufficiently permeated into the hairless mouse skin owing to their accumulative property, and SEE enhanced the permeability of the moderately lipophilic compounds into the skin.

Serrano, G., C. Pujol, et al. (1989). “Riehl's melanosis: pigmented contact dermatitis caused by fragrances.” J Am Acad Dermatol 21(5 Pt 2): 1057-60. We report a case of a 27-year-old woman with a patchy, dark brown hyperpigmentation on the face. Patch tests were positive to lemon oil, geraniol, and hydroxycitronellal. A compact face powder that the patient used contained two of these chemicals. Hyperpigmentation disappeared within 6 months after the patient avoided contact with cosmetics containing these fragrances.

Sharma, J. N., K. C. Srivastava, et al. (1994). “Suppressive effects of eugenol and ginger oil on arthritic rats.” Pharmacology 49(5): 314-318. This study examined the effect of eugenol and ginger oil on severe chronic adjuvant arthritis in rats. Severe arthritis was induced in the right knee and right paw of male Sprague-Dawley rats by injecting 0.05 ml of a fine suspension of dead Mycobacterium tuberculosis bacilli in liquid paraffin (5 mg/ml). Eugenol (33 mg/kg) and ginger oil (33 mg/kg), given orally for 26 days, caused a significant suppression of both paw and joint swelling. These findings suggest that eugenol and ginger oil have potent antiinflammatory and/or antirheumatic properties.

Shureiqi, I., P. Reddy, et al. (2000). “Chemoprevention: General perspective.” Critical Reviews in Oncology/Hematology 33(3): 157-167. Chemoprevention is the use of natural or synthetic compounds to block, reverse, or prevent the development of invasive cancers. Cellular carcinogenesis forms the biologic basis for the identification of chemopreventives, assessment of their activity, and ultimately the success or failure of a chemopreventive. Chemopreventive agents undergo multistep evaluations to assess efficacy that are similar in concept but vastly different in practice to standard ablative oncologic therapeutics. In vitro assessments of potential anticarcinogenesis efficacy include measurements of an agent's antioxidant activity, induction of phase II

metabolizing enzymes and effects upon cellular proliferation and apoptotic control pathways. In vivo efficacy is assessed primarily in rodent models of carcinogenesis that are specific for a given organ target. The role of genetically modified animal models in the in vivo assessment of chemoprevention agents remains unclear. Clinical assessment of chemopreventive agent efficacy consists of a multistep process of identification of an optimal chemopreventive agent (phase 1), demonstration of efficacy in humans through the modulation of reversal of a tissue, biochemical, and molecular surrogates for neoplastic transformation and invasion (phase 2) and cancer risk reduction in large cohort trials (phase 3). Opportunities and future needs include the development of reliable, predictive in vivo models of carcinogenesis, careful exploration of the preventive pharmacology of therapeutic agents being used for non-cancer prevention indications, and the incorporation of genetic risk cohorts to define cancer chemopreventive efficacy. Copyright (C) 2000 Elsevier Science Ireland Ltd.

Sidibe, L., J. C. Chalchat, et al. (2001). “Aromatic plants of Mali (IV): chemical composition of essential oils of Cymbopogon citratus (DC) Stapf and C. giganteus (Hochst.) Chiov.” Journal of Essential Oil Research 13(2): 110-112. The composition of the essential oils of C. citratus and C. giganteus from Mali and Cote d'Ivoire, collected in 1993 and 1994, was determined by GC and GC/MS, and they were found to contain 19 and 27 constituents, respectively. C. citratus oil from Mali contained a high proportion of citral (approximately 75%) (geranial/neral ca 2/1), some myrcene (6.2-9.1%) and geraniol (3.0-5.6%). It differed from the oil of the Ivory Coast in which the contents of geranial, neral and myrcene each ranged between 18-35%. C. giganteus oil was characterized by high proportions of cis- and trans-p-mentha-1(7), 8- dien-2-ols (approx. 50%) and p-mentha-2,8-dien-1-ols (approx.25%) together with isopiperitenol-carveol (approx. 10%) and traces of carvone (<5%).

Tamimi, R. M., P. Lagiou, et al. (2002). “Prospects for chemoprevention of cancer.” Journal of Internal Medicine 251(4): 286-300. The recent progress in molecular biology and pharmacology has increased the likelihood that cancer prevention will rely increasingly on interventions collectively termed 'chemoprevention'. Cancer chemoprevention is the use of agents to inhibit, delay or reverse carcinogenesis. A number of potential targets for chemoprevention have recently been identified. Many classes of agents including antioestrogens, anti-inflammatories, antioxidants and other diet-derived agents have shown a great deal of promise. In this review, we will begin by describing the general classes of chemopreventive agents and the mechanisms by which these agents act. We will then describe the opportunities that presently exist for chemoprevention of specific cancers.

Tomassoni, A. J. and K. Simone (2001). “Herbal medicines for children: An illusion of safety?” Current Opinion in Pediatrics 13(2): 162-169. Herbal medicaments are in common use. In general, the judicious use of carefully selected and prepared herbal medications seems to cause few adverse effects and may be beneficial. However, toxic effects of these products have been reported with increasing frequency. Infants and children may be even more susceptible to some of the adverse effects and toxicity of these products because of differences in physiology, immature metabolic enzyme

systems, and dose per body weight. Although information promoting the use of herbal medicine is widespread, true evidence-based information about the efficacy and safety of herbal medications is limited. Although the most conservative approach is to recommend against use of herbal medicine until such evidence is available, some patients are not receptive to this approach. A reasonable approach for health care providers may be to follow such use closely, assist in herbal therapeutic decisions, and monitor for adverse effects and interactions. This manuscript discusses general concepts about herbal medicines, public health implications, and a framework for mechanisms of adverse effects from the use of botanicals. Adverse effects and toxicity of selected herbal products, including Chinese herbal medicines, are presented. The authors propose a risk reduction approach in which physicians actively seek information about the use of complementary or alternative medicine while taking medical histories. (c) 200 Lippincott Williams & Wilkins, Inc.

Turini, M. E. and R. N. DuBois (2002). “Primary prevention: Phytoprevention and chemoprevention of colorectal.” Hematology/Oncology Clinics of North America 16(4): 811-840. Considering the various stages of carcinogenesis and the numerous tumor types and available chemoprevention agents, knowledge of the etiology and the type of cancer to be treated, or possibly prevented, and understanding of the mechanisms by which agents exert their chemoprevention benefits may provide for improved strategy in designing therapeutic regimens. Because cancer usually develops over a 10- to 20-year period, it may be necessary for some agents to be provided before or early in the initiation steps of carcinogenesis to have beneficial effects. On the other hand, some agents may be more suitable for CRC prevention if provided at a later stage of carcinogenesis. Gene array, genomics, and proteomics are useful tools in advancing our understanding of the molecular events involved in carcinogenesis and in identifying markers of risk and surrogate end-points for colorectal cancer progression. These techniques may also serve for screening, identifying, and providing treatment targets for high-risk patient populations. Treatment could be developed depending on a patient's individual needs and genomic tumor profile. Clinical markers and surrogate end-points should be considered, together with molecular measurements, to more accurately assess risk. NSAIDs and COXIBs are clinically recognized as chemoprevention agents, and clinical trials evaluating their efficacy are ongoing. Treatment protocols, including dose and timing, remain to be determined, however. DFMO may best be used in combination with other chemoprevention agents. Dietary fiber and calcium supplements, as part of an overall low-fat diet, may decrease CRC risk. Long-term compliance with this regimen may be necessary to effect a beneficial outcome. Folate holds promise but needs further investigation, especially because its beneficial effects may depend on cancer type. Phytochemicals have been identified as strong candidates for use as agents to prevent colorectal cancer in cell culture and in rodent models of carcinogenesis. Their potential as chemoprevention agents must be demonstrated in clinical trials. In vitro and animal studies indicate that combination therapy may be a promising strategy over the monotherapy approach; clinical trials addressing the safety and efficacy of some combinations (DFMO/sulindac, fiber/calcium) are underway. The gastrointestinal tract and other organs are constantly exposed to a mixture of potentially toxic compounds and molecules considered favorable to health. Homeostasis between stress-mediated by toxic

compounds and defensive mechanisms, is key for the maintenance of health and the prevention of disease. Whereas aggressive pharmacologic treatment may be necessary for patients at high risk for cancer, dietary supplements may be useful for populations at normal risk. The message for cancer prevention in the general population may well remain: keep a balanced healthy diet, eating a variety from all food groups, as part of a healthy lifestyle that includes moderate exercise.

Vaillend, C., C. Rampon, et al. (2002). “Gene control of synaptic plasticity and memory formation: Implications.” Current Molecular Medicine 2(7): 613-628. There has been nearly a century of interest in the idea that information is stored in the brain as changes in the efficacy of synaptic connections between neurons that are activated during learning. The discovery and detailed report of the phenomenon generally known as long-term potentiation opened a new chapter in the study of synaptic plasticity in the vertebrate brain, and this form of synaptic plasticity has now become the dominant model in the search for the cellular and molecular bases of learning and memory. Accumulating evidence suggests that the rapid activation of the genetic machinery is a key mechanism underlying the enduring modification of neural networks required for the laying down of memory. Here we briefly review these mechanisms and illustrate with a few examples of animal models of neurological disorders how new knowledge about these mechanisms can provide valuable insights into identifying the mechanisms that go awry when memory is deficient, and how, in turn, characterisation of the dysfunctional mechanisms offers prospects to design and evaluate molecular and biobehavioural strategies for therapeutic prevention and rescue.

Van Lieshout, E. M. M., G. H. Posner, et al. (1998). “Effects of the sulforaphane analog compound 30, indole-3-carbinol.” Biochimica et Biophysica Acta - General Subjects 1379(3): 325-336. Several dietary compounds have been demonstrated to reduce gastrointestinal cancer rates in both humans and animals. We showed that high human gastrointestinal tissue levels of glutathione S-transferase (GST), a family of detoxification enzymes consisting of class Alpha, Mu, Pi and Theta isoforms, were inversely correlated with cancer risk. We now investigated whether the sulforaphane analog compound 30, indole-3-carbinol, D-limonene or relafen, supplemented in the diet for two weeks at 1450, 250, 10,000, and 200 ppm, respectively, influenced (i) GST activity, (ii) GST isoenzyme levels, (iii) GSH levels, or (iv) glutathione peroxidase (GPx) activity in the gastrointestinal tract of male Wistar rats. Sulforaphane analog compound 30 enhanced GST activity in all organs studied (1.2-2.4 X). It induced GST Alpha levels in small intestine and liver, GST Mu levels in stomach and small intestine, GST Pi levels in stomach and small and large intestine, and GSH levels in stomach and proximal and middle small intestine. Indole-3-carbinol induced gastric GST Mu and hepatic GST Alpha levels. D-limonene induced hepatic GST Alpha, colonic GST Pi levels and proximal small intestinal GST enzyme activity and GST Pi levels. Relafen induced hepatic GST Alpha levels, distal small intestinal and gastric GST Pi levels, and oesophageal and proximal small intestinal GSH levels. GPx activity was enhanced by relafen in oesophagus, and in distal small intestine by sulforaphane analog compound 30. Enhancement of GSTs and to a lesser extent GPx and GSH, resulting in a more efficient

detoxification. may explain at least in part the anticarcinogenic properties of sulforaphane analog compound 30, and to a much lesser extent of indole-3-carbinol and D-limonene.

Venkatesan, N. (1999). “Pulmonary protective effects of curcumin against paraquat toxicity.” Life Sciences 66(2): L-21-PL-28. An early feature of paraquat (PQ) toxicity is the influx of inflammatory cells, releasing proteolytic enzymes and oxygen free radicals, which can destroy the lung epithelium and result in pulmonary fibrosis. Therefore, the ability to suppress early lung injury seems to be an appropriate therapy of pulmonary damage before the development of irreversible fibrosis. Here I show curcumin confers remarkable protection against PQ lung injury. A single intraperitoneal injection of PQ (50 mg/kg) resulted in a significant rise in the levels of protein, angiotensin converting enzyme (ACE), alkaline phosphatase (AKP), N-acetyl-beta-D-glucosaminidase (NAG) and thiobarbituric acid reactive substances (TBARS), and neutrophils in the bronchoalveolar lavage fluid (BALF), while a decrease in glutathione levels. In paraquat rats bronchoalveolar lavage (BAL) cell TBARS concentration was increased with a simultaneous decrease in glutathione content. In addition, the data also demonstrated that PQ caused a decrease in ACE and glutathione levels and an increase in levels of TBARS and myeloperoxidase (MPO) activity in the lung. Interestingly, curcumin prevented the general toxicity and mortality induced by PQ and blocked the rise in BALF protein, ACE, AKP, NAG TBARS and neutrophils. Similarly, curcumin prevented the rise in TBARS content in both BAL cell and lung tissue and MPO activity of the lung. In addition, PQ induced reduction in lung ACE and BAL cell and lung glutathione levels was abolished by curcumin treatment. These findings indicate that curcumin has important therapeutic implications in facilitating the early suppression of PQ lung injury.

Viana, G. S. B., T. G. Do Vale, et al. (1998). “Analgesic and antiinflammatory effects of two chemotypes of Lippia.” Pharmaceutical Biology 36(5): 347-351. The antinociceptive and antiedematogenic effects of essential oils (EO, types I and II) from the leaves of two chemotypes of Lippia alba were studied with mice using the hot plate test, acetic acid-induced writhing, and the formalin test, and with rats using paw edema induced by carrageenan or dextran. The results showed dose-dependent inhibition of writhing with doses of 0.5 and 1 mg/kg, i.p., and 1 and 2 mg/kg, p.o., with chemotypes I and II, respectively. A similar but less intense effect was detected in the formalin test, where the two chemotypes (0.5 and 1 mg/kg, i.p.) predominantly inhibited the 2nd phase of the response, and only the effect of the EO I was reversed by the opioid antagonist, naloxone. Latency time to the thermic stimulus as detected by the hot plate test was increased with I but not with II, at doses higher than 10 mg/kg, p.o. A significant antiedematogenic effect was seen at 2 h with 10 and 50 mg/kg, p.o., of I, in paw edema induced by carrageenan or dextran. However, in the same dose range, II was more effective against dextran-induced edema, but no effect was seen with the carrageenan model. The essential oils of the two types of L. alba are chemically distinct, with I containing a high content of citral and II a high content of carvone with no citral, which could explain the observed differences in their pharmacological actions.

Viollon, C. and J. P. Chaumont (1994). “Antifungal properties of essential oils and their main components upon Cryptococcus neoformans.” Mycopathologia 128(3): 151-3.

Cryptococcus neoformans opportunistic fungus met in the last phasis of AIDS is inhibited in vitro by several essential oils on natural volatile compounds. The minimal inhibitory concentration may reach 100 microliters/l and minimal fungicidal concentration 200 microliters/l with Palmarosa or Cinnamon oils. Among phenolic compounds, thymol and carvacrol are most fungitoxic. Terpenoids, citral, geraniol, and citronellol show best activities.

Wargovich, M. J., C. D. Chen, et al. (1996). "Aberrant crypts as a biomarker for colon cancer: Evaluation of." Cancer Epidemiology Biomarkers and Prevention 5(5): 355-360. We assessed the effects of 41 potential chemopreventive agents in the F344 rat using the inhibition of carcinogen-induced aberrant crypt foci (ACF) in the colon as the measure of efficacy. ACF were induced by the carcinogen azoxymethane in F344 rats by two sequential weekly injections at a dose of 15 mg/kg. Two weeks after the last azoxymethane injection, animals were evaluated for the number of aberrant crypts detected in methylene blue- stained whole mounts of rat colon. The 41 agents were derived from a priority listing that was based on reports of chemopreventive activity in the literature and/or efficacy data from in vitro models of carcinogenesis. The list of agents included representative examples of phytochemicals, vitamins, minerals, inhibitors of proliferation, inducers of Phase 1 and Phase 2 metabolism systems, nonsteroidal anti-inflammatory agents, and differentiation agents. Eighteen agents were positive in the assay, significantly reducing the incidence of ACF at least in one of two doses tested. As a chemical class, the nonsteroidal anti-inflammatory drugs, which included ibuprofen, ketoprofen, piroxicam, and indomethacin, were most active; other less potent agents were arginine, butylated hydroxyanisole, curcumin, diallyl sulfide, difluoromethylornithine, 18beta-glycyrrhetinic acid, indole-3-carbinol, oltipraz, purpurin, rutin, and the sodium salts of butyrate, selenite, and thiosulfate. Twenty-three agents did not inhibit ACF; included among these were several agents that promoted the development of ACF at one or both doses tested: benzyl isothiocyanate, calcium glucarate, catechin, dihydroepiandosterone, fluocinolone acetonide, folio acid, levamisole, 2-mercaptoethanesulfonic acid, nordihydroguiaretic acid, potassium glucarate, propyl gallate, beta-sitosterol, sodium cromolyn, sodium molybdate, and sulfasalazine. The aberrant crypt assay demonstrates reasonable specificity and sensitivity in predicting which agents are likely to prevent colon cancer.

Wargovich, M. J., C. Harris, et al. (1992). "Growth kinetics and chemoprevention of aberrant crypts in the rat colon." Journal of Cellular Biochemistry 50(SUPPL. G): 51-54. Single and multiple colonic crypts exhibiting dysplasia that are detectable in situ by staining of rat colon with methylene blue are called aberrant crypts (AC) and may serve as an intermediate marker for colon cancer. In a characterization study, we have established the kinetics of AC growth and development over a period of 20 d following injection of rats with the carcinogen azoxymethane (AOM). AC are not present at 5 d post-injection, but are a constant feature at 10 d and thereafter. Multiple AC, presumably clonal, begin to evolve at 10 d and are consistent by 20 d, forming incipient microadenomata. We have examined 20 candidate chemopreventive agents for inhibition of AC. All agents were given in AIN-76 diet, at two dose levels, with injections of AOM. AC were measured after 5 weeks of growth. Among the most active AC-inhibiting agents

were BHA, DFMO, quercetin, diallyl sulfide, 18beta-glycyrrhetinic acid, and ascohbyl palmitate. In a postinitiation study, the differentiating agent sodium butyrate was ineffective, but piroxicam was highly effective in modulating AC growth. Further, piroxicam inhibited AC development at all stages of growth from single to polycryptal clusters of AC. The AC assay shows marked sensitivity and specificity for screening agents for chemoprevention of colon cancer.

Zivanovic, L. J., M. Jovanovic, et al. (1990). “Densitometric determination of monoterpenoids in Melissa extracts.” Fitoterapia 61(1): 82-83. Melissa fluid extract is used as a diaphoretic and a stimulant in pharmaceutical preparations, as a skin antiirritant in emulsions and lotions, and because of its antiviral effect in some pharmaceutical ointments. A densitometric method for the determination of geraniol, linalool, citral and citronellal in Melissa officinalis leaf extracts is presented.

Geraniol acetate CAS# 105-87-3 (Geranyl acetate, Geranyl ethanoate, 2,6-octadien dimethyl acetate, Dimethyl 2,6-octadien ethanoate)

Binder, G., T. v. d. Berg, et al. (1996). "Regeneration of plants and production of volatiles from callus cultures of Melissa officinalis L. 3. Effect of exogenous growth regulators on essential oil composition." Angewandte Botanik 70(5/6): 181-184. Regenerates of lemon balm (M. officinalis) were established as an in vitro-system to study the effects of exogenous growth regulators (NAA, BAP [benzyladenine] and ABA) on morphology and essential oil composition. Long term cultivation resulted only in changes of minor essential oil constituents; citronellal, citronellol, nerol and geraniol contents were elevated, whereas geranyl acetate content was reduced. The essential oil composition of plants growing in the presence of NAA exhibited similar changes in citronellal, citronellol and geranyl acetate, i.e. like plants at an advanced stage of development. Supplementation of the medium with a high BAP concentration induced plants to accumulate >10 % alloaromadendrene, a sesquiterpene hydrocarbon found only as a trace compound in control plants and not described before for the leaf essential oil of naturally grown M. officinalis. ABA had a slight effect on the production of some minor compounds, and showed a synergistic effect in combination with BAP.

Chagonda, L. S., C. Makanda, et al. (2000). "The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe." Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Chinou, I. B., V. Roussis, et al. (1996). "Chemical and biological studies on two Helichrysum species of Greek origin." Planta Medica 62(4): 377-379. The essential oils obtained from the aerial parts of H. amorginum and H. italicum (collected from Amorgos, Greece) were analysed by GC and GC-MS. From the 25 identified constituents representing 89.98 and 82.06% of the 2 oils respectively, geraniol, geranyl acetate, neryl acetate and nerolidol were the major components. The essential oils and geraniol, geranyl acetate and neryl acetate exhibited antibacterial activity against Staphylococcus aureus, S.

epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae and Escherichia coli.

Chowdhury, A. R. and V. P. Kapoor (2000). “Essential oil from the fruit of Apium graveolens.” Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Inouye, S., K. Uchida, et al. (2001). “Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact.” Journal of Essential Oil Research 13(1): 68-72. The flowers of Perovskia abrotanoides and Nepeta juncea and the leaves and flowers of Thymus linearis were collected at full bloom from different areas of Karakoram district, Pakistan, then dried and examined for essential oil composition. Dried plant parts were placed in air-tight boxes with 3 species of fungi (Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes or Trichophyton rubrum) for one, 3 and 5 days, respectively. P. abrotanoides extracts contained high concentrations of 1,8-cineole (24-27%) and alpha-pinene (18-23%) and low concentrations of borneol and bornyl acetate. N. juncea essential oils contained nepetalactone (5.8 plus or minus 0.54 mg per g of dried flowers), and several minor constituents such as nerol, 1,8-cineole, neryl acetate and an unidentified component. Two Thymus linearis chemotypes were collected; that from Hunza and Rupal valley had thymol and carvacrol as major components, and the other chemotype from the Rakaposhi area had geranyl acetate and geraniol as major components. N. juncea and Thymus linearis essential oils inhibited fungal growth by vapour contact, while P. abrotanoides showed no activity. The 2 Trichophyton species were the most susceptible and C. albicans was the least susceptible to the toxic effects of plant aromatic compounds, while A. fumigatus showed intermediate susceptibility.

Kulevanova, S., M. Ristic, et al. (1996). “Composition of the essential oil from Thymus moesiacus from Macedonia.” Planta Medica 62(1): 78-79. T. moesiacus is used in traditional medicine to treat coughs, flu, pulmonary infections and abdominal disorders. The essential oil, hydrodistilled from plant material of T. moesiacus (collected from Macedonia), was examined by GC and GC-MS. The main constituents were geraniol (14.87-33.27%), linalool (8.14-25.02%), geranyl acetate (4.11-16.75%) and carvacrol (12.31-13.31%).

Marcos Becerro, J. F. and F. J. Rodriguez Gorostiza (1968). “[The effect of farnesyl geranyl acetate on duodenal ulcer].” Rev Esp Enferm Apar Dig 27(4): 633-50.

Padula, L. Z., A. M. Collura, et al. (1977). “Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil.” Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Sangalli, B. C. and W. Chiang (2000). “Toxicology of nutmeg abuse.” Journal of Toxicology - Clinical Toxicology 38(6): 671-678. Background: Unpleasant and frightening side effects associated with the abuse of nutmeg occasionally generate emergency department referrals. We report a young patient's first-time experience with nutmeg and review the mechanisms of its toxicity. Case Report: A 13-year-old female ingested 15-24 g of nutmeg over a 3-hour period and smoked and shared 2 joints of marijuana. To facilitate ingestion, the nutmeg was put into 00-000 gelatin capsules. Bizarre behavior and visual, auditory, and tactile hallucinations developed. She also experienced nausea, gagging, hot/cold sensations, and blurred vision followed by numbness, double, and 'triple' vision, headache, and drowsiness. Nystagmus, muscle weakness, and ataxia were present. Her vital signs and laboratory tests were normal. She received 50 g of activated charcoal and except for complaints of dizziness and visual changes, her 2-day admission was uneventful. The central nervous system activity of nutmeg is often postulated to result from biotransformation of its chemical components to amphetamine-like compounds, but this has not been proven. Nutmeg contains several compounds with structural similarities to substances with known central nervous system neuromodulatory activity.

Balazs, T. (2000). "Research reports." International Journal of Aromatherapy 10(1-2): 68-71.

Barnes, J. (1998). "Complementary medicine: Aromatherapy." Pharmaceutical Journal 260(6998): 862-867.

Buchbauer, G., L. Jirovetz, et al. (1993). "Fragrance compounds and essential oils with sedative effects upon inhalation." Journal of Pharmaceutical Sciences 82(6): 660-664. In experiments with female 6- to 8-week-old Swiss mice [see also Planta Medica (1987) 53, 315-318], a total of 44 fragrance compounds and essential oils, obtained from Dragoco Company (Vienna, Austria) and known to possess sedative properties, were screened for their potential aromatherapeutic value when administered by inhalation. The motility of untreated mice was compared with that of mice exposed to a specific compound after no pretreatment or after a caffeine-induced overagitation treatment. Compared with the motility of untreated mice (100%) that of mice exposed to lavender [Lavandula sp.] oil, neroli [Citrus aurantium] oil, linalool, linalyl acetate, citronellal, benzaldehyde, 2-phenylethyl acetate, alpha-terpineol and sandalwood [Santalum album] oil was decreased by 78.4, 65.3, 73.0, 69.1, 49.8, 43.7, 45.0, 45.0 and 40.0%, respectively. In contrast, an increased motility was observed after exposure to geraniol, isoborneol, isoeugenol, orange [Citrus sp.] terpenes and thymol. The sedative effect of lavender oil, isoeugenol, linalool, maltol, carvone and linalyl acetate counteracted caffeine-induced overagitation; overagitation was amplified by anthranilic acid methyl ester, farnesol, lime (Tilia sp.) blossom oil and nerol inhalation. Serum samples, taken shortly after the inhalation treatment, were analysed by GC-MS, GC-fourier transform infrared and GC-flame inonization techniques in order to identify active constituents. A total of 21 substances were identified at concentrations of up to 0.1 ng/ml serum. Correlations of the aroma detection thresholds and sedative properties associated with these substances indicated that there might be a direct pharmacological interaction of fragrance molecules with body tissues rather than a reflective interaction caused by a pleasant feeling.

Ceschel, G. C., P. Maffei, et al. (2000). "In vitro permeation through porcine buccal mucosa of Salvia desoleana." International Journal of Pharmaceutics 195(1-2): 171-177. In the light of recent studies, which have shown that the essential oil derived from some Lamiaceae species has appreciable anti-inflammatory activity, moderate anti-microbial action and the ability to inhibit induced hyperalgesia, an assessment of the diffusion and permeation of Salvia desoleana Atzei and Picci (S. desoleana) essential oil through porcine buccal mucosa was considered useful for a possible application in the stomatological field. Topical formulations (microemulsions, hydrogels and microemulsion- hydrogels) were prepared for application to the buccal mucosa. The mucosa permeation of the oil from the formulations was evaluated using Franz cells, with porcine buccal mucosa as septum between the formulations (donor compartment) and the receptor phase chambers. The study also aimed at optimising the permeability of the S. desoleana essential oil by means of an enhancer, the diethylene glycol monoethyl ether

Transcutol(R). The diffusion of the oil through the membrane was determined by evaluating the amount of essential oil components present in the receiving solution, the flux and the permeation coefficient (at the steady state) in the different formulations at set intervals. Qualitative and quantitative determinations were done by gas chromatographic analysis. All the formulations allow a high permeability coefficient in comparison with the pure essential oil. In particular, the components with a terpenic structure (beta-pinene, cineole, alpha-terpineol and linalool) have the highest capacity to pass through the porcine buccal mucosa when compared to the other components (linalyl acetate and alpha-terpinil acetate). Moreover, the enhancer, diethylene glycol monoethyl ether largely increases the permeation of the essential oil components in relation to the concentration. (C) 2000 Elsevier Science B.V.

Chadha, A. and K. M. Madyastha (1984). "Metabolism of geraniol and linalool in the rat and effects on liver and lung microsomal enzymes." Xenobiotica 14(5): 365-74. Metabolites isolated from the urine of rats after oral administration of geraniol (I) were: geranic acid (II), 3-hydroxy-citronellic acid (III), 8-hydroxy-geraniol (IV), 8-carboxy-geraniol (V) and Hildebrandt acid (VI). Metabolites isolated from urine of rats after oral administration of linalool (VII) were 8-hydroxy-linalool (VIII) and 8-carboxy-linalool (IX). After three days of feeding rats with either geraniol or linalool, liver-microsomal cytochrome P-450 was increased. Both NADH- and NADPH-cytochrome c reductase activities were not significantly changed during the six days of treatment. Oral administration of these two terpenoids did not affect any of the lung-microsomal parameters measured.

Chagonda, L. S., C. Makanda, et al. (2000). "The essential oils of wild and cultivated Cymbopogon validus (Stapf) Stapf ex Burtt Davy and Elionurus muticus (Spreng.) Kunth from Zimbabwe." Flavour and Fragrance Journal 15(2): 100-104. The steam-distilled oils from wild and cultivated Cymbopogon validus and Elionurus muticus, both of which are used medicinally, were analysed by GC and GC-MS. The major components from C. validus in the wild (collected from Nyanga) were: myrcene (23.1-35.6%), (E)-beta-ocimene (10.3-11.5%), geraniol (3.4-8.3%), linalol (3.2-3.7%) and camphene (5.2-6.0%). Cultivated mature plants contained myrcene (11.6-20.2%), (E)-beta-ocimene (6.0-12.2%), borneol (3.9-9.5%) and geraniol (1.7-5.0%) and camphene (3.3-8.3%) as the major components. Young nursery crop/seedlings (20-30 cm high) contained oil with myrcene (20.6%), geraniol (17.1%) and germacrene-D-4-ol (8.3%) as the major components. Geranyl acetate (4.5%), linalol (4.5%) and borneol (2.9%) were notable minor components. The major components from wild (collected near Harare) and cultivated E. muticus were geranial (40.1-44.8%), neral (26.0-35.4%) and geranyl acetate (1.8-8.6%). Dried lower parts from cultivated E. muticus contained oil rich in geranial (29.6%), neral (20.2%) and geranyl acetate (18.8%), whilst the upper aerial parts contained geranial (41.9%), neral (26.4%) and geranyl acetate (4.7%) as the main components.

Chang, C. W., J. P. Wang, et al. (1999). "Terpenoids from Ocimum basilicum." Chinese Pharmaceutical Journal 51(2): 181-189. Eight known terpenoids were isolated from the roots and stems of Ocimum basilicum (Labiatae). These compounds included a

sesquiterpene, T-cadinol (1), a monoterpene, linalool (2), and six triterpenoids, betulinic acid (3), betulin (4), pomolic acid (5), alphitolic acid (6), ursolic acid (7) and tormentic acid (8). Their structures were determined on the basis of spectral and physical data. Except for linalool, the other seven terpenoids were isolated for the first time from this plant. The anti-inflammatory activities of the triterpenoids were investigated.

Chowdhury, A. R. and V. P. Kapoor (2000). "Essential oil from the fruit of Apium graveolens." Journal of Medicinal and Aromatic Plant Sciences 22(1B): 621-623. Apium graveolens, although exotic, has been naturalized in India. The fruits of A. graveolens on hydrodistillation gave 2.2% dry weight basis golden yellow essential oil. On GC-MS examination, the oil was found to contain limonene, beta-phellandrene, alpha-pinene, beta- pinene, beta-elemen, alpha-humulene, patchoulene, beta-selinene, pentyl benzene, benzyl alcohol, carveol, eudesmol, geraniol, limonene glycol, linalool, menthol, terpineol, thujol, caryophyllene oxide, citral, methyl heptanal, carvone, dihydrocarvone, menthone, phenyl ethyl ketone, butyl phthalide, geranyl acetate and exobornyl acetate. The composition suggests that the oil may be used for perfuming soaps, detergents and as flavouring material in foods.

Cometto-Muniz, J. E., W. S. Cain, et al. (1998). "Trigeminal and olfactory chemosensory impact of selected terpenes." Pharmacol Biochem Behav 60(3): 765-70. In Experiment 1, four normosmics and four anosmics (three congenital, one idiopathic) provided odor and nasal pungency thresholds, respectively, for the following terpenes: delta3-carene, p-cymene, linalool, 1.8-cineole, and geraniol, plus the structurally related compound cumene. Additionally, all subjects provided nasal localization (i.e., right/left) and eye irritation thresholis. Trigeminally mediated thresholds (i.e., nasal pungency, nasal localization, and eye irritation) lay about three orders of magnitude above odor thresholds, which ranged between 0.1 and 1.7 ppm. The results implied uniform chemesthetic sensitivity across tasks and sites of impact. In Experiment 2, normosmics and anosmics provided odor and nasal pungency thresholds, respectively, for three pairs of isomeric terpenes: alpha- and gamma-terpinene, alpha- and beta-pinene, and R(+)- and S(-)-limonene. Odor thresholds ranged between 1.4 and 19 ppm, that is, about an order of magnitude higher than those of the previous terpenes, with no substantial differences between odor thresholds of members of a pair. Regarding chemesthetic impact, only alpha-terpinene evoked nasal pungency. The overall outcome suggests comparable trigeminal chemosensitivity between nose and eyes and between normosmics and anosmics, as shown before for homologous n-alcohols. It also lends support to a previously derived solvation model of the chemesthetic potency of airborne substances, and indicates the likely importance of certain molecular-size restrictions for effective trigeminal impact.

Gurdip Singh, I. P. S. Kapoor, et al. (2000). "Studies on essential oils, part 28: chemical composition, antifungal and insecticidal activities of rhizome volatile oil of Homalomena aromatica Schott." Flavour and Fragrance Journal 15(4): 278-280. HPLC and GC-MS analysis of rhizome oil of Homalomena aromatica showed the presence of 39 components accounting for 96.9% of the total oil. The major component was linalool (62.1%), followed by terpinen-4-ol (17.2%), alpha-terpineol (2.4%), gamma-terpinene (1.9%),

alpha- cadinol (1.5%), geraniol (1.4%), nerol (1.4%), alpha-terpinene (1.0%), spatulenol (1.0%) and T-cadinol (1.0%). However, the higher percentage of linalool (87.5%) was obtained in HPLC studies. This oil showed good antifungal activity against Curvularia pallescens [Cochliobolus pallescens], Aspergillus niger and Fusarium graminearum [Gibberella zeae] as well as also showing insecticidal behaviour against white termite (Odontotermes obesus).

Hossain, S. J., K. Hamamoto, et al. (2002). "Effects of tea components on the response of GABA(A) receptors expressed in Xenopus Oocytes." J Agric Food Chem 50(14): 3954-60. To study the effects of tea components on ionotropic gamma-aminobutyric acid (GABA) receptor response, ionotropic GABA receptors (GABA(A) receptors) were expressed in Xenopus oocytes by injecting cRNAs synthesized from cloned cDNAs of the alpha(1) and beta(1) subunits of the bovine receptors, and their electrical responses were measured by a voltage clamping method. Extracts of green tea, black tea, and oolong tea in an aqueous solution induced the GABA-elicited response, which showed that these teas contain GABA, whereas coffee does not. Caffeine weakly inhibited the response in a competitive manner (K(i) = 15 mM), and (+)-catechin inhibited it in a noncompetitive one (K(i) = 1.7 mM). Especially, two catechin derivatives, (-)-epicatechin gallate and (-)-epigallocatechin gallate, inhibited the response strongly. Alcohols such as leaf alcohol or linalool potentiated the response, possibly because their binding to the potentiation site enhances the GABA-binding affinity to GABA(A) receptors when they bind. Extracts of green tea made with ethyl ether, which must contain lipophilic components of green tea, inhibited the response elicited by GABA, possibly because the amounts of caffeine and catechin derivatives were much larger than fragrant alcohols in such extracts of tea.

Houmani, Z., S. Azzoudj, et al. (2002). "The essential oil composition of Algerian zaatar: Origanum spp. and." Journal of Herbs, Spices and Medicinal Plants 9(4): 275-280. Origanum spp. and Thymus spp. growing spontaneously in Algeria are collected from wild populations and are sold in the local markets under the same or similar vernacular name, zaatar (Origanum) or zhitra (Thymus). Thymus willdenowii Boiss. and Thymus algeriensis Boiss. & Reuter are mostly used as condiments while Origanum floribundun Munby and Origanum vulgare L. ssp. gladulosum (Desf.) Ietswaart are used against diarrhoea and other digestive and respiratory system disorders, as well as additive to forrage as an appetite stimulant. All four species were quite rich in essential oils; the analyses of the oils showed that all were rich in the compounds of the carvacrol pathway (p-cymene, gamma-terpinene, carvacrol, thymol, and their methyl-ethers). Only minor qualitative, but considerable quantitative, variation was found within and between the species that comprise zaatar: the major compounds of O. floribundum were p-cymene (31%), thymol (9.9%) and carvacrol (35.0%); the major compounds of O. vulgate ssp. gladulosum were gamma-terpinene (13.6%), thymol-methylether (16.3%), carvacrol-methylether (11.4%) and thymol (26.1%); the major compounds of T. willdenowii were p-cymene (15.2%), thymol (15.1%) and carvacrol (51.3%); finally, from the two samples of T. algeriensis analyzed, one was rich in linalool (78.8%) and the other was rich in thymol (62.7%).

Isaac, O. (1994). "Calendula officinalis L. - Marigold." Zeitschrift fur Phytotherapie 15(6): 356-370. Marigold (Calendula officinalis L.) in the process of rediscovering natural healing forces has gained importance. Increasing significance is contributed to calendula ointments, which have been used traditionally for a long time. The range of the calendula constituents is characterized by a high level of terpenoids e.g. saponosides in the form of oleanolglycosides and triterpene alcohols. The triterpenediol-3-monoesters consist for 85% of faradiolesters. The colour of the flowers depends on their content of carotinoids. Orange flowers contain carotins, especially lycopin, while the yellow varieties predominately contain xanthophylls. Characteristic calendula-flavonoids are the isorhamnetin glycosides. Contrary to other asteraceae marigold contains no sesquiterpene lactons. Also remarkable is the fat oil of the seeds which predominately consists of the conjugated trienoic acid calendula acid. Calendula preparations are mainly used for the treatments of wounds and for cosmetical purposes. The antiinflammatory principle can be isolated by lipophilic extraction, e.g. by extraction with hypercritical carbondioxide and used as ingredient of creams and ointments.

Kahlos, K., J. L. Kiviranta, et al. (1994). "Volatile constituents of wild and in vitro cultivated Gloeophyllum odoratum." Phytochemistry 36(4): 917-22. The brown-rot fungus Gloeophyllum odoratum was collected from spruce stumps in southern Finland. The volatiles in the fruiting body and fungal cultures grown in malt extract and liquid medium were investigated. Chitin, chitosan and D-(+)-glucosamine at a concentration of 450 mgl-1 medium were used as elicitors. Chitosan completely inhibited growth in the solid medium. The main volatile(s) according to GC and GC-MS analysis were either linalool, citronellol, geraniol and methyl p-methoxyphenylacetate or drimenol depending on the culture type and elicitor. The composition of volatiles in the natural fungus differed slightly from that of the cultivated fungus since the major compound was methyl p-methoxyphenylacetate. The volatile oils were toxic to larvae of the brine shrimp, Artemia salina, indicating that they may possess insecticidal and cytotoxic activity.

Kohlert, C., I. Van Rensen, et al. (2000). "Bioavailability and pharmacokinetics of natural volatile terpenes in." Planta Medica 66(6): 495-505. Herbal medicinal products containing natural volatiles are used in the treatment of gastrointestinal diseases, pain, colds and bronchitis. Many pharmacological studies report a wide variety of in vitro effects, with anti-inflammatory and antimicrobial activities investigated most frequently. In comparison, relatively few studies on the bioavailability and pharmacokinetics have been carried out. Thus, the relevance of the in vitro activity to the therapeutic effects found in individual studies or documented in textbooks of phytotherapy is still not established. Further studies with essential oils and their single compounds providing supporting evidence of efficacy and demonstrating systemic availability are necessary. Such data could also be important in the context of safety.

Kulevanova, S., A. Kaftandzieva, et al. (2000). "Investigation of antimicrobial activity of essential oils of several Macedonian Thymus L. species (Lamiaceae)." Boll Chim Farm 139(6): 276-80. Antimicrobial activity of twenty specimens of essential oils of eleven Thymus species, naturally occurring in the Macedonian flora, was investigated by agar diffusion and broth dilution methods. Inhibition of growth and microbicidal action was

examined on three Gram positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumoniae). In spite of wide variability in essential oil composition, ranging from traces of thymol to the amount of about 50% thymol in oils, all examined samples of Thymus essential oil possessed strong antibacterial activity. Zones of inhibition of growth (for 25% dilution of oils) was from 10-54 mm in diameters. MICs ranging from 0.012-0.1% while MMCs were from 0.025-0.4% for essential oils that contained large amounts of phenols and 0.2-1.6% for those which contained traces of phenols and large amounts of geraniol, linalool and (Z + E)-citral.

Kulevanova, S., M. Ristic, et al. (1996). "Composition of the essential oil from Thymus moesiacus from Macedonia." Planta Medica 62(1): 78-79. T. moesiacus is used in traditional medicine to treat coughs, flu, pulmonary infections and abdominal disorders. The essential oil, hydrodistilled from plant material of T. moesiacus (collected from Macedonia), was examined by GC and GC-MS. The main constituents were geraniol (14.87-33.27%), linalool (8.14-25.02%), geranyl acetate (4.11-16.75%) and carvacrol (12.31-13.31%).

Kulevanova, S., M. Ristic, et al. (1997). "Composition of essential oils of Thymus tosevii ssp. tosevii and Thymus tosevii ssp. substriatus from Macedonia." Pharmazie 52(5): 382-386. T. tosevii subsp. tosevii and T. tosevii subsp. substriatus are used in traditional medicine in Macedonia against cold, flu, pulmonary infection and abdominal throes. The essential oils of T. tosevii subsp. tosevii and T. tosevii subsp. substriatus, growing wild in Macedonia, were investigated by means of GC and GC-MS. The main components of the oils were thymol, carvacrol, linalool, geraniol, terpenyl acetate, p-cymene and gamma-terpinene. The essential oil composition varied according to the origin and the year of plant collection.

Limberger, R. P., M. E. G. Sobral, et al. (2001). "Biological activities and essential oil composition of leaves of." Pharmaceutical Biology 39(4): 308-311. Infusions obtained from dried and fresh leaves of Blepharocalyx salicifolius were assessed in antibacterial (with S. aureus and E. coli), antiinflammatory, antinociceptive, antispasmodic and intestinal transit models. All samples analyzed showed significant antibacterial activity against Gram-positive and Gram-negative bacteria. The highest activity was observed with the dried leaves against E. coli. An infusion from fresh leaves inhibited the stimulating action of acetylcholine on intestinal musculature (average inhibition 45%). Other biological assays gave no significant results with doses up to 300 and 600 mg/kg for dried and fresh material, respectively. The essential oil obtained from fresh leaves by hydrodistillation (0.9%) was analyzed by GC and GC/MS, where 42 components were identified. The main components were 1,8-cineole (25.2%), linalool (20.4%) and beta-caryophyllene (22.9%).

Lis-Balchin, M. T. and S. L. Hart (1994). "A pharmacological appraisal of the folk medicinal usage of Pelargonium grossularioides and Erodium cicutarium." Journal of Herbs, Spices & Medicinal Plants 2(3): 41-48. In Africa, P. grossularioides and E. cicutarium are used in traditional medicine for their abortifacient properties, and to treat fevers, dysentery, wounds and worm infestations. The pharmacological effects of extracts

(hexane, methanol and water) of leaves of P. grossularioides (obtained from South Africa) and E. cicutarium (obtained from Cambridge University, UK), were studied in vitro using guinea pig ileum, rat uterus, rat phrenic nerve preparations, and rabbit hearts. Extracts from both plants increased the tone, and reduced the strength or inhibited contraction, of guinea pig ileum. Extracts stimulated contractions of the rat uterus, increased the tension of the isolated diaphragm muscle in phrenic nerve preparations, and produced a negative ionotropic action in the rabbit heart. Hexane extracts were the most active, followed by methanol extracts. The compositions of the essential oils from both species were compared. Both species contained methyl eugenol, geraniol, citronellol, isomenthone and linalool. Sesquiterpenes, which accounted for 10% of the E. cicutarium essential oil, were absent from P. grossularioides.

Lopes, D., M. Koketsu, et al. (1999). "Chemical composition of Pourouma guianensis Aublet essential oils." Flavour and Fragrance Journal 14(4): 233-236. The essential oils from leaves, stem bark and pistillate flowers of P. guianensis (collected in Rio de Janeiro, Brazil; species used medicinally and for edible fruits) were isolated by hydrodistillation and the volatile constituents were determined by HRGC and HRGC-MS. Methyl salicylate was the major compound identified in all oils studied and was present in yields of 20.8% (leaves), 31.2% (stem bark) and 62.2% (pistillate flowers). Altogether, 50 constituents were identified in the essential oil obtained from leaves, representing 76.6% of the total oil. Aliphatic C6 alcohols and esters were, in number and in quantity, the principal constituents (29.5%). Oxygenated monoterpenes were found to be an important group of compounds and the most representative compound was linalol [linalool] (2.4%). Thirty-eight components were identified in the essential oil from stem bark, representing 79.3% of the total oil. Among the monoterpenes identified, linalol was the principal compound (0.8%). The total content of fatty acids amounted to 40.0%. Analysis of the essential oil from pistillate flowers allowed the identification of 36 compounds, representing 88.5% of the oil. Ten oxygenated monoterpenes were identified, whereas linalol and its furan derivatives (9.7%), nerol (0.4%) and geraniol (1.3%) were the most abundant. Five aromatic derivatives were identified in the pistillate flower essential oil: methyl salicylate (62.2%), ethyl salicylate (0.1%), benzyl salicylate (0.2%), benzyl benzoate (0.3%) and benzaldehyde (0.1%).

Mashanov, V. I. and I. E. Logvinenko (1979). "Artemisia balchanorum under cultivation." Doklady Vsesoyuznoi Ordena Lenina Akademii Sel'skokhozyaistvennykh Nauk Imeni V.I. Lenina(1): 23-24. In 1972, the high-yielding varieties Krymchanka [Crimean], Balkhanka, Yuzhanka [Southerner], Evrika [Eurika] and Slavyanka were selected from a population in the Ukraine for high content of citral, linalool and geraniol. During 1975-77, they were studied in various zones of the southern Ukraine. The best for yield of essential oil in all zones were Evrika and Balkhanka.

Moretti, M. D. L., A. T. Peana, et al. (1997). "A study on anti-inflammatory and peripheral analgesic action of Salvia sclarea oil and its main components." Journal of Essential Oil Research 9(2): 199-204. Activity against the acute inflammatory process induced by carrageenan and histamine in rats and the antinociceptive effect in mice were investigated after administration of S. sclarea essential oil (obtained from leaves grown in

Sardinia, Italy) and some of its constituents. The essential oil (250 mg/kg, s.c.) showed significant antiinflammatory and moderate analgesic properties. The antiinflammatory action was more conspicuous against carrageenan- induced oedema (equivalent to a 5 mg/kg dose of indometacin) than against histamine-induced oedema. The effect was correlated to the presence of methyl chavicol, linalool, alpha-terpineol and linalyl acetate. These constituents were less active when administered separately than when combined in the essential oil. They were also less effective than the oxygenated fractions obtained by Flash chromatography. The moderate peripheral analgesia (evaluated by the formic acid-induced writhing test) produced by S. sclarea essential oil appeared to be mainly attributable to its alcoholic component.

Moretti, M. D. L., A. T. Peana, et al. (1997). "A study on anti-inflammatory and peripheral analgesic action of Salvia sclarea oil and its main components." Journal of Essential Oil Research 9(2): 199-204. Activity against the acute inflammatory process induced by carrageenan and histamine in rats and the antinociceptive effect in mice were investigated after administration of S. sclarea essential oil (obtained from leaves grown in Sardinia, Italy) and some of its constituents. The essential oil (250 mg/kg, s.c.) showed significant antiinflammatory and moderate analgesic properties. The antiinflammatory action was more conspicuous against carrageenan- induced oedema (equivalent to a 5 mg/kg dose of indometacin) than against histamine-induced oedema. The effect was correlated to the presence of methyl chavicol, linalool, alpha-terpineol and linalyl acetate. These constituents were less active when administered separately than when combined in the essential oil. They were also less effective than the oxygenated fractions obtained by Flash chromatography. The moderate peripheral analgesia (evaluated by the formic acid-induced writhing test) produced by S. sclarea essential oil appeared to be mainly attributable to its alcoholic component.

Mumcuoglu, K. Y., R. Galun, et al. (1996). "Repellency of essential oils and their components to the human body louse, Pediculus humanus humanus." Entomologia Experimentalis et Applicata 78(3): 309-314. Five essential oils and 9 of their components were compared with diethyltoluamide (DEET) for their repellent activity against P. humanus humanus [P. humanus]. The absolute or intrinsic repellency of the compounds was tested by applying the repellent to corduroy patches and comparing them with untreated patches. It was found that the most effective repellents were DEET and citronella, whose activity lasted at least 29 days. The activity of rosemary lasted at least 18 days and that of eucalyptus more than 8 days. The repellent activity of the oil components such as citronellal and geraniol lasted more than 15 and 8 days, respectively. DEET remained effective at a dilution of 1:32, geraniol at 1:8, citronella at 1:4 and rosemary and citronellal at 1:1. The comparative or standard repellency of the candidate repellents was examined with the aid of a new screening technique using hairs treated with ammonium bicarbonate which is attractive to lice. Using this technique it could be shown that the repellent activity of citronella and geraniol lasted 2 days and that of rosemary and citronellal for only 1 day. DEET was active for <1 day. Serial dilutions of these substances also revealed that citronella was the most potent repellent for lice, followed by citronellal, rosemary, geraniol and DEET. The differences however, were not significant.

Nin, S., P. Arfaioli, et al. (1995). "Quantitative determination of some essential oil components of selected Artemisia absinthium plants." Journal of Essential Oil Research 7(3): 271-277. In traditional medicine, A. absinthium is used as an anthelmintic, insecticide, stomachic, and tonic. The essential oils, steam- distilled from leaves and flowers of plants propagated from 49 mother plants obtained from Italy (21 plants), Austria (10), Germany (5), France (4) or USA (9), were analysed by GC. More than 90 compounds were detected, most of which occurred only in trace amounts. Quantitative and qualitative differences were observed in the contents of 8 antibacterial components (alpha- and beta-thujone, terpinen-4-ol, linalool, nerol, geraniol, alpha-pinene, and 1,8-cineole [eucalyptol]). These variations were observed between individual accessions, and between plants obtained from the same geographical location. The essential oils of some genotypes were characterized by particularly high percentages of active principles.

Nishikitani, M., K. Kubota, et al. (1996). "Geranyl 6-O-alpha-L-arabinopyranosyl-beta-D-glucopyranoside isolated as an aroma precursor from leaves of a green tea cultivar." Biosci Biotechnol Biochem 60(5): 929-31. A new glycosidic aroma precursor was isolated from green tea leaves (Camellia sinensis var. sinensis cv. Yabukita) along with the known primeverosides of cis-linalool 3,6-oxide, linalool and geraniol. These glycosides were separated by chromatographic isolation on Amberlite XAD-2, ODS flash chromatography, and finally HPLC. The chemical structure of the new unknown glycoside was confirmed as geranyl 6-O-alpha-L-arabinopyranosyl-beta-D-glucopyranoside (geranyl beta-vicianoside) by spectrometric analyses and by an enzymatic hydrolysis with glycosidase followed by GC-MS and HPLC analyses. Moreover the vicianoside was hydrolyzed with acetone powder obtained from fresh tea leaves to generate the same compounds, suggesting this glycoside to be a tea aroma precursor.

Onisei, T., E. T. Toth, et al. (1995). "Growth and volatile oil production of two different vitroclones of Pelargonium roseum Ait." Rivista Italiana EPPOS(16): 13-19. In vitro-cultured plants of P. roseum, derived by direct organogenesis from stem nodes taken from 2 plants of different origin (Fundulea and Chisinau), were transplanted in the field in June. At the end of July and in mid Sep., plants were assessed for growth parameters and essential oil yield (data presented). Differences in in vitro culture establishment, shoot regeneration, shoot multiplication, root induction, plant acclimatization and essential oil production are discussed in relation to the explant source. The plants retained the characteristics of the parental genotype. Fundulea plants were more productive with regard to all the parameters assessed (plant height, number of branches, number of axillary buds, and plant FW and DW). The major essential oil constituents were geraniol, linalool and citronellal; these were present in higher quantities in plants derived from Fundulea explants, compared with those of Chisinau origin. Citronellol was only detected in plants of Fundulea origin.

Padula, L. Z., A. M. Collura, et al. (1977). "Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil." Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E.

muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Passet, J., J. P. Laget, et al. (1994). "[Systemic emulsions. 2. Use of different methods of formulation: the effect of essential oils of thyme on stability]." J Pharm Belg 49(6): 469-78. As part of an ongoing investigation on emulsifying techniques, we studied the influence of different essential oils from Thymus vulgaris on emulsion stability. All four chimiotypes tested (geraniol, linalol, carvacrol, and thymol) caused a marked decrease in stability. This instability cannot be explained by a change in the hydrophilic lipophilic balance since the HLBc of the new oil phase (essential oil + paraffine oil) was not significantly different from that of paraffine alone.

Pattnaik, S., V. R. Subramanyam, et al. (1997). "Antibacterial and antifungal activity of aromatic constituents of essential oils." Microbios 89(358): 39-46. Five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) were tested for antimicrobial activity against 18 bacteria (including Gram positive cocci and rods, and Gram negative rods) and 12 fungi (Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, A. oryzae, Alternaria citrii, Fusarium oxysporum, F. solani, Helminthosporium compactum, Macrophoma phaseolina, Sclerotium rolfsii, Sporothrix schenckii and Trichophyton mentagrophytes). In terms of antibacterial activity linalool was the most effective and inhibited 17 bacteria, followed by cineole, geraniol (each of which inhibited 16 bacteria), menthol and citral aromatic compounds, which inhibited 15 and 14 bacteria, respectively. Citral and geraniol oils were the most effective against fungi (inhibiting all 12 fungi), followed by linalool (which inhibited 10 fungi), cineole and menthol (each of which inhibited 7 fungi) compounds.

Perry, N. S. L., P. J. Houghton, et al. (2000). "In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes." Journal of Pharmacy and Pharmacology 52(7): 895-902. The effects of S. lavandulaefolia [S. lavandulifolia] essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole [eucalyptol] (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma- terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha- pinene and were 0.03 micro g/ml, 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma- terpinene) were less potent (camphor IC50 of >10 mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro

cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Perry, N. S., P. J. Houghton, et al. (2000). "In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes." J Pharm Pharmacol 52(7): 895-902. Sage (Salvia spp) is reputed in European herbal encyclopaedias to enhance memory, and current memory-enhancing/anti-dementia drugs are based on enhancing cholinergic activity by inhibiting cholinesterase. In this study the effects of Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and some of its constituent terpenes on human erythrocyte acetylcholinesterase were examined in-vitro. The main constituents in the essential oil batch used for analysis of cholinesterase inhibition were camphor (27%), 1,8-cineole (13%), alpha- and beta-pinene (10-15%) and bornyl acetate (10%) with other minor constituents (1% or less) including geraniol, limonene, linalool, terpineol and gamma-terpinene. Using the Ellman spectrophotometric method, kinetic analysis was conducted on the interaction of the essential oil and the main monoterpenoids, camphor, 1,8-cineole and alpha-pinene. IC50 values were obtained for the essential oil, 1,8-cineole and alpha-pinene and were 0.03 microL [corrected] mL(-1), 0.67 mM and 0.63 mM, respectively. Camphor and other compounds tested (geraniol, linalool and gamma-terpinene) were less potent (camphor IC50: >10mM). The essential oil, alpha-pinene, 1,8-cineole and camphor were found to be uncompetitive reversible inhibitors. These findings suggest that if the inhibitory activity of the essential oil is primarily due to the main inhibitory terpenoid constituents identified, there is a major synergistic effect among the constituents. Since no single constituent tested was particularly potent, it remains to be determined whether these in-vitro cholinesterase inhibitory activities are relevant to in-vivo effects of the ingestion of S. lavandulaefolia essential oil on brain acetylcholinesterase activity.

Rastogi, S. C., S. Heydorn, et al. (2001). "Fragrance chemicals in domestic and occupational products." Contact Dermatitis 45(4): 221-5. Epidemiological studies have described an increasing prevalence of fragrance allergy and indicated an association with hand eczema. 59 domestic and occupational products intended for hand exposure were subjected to gas chromatography-mass spectrometric (GC-MS) analyses to test the hypothesis that fragrance chemicals known to have the potential to cause contact allergy but not included in fragrance mix (FM) may be common ingredients in these products. A quantitative analysis of 19 selected fragrances was performed by GC-MS. Further analysis of GC-MS data revealed the presence of 43 other fragrance chemicals/groups of fragrance chemicals in the products investigated. Among the 19 target substances the most commonly detected were limonene in 78%, linalool in 61% and citronellol in 47% of the products investigated. The FM ingredients were present in these products with the following frequencies: oak moss (evernic acid methylester) 2%, cinnamic alcohol 2%, cinnamic aldehyde (cinnamal) 3%, isoeugenol 5%, alpha-amylcinnamic aldehyde (amyl cinnamal) 8%, hydroxycitronellal 12%, eugenol 27%, and geraniol 41%. Thus, the chemical analyses of domestic and occupational products indicates that investigation of potential contact allergy related to these products types should consider fragrance allergens additional to those in the FM, since these may occur with high frequency.

Riou, C., J. M. Salmon, et al. (1998). "Purification, characterization, and substrate specificity of a novel highly glucose-tolerant beta-glucosidase from Aspergillus oryzae." Appl Environ Microbiol 64(10): 3607-14. Aspergillus oryzae was found to secrete two distinct beta-glucosidases when it was grown in liquid culture on various substrates. The major form had a molecular mass of 130 kDa and was highly inhibited by glucose. The minor form, which was induced most effectively on quercetin (3,3',4',5,7-pentahydroxyflavone)-rich medium, represented no more than 18% of total beta-glucosidase activity but exhibited a high tolerance to glucose inhibition. This highly glucose-tolerant beta-glucosidase (designated HGT-BG) was purified to homogeneity by ammonium sulfate precipitation, gel filtration, and anion-exchange chromatography. HGT-BG is a monomeric protein with an apparent molecular mass of 43 kDa and a pI of 4.2 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing polyacrylamide gel electrophoresis, respectively. Using p-nitrophenyl-beta-D-glucoside as the substrate, we found that the enzyme was optimally active at 50 degreesC and pH 5.0 and had a specific activity of 1,066 &mgr;mol min-1 mg of protein-1 and a Km of 0.55 mM under these conditions. The enzyme is particularly resistant to inhibition by glucose (Ki, 1. 36 M) or glucono-delta-lactone (Ki, 12.5 mM), another powerful beta-glucosidase inhibitor present in wine. A comparison of the enzyme activities on various glycosidic substrates indicated that HGT-BG is a broad-specificity type of fungal beta-glucosidase. It exhibits exoglucanase activity and hydrolyzes (1-->3)- and (1-->6)-beta-glucosidic linkages most effectively. This enzyme was able to release flavor compounds, such as geraniol, nerol, and linalol, from the corresponding monoterpenyl-beta-D-glucosides in a grape must (pH 2.9, 90 g of glucose liter-1). Other flavor precursors (benzyl- and 2-phenylethyl-beta-D-glucosides) and prunin (4',5,7-trihydroxyflavanone-7-glucoside), which contribute to the bitterness of citrus juices, are also substrates of the enzyme. Thus, this novel beta-glucosidase is of great potential interest in wine and fruit juice processing because it releases aromatic compounds from flavorless glucosidic precursors.

Saxena, V. K. and R. N. Sharma (1998). "Constituents of the essential oil from Commiphora mukul gum resin." Journal of Medicinal and Aromatic Plant Sciences 20(1): 55-56. The gum-resin consisted of alpha-pinene (4.75%), myrcene (3.50%), eugenol (14.70%), cadinene (5.50%), geraniol (6.20%), methyl heptanone (17.50%), (+)-alpha-phellandrene (5.10%), (+)-limonene (6.50%), (plus or minus)-bornyl acetate (7.30%), 1,8-cineole [eucalyptol] (3.50%), (plus or minus)-linalool (8.70%), methyl chavicol (5.40%), alpha-terpineol (4.00%) and several unidentified compounds.

Seidakhmetova, R. B., A. A. Beisenbaeva, et al. (2002). "Chemical composition and biological activity of the essential oil from." Pharmaceutical Chemistry Journal 36(3): 135-138.

Sharma, J. N., K. C. Srivastava, et al. (1994). "Suppressive effects of eugenol and ginger oil on arthritic rats." Pharmacology 49(5): 314-318. This study examined the effect of eugenol and ginger oil on severe chronic adjuvant arthritis in rats. Severe arthritis was induced in the right knee and right paw of male Sprague-Dawley rats by injecting 0.05 ml of a fine suspension of dead Mycobacterium tuberculosis bacilli in liquid paraffin (5

mg/ml). Eugenol (33 mg/kg) and ginger oil (33 mg/kg), given orally for 26 days, caused a significant suppression of both paw and joint swelling. These findings suggest that eugenol and ginger oil have potent antiinflammatory and/or antirheumatic properties.

Szabo, K., E. Nemeth, et al. (1996). "Morphological and chemical variability of basil genotypes." Beitrage zur Zuchtungsforschung - Bundesanstalt fur Zuchtungsforschung an Kulturpflanzen 2(1): 76-79. The morphological and chemotaxonomic variability of 13 basil (Ocimum basilicum) genotypes was investigated. The genotypes were classified into 3 main groups on the basis of essential oil composition: (1) linalool; (2) linalool-estragole; and (3) linalool-geraniol-eugenol- gamma-murolene chemotypes. Data on plant height, secondary branches, leaves : stem : flower ratio and essential oil contents in different plant organs are also presented.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). "Constituents of essential oil of Myrtus communis." Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Wang, G., X. Zhu, et al. (1992). "[Analysis of chemical constituent of essential oil in Lonicera japonnica Thunb. cultivated on the northern plain of Henan Province]." Zhongguo Zhong Yao Za Zhi 17(5): 268-70, 319. The chemical constituents of the essential oil in the dry flower and fresh flower of Lonicera japonica were analyzed by the GC-MS-DS technique and the superimposition of authentic samples. 27 and 30 monoterpenoids and sesquiterpenoids were identified from the essential oil of the dry flower and fresh flower respectively. The major constituents have been found to be linalool, geraniol, aromadendrene and eugenol etc.

Yanai, T. and M. Sato (2000). "Purification and characterization of an alpha-L-rhamnosidase from Pichia angusta X349." Biosci Biotechnol Biochem 64(10): 2179-85. An intracellular alpha-L-rhamnosidase from Pichia angusta X349 was purified to homogeneity through four chromatographic steps. The alpha-L-rhamnosidase appeared to be a monomeric protein with a molecular mass of 90 kDa. The enzyme had an isoelectric point at 4.9, and was optimally active at pH 6.0 and at around 40 degrees C. The Ki for L-rhamnose inhibition was 25 mM. The enzyme was inhibited by Cu2+, Hg2+, and p-chloromercuribenzoate. The alpha-L-rhamnosidase was highly specific for alpha-L-rhamnopyranoside and liberated rhamnose from naringin, rutin, hesperidin, and 3-quercitrin. The alpha-L-rhamnosidase was active at the ethanol concentrations of wine. It efficiently released monoterpenols, such as linalool and geraniol, from an aroma precursor extracted from Muscat grape juice.

Zivanovic, L. J., M. Jovanovic, et al. (1990). "Densitometric determination of monoterpenoids in Melissa extracts." Fitoterapia 61(1): 82-83. Melissa fluid extract is used as a diaphoretic and a stimulant in pharmaceutical preparations, as a skin antiirritant in emulsions and lotions, and because of its antiviral effect in some pharmaceutical

ointments. A densitometric method for the determination of geraniol, linalool, citral and citronellal in Melissa officinalis leaf extracts is presented.

Linalool acetate CAS# 115-95-7 (Bergamiol, Bergamol, Linalyl acetate, Licareol acetate, Linalol acetate, Lynalyl acetate, 3,7-Dimethyl 1,6-octadien acetate)

Buchbauer, G., L. Jirovetz, et al. (1993). "Fragrance compounds and essential oils with sedative effects upon inhalation." Journal of Pharmaceutical Sciences 82(6): 660-664. In experiments with female 6- to 8-week-old Swiss mice [see also Planta Medica (1987) 53, 315-318], a total of 44 fragrance compounds and essential oils, obtained from Dragoco Company (Vienna, Austria) and known to possess sedative properties, were screened for their potential aromatherapeutic value when administered by inhalation. The motility of untreated mice was compared with that of mice exposed to a specific compound after no pretreatment or after a caffeine-induced overagitation treatment. Compared with the motility of untreated mice (100%) that of mice exposed to lavender [Lavandula sp.] oil, neroli [Citrus aurantium] oil, linalool, linalyl acetate, citronellal, benzaldehyde, 2-phenylethyl acetate, alpha-terpineol and sandalwood [Santalum album] oil was decreased by 78.4, 65.3, 73.0, 69.1, 49.8, 43.7, 45.0, 45.0 and 40.0%, respectively. In contrast, an increased motility was observed after exposure to geraniol, isoborneol, isoeugenol, orange [Citrus sp.] terpenes and thymol. The sedative effect of lavender oil, isoeugenol, linalool, maltol, carvone and linalyl acetate counteracted caffeine-induced overagitation; overagitation was amplified by anthranilic acid methyl ester, farnesol, lime (Tilia sp.) blossom oil and nerol inhalation. Serum samples, taken shortly after the inhalation treatment, were analysed by GC-MS, GC-fourier transform infrared and GC-flame inonization techniques in order to identify active constituents. A total of 21 substances were identified at concentrations of up to 0.1 ng/ml serum. Correlations of the aroma detection thresholds and sedative properties associated with these substances indicated that there might be a direct pharmacological interaction of fragrance molecules with body tissues rather than a reflective interaction caused by a pleasant feeling.

Cain, W. S., F. T. Schiet, et al. (1995). "Comparison of models of odor interaction." Chem Senses 20(6): 625-37. Subjects rated the overall perceived intensity of concentrations of the odorants cineole, geraniol, hexyl salicylate, and linalyl acetate smelled alone and in binary mixtures. The subjects also rated intensity of specified constituents (e.g. amount of cineole in cineole, and in mixtures of cineole and linalyl acetate). The intensity of the stronger component alone offered a close description of perceived intensity. In addition to the Stronger Component model, two other psychological models (Vector and U model) and two psychophysical models (UPL2 and Equiratio Mixture model) offered descriptions ranging from fair to very good. Psychological models gave better fits, but lack explanatory power. Some results indicated that weaker odors add more potently than stronger odors, an outcome incompatible with these models. The psychophysical models, based on the additivity of single components, generally overestimated perceived intensity. Judgments of individual qualities gave only slight encouragement to any expectation of differences in masking or maskability among odorants. The results highlight the need to test particular critical hypotheses regarding how people perceive mixtures.

Ceschel, G. C., P. Maffei, et al. (2000). "In vitro permeation through porcine buccal mucosa of Salvia desoleana." International Journal of Pharmaceutics 195(1-2): 171-177.

In the light of recent studies, which have shown that the essential oil derived from some Lamiaceae species has appreciable anti-inflammatory activity, moderate anti-microbial action and the ability to inhibit induced hyperalgesia, an assessment of the diffusion and permeation of Salvia desoleana Atzei and Picci (S. desoleana) essential oil through porcine buccal mucosa was considered useful for a possible application in the stomatological field. Topical formulations (microemulsions, hydrogels and microemulsion- hydrogels) were prepared for application to the buccal mucosa. The mucosa permeation of the oil from the formulations was evaluated using Franz cells, with porcine buccal mucosa as septum between the formulations (donor compartment) and the receptor phase chambers. The study also aimed at optimising the permeability of the S. desoleana essential oil by means of an enhancer, the diethylene glycol monoethyl ether Transcutol(R). The diffusion of the oil through the membrane was determined by evaluating the amount of essential oil components present in the receiving solution, the flux and the permeation coefficient (at the steady state) in the different formulations at set intervals. Qualitative and quantitative determinations were done by gas chromatographic analysis. All the formulations allow a high permeability coefficient in comparison with the pure essential oil. In particular, the components with a terpenic structure (beta-pinene, cineole, alpha-terpineol and linalool) have the highest capacity to pass through the porcine buccal mucosa when compared to the other components (linalyl acetate and alpha-terpinil acetate). Moreover, the enhancer, diethylene glycol monoethyl ether largely increases the permeation of the essential oil components in relation to the concentration. (C) 2000 Elsevier Science B.V.

Moretti, M. D. L., A. T. Peana, et al. (1997). “A study on anti-inflammatory and peripheral analgesic action of Salvia sclarea oil and its main components.” Journal of Essential Oil Research 9(2): 199-204. Activity against the acute inflammatory process induced by carrageenan and histamine in rats and the antinociceptive effect in mice were investigated after administration of S. sclarea essential oil (obtained from leaves grown in Sardinia, Italy) and some of its constituents. The essential oil (250 mg/kg, s.c.) showed significant antiinflammatory and moderate analgesic properties. The antiinflammatory action was more conspicuous against carrageenan- induced oedema (equivalent to a 5 mg/kg dose of indometacin) than against histamine-induced oedema. The effect was correlated to the presence of methyl chavicol, linalool, alpha-terpineol and linalyl acetate. These constituents were less active when administered separately than when combined in the essential oil. They were also less effective than the oxygenated fractions obtained by Flash chromatography. The moderate peripheral analgesia (evaluated by the formic acid-induced writhing test) produced by S. sclarea essential oil appeared to be mainly attributable to its alcoholic component.

Moretti, M. D. L., A. T. Peana, et al. (1997). “A study on anti-inflammatory and peripheral analgesic action of Salvia sclarea oil and its main components.” Journal of Essential Oil Research 9(2): 199-204. Activity against the acute inflammatory process induced by carrageenan and histamine in rats and the antinociceptive effect in mice were investigated after administration of S. sclarea essential oil (obtained from leaves grown in Sardinia, Italy) and some of its constituents. The essential oil (250 mg/kg, s.c.) showed significant antiinflammatory and moderate analgesic properties. The antiinflammatory

action was more conspicuous against carrageenan- induced oedema (equivalent to a 5 mg/kg dose of indometacin) than against histamine-induced oedema. The effect was correlated to the presence of methyl chavicol, linalool, alpha-terpineol and linalyl acetate. These constituents were less active when administered separately than when combined in the essential oil. They were also less effective than the oxygenated fractions obtained by Flash chromatography. The moderate peripheral analgesia (evaluated by the formic acid-induced writhing test) produced by S. sclarea essential oil appeared to be mainly attributable to its alcoholic component.

Oh, K., H. Matsuoka, et al. (1993). "Automatic evaluation of antifungal volatile compounds on the basis of the dynamic growth process of a single hypha." Appl Microbiol Biotechnol 38(6): 790-4. An automatic analysing system was developed and employed for the evaluation of antifungal activity of volatile compounds in the gas phase. Aspergillus niger was inoculated on agar medium in the reaction vessel. The reaction vessel was incubated at 28 degrees C for 24 h and then a volatile compound was introduced into the vessel either in a batch or flow manner. The antifungal activity of the respective compounds estimated in situ was expressed by the dynamic response parameters of a single hypha. All volatiles tested in the present system inhibited hyphal growth, except linalyl acetate: Limone and geraniol were the most inhibitory. In contrast, linalyl acetate promoted hyphal growth. By definition of the parameters, the fungicidal and fungistatic effects could be distinguished.

Padula, L. Z., A. M. Collura, et al. (1977). "Experimental cultivation of Elyonurus muticus in Argentina. Qualitative and quantitative analysis of the essential oil." Riv. Ital. Essenze, Profumi, Piante Offic., Aromi, Saponi, Cosmet., Aerosol 59(2): 58-63. E. muticus differs from Cymbopogon citratus (previously cultivated), by greater frost resistance, more vigorous aerial growth and higher essential oil contents and yields/unit area. It is possible to harvest 2 crops/year. From the Elyonurus essential oil alpha -pinene, myrcene, limonene, methyleptenone, linalool, linalyl acetate, terpineol, nerol, geranyl acetate, neral and geranial were isolated.

Vanhaelen, M. and R. Vanhaelen-Fastre (1980). "Constituents of essential oil of Myrtus communis." Planta Medica 39(2): 164-167. beta -Pinene, myrcene, phellandrene, limonene, gamma -terpinene, p- cymene, linalool, linalyl acetate, beta -caryophyllene, alpha - terpineol and methyl eugenol were identified. The presence of earlier reported constituents (alpha -pinene, camphene, dipentene, 1:8- cineol, myrtenyl acetate, myrtenol, nerol and geraniol) was confirmed.

Neryl acetate CAS# 141-12-8 (2,6-Octadien 3,7-dimethyl acetate, Nerol acetate, Neryl acetate)

Chinou, I. B., V. Roussis, et al. (1996). "Chemical and biological studies on two Helichrysum species of Greek origin." Planta Medica 62(4): 377-379. The essential oils obtained from the aerial parts of H. amorginum and H. italicum (collected from Amorgos, Greece) were analysed by GC and GC-MS. From the 25 identified constituents representing 89.98 and 82.06% of the 2 oils respectively, geraniol, geranyl acetate, neryl acetate and nerolidol were the major components. The essential oils and geraniol, geranyl acetate and neryl acetate exhibited antibacterial activity against Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae and Escherichia coli.

Inouye, S., K. Uchida, et al. (2001). "Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact." Journal of Essential Oil Research 13(1): 68-72. The flowers of Perovskia abrotanoides and Nepeta juncea and the leaves and flowers of Thymus linearis were collected at full bloom from different areas of Karakoram district, Pakistan, then dried and examined for essential oil composition. Dried plant parts were placed in air-tight boxes with 3 species of fungi (Candida albicans, Aspergillus fumigatus and Trichophyton mentagrophytes or Trichophyton rubrum) for one, 3 and 5 days, respectively. P. abrotanoides extracts contained high concentrations of 1,8-cineole (24-27%) and alpha-pinene (18-23%) and low concentrations of borneol and bornyl acetate. N. juncea essential oils contained nepetalactone (5.8 plus or minus 0.54 mg per g of dried flowers), and several minor constituents such as nerol, 1,8-cineole, neryl acetate and an unidentified component. Two Thymus linearis chemotypes were collected; that from Hunza and Rupal valley had thymol and carvacrol as major components, and the other chemotype from the Rakaposhi area had geranyl acetate and geraniol as major components. N. juncea and Thymus linearis essential oils inhibited fungal growth by vapour contact, while P. abrotanoides showed no activity. The 2 Trichophyton species were the most susceptible and C. albicans was the least susceptible to the toxic effects of plant aromatic compounds, while A. fumigatus showed intermediate susceptibility.

Zingerone CAS# 122-48-5 (Gingerone, Zingiberone, Paradol, Vanillyl acetone, 4-Hydroxy-3-methoxyphenyl ethyl methyl ketone, Methoxy hydroxy benzyl acetone, 4-Hydroxy 3-methoxyphenyl butanone)

Afzal, M., D. Al-Hadidi, et al. (2001). "Ginger: An ethnomedical, chemical and pharmacological review." Drug Metabolism and Drug Interactions 18(3-4): 159-190. Powerful medicinal properties have been recorded for Zingiber officinale, commonly known as ginger. All of these medicinal activities have been compiled with 99 references to the present status of the plant in the literature. Volatile components and the presence of trace metals are included. In addition, details of individual medicinal activities are given and the molecular structures of identified organic metabolites and their synthesis are described.

Jancso, G. and E. Kiraly (1981). "Sensory neurotoxins: Chemically induced selective destruction of." Brain Research 210(1-2): 83-89. Neonatal capsaicin treatment has been shown to cause selective degeneration of chemosensitive primary sensory neurons involved in the mediation of chemogenic pain and in neurogenic inflammatory responses. In the present study the neurotoxic effect of capsaicin congeners was investigated in the newborn rat. Some quantitative data on the selective neurotoxic action of capsaicin are also reported. Electron microscopy indicates that some pungent congeners of capsaicin also induce the selective degeneration of type 'B' sensory ganglion cells. At high doses the distribution pattern of axon terminal degeneration within the spinal cord and brain stem was equivalent to that observed after neonatal capsaicin treatment. The neurotoxic potency of capsaicin congeners, unlike desensitizing activity, is closely related to the sensory irritant property of these compounds. It is concluded that primary sensory neurons degenerating after the administration of these capsaicin congeners may correspond to substance P-containing chemosensitive primary sensory neurons involved in the transmission of nociceptive impulses.

Jayasekhar, P., S. B. Rao, et al. (1998). "Synthesis and pharmacological activity of some Mannich bases of." Indian Journal of Pharmaceutical Sciences 60(4): 191-195. Mannich bases of dehydrozingerone were synthesised by the Mannich reaction with dialkylamine hydrochlorides and also by aldol condensation of vanillin with dialkylaminobutan-2-one. They displayed antiinflammatory, analgesic and antipyretic activities.

Mustafa, T., K. C. Srivastava, et al. (1993). "Pharmacology of ginger, Zingiber officinale." Journal of Drug Development 6(1): 25-39. In traditional medicine the rhizome of ginger was held to possess medicinal properties. The scientific investigations relating to consumption of fresh or powdered rhizome by humans and in vitro effects of aqueous and organic extracts and of volatile oils are reviewed. Pungent components of ginger inhibit cyclooxygenase and lipoxygenase activity in the arachidonic acid metabolic pathway and thereby probably reduce inflammation and relieve pain in rheumatic disorders and migraine headache. Consumption of ginger reduces plasma thromboxane Binf 2 (TXBinf 2) levels in humans. Ginger is reported to reduce nausea vertigo and vomiting for which the mechanism of action is however not yet understood. Effects on the gastrointestinal system include increase in bile secretion and anti-emetic

action. An acetone extract of ginger and (6)-shogaol given orally, accelerate gastroinstestinal movement in mice while given i.v. (6)-shogaol inhibits such movement. Galanolactone antagonises 5-HTinf 3 receptors which may explain the anti-emetic and gastrointestinal movement enhancing effects. Zingiberone and(6)-gingerol are reported to protect against gastric mucosal lesions. (6)-Shogaol is known to reduce blood pressure by both a central and a peripheral action. (8)-Gingerol has a cardiotonic action via enhancement of the Ca-ATPase in the sarcoplasmic reticulum. Ginger contains mutagenic (gingerol and shogaol) and anti-mutagenic (zingiberone) compounds. Ginger extract exhibits cytotoxic effects in cultured plant cells but it is not known whether ginger can suppress tumour growth in experimental animals or humans. Some of the chemical compounds from ginger may prove to have anti-inflammatory, anti-emetic, cardiotonic and gastroprotective properties in humans without side effects.

Opletalova, V. (1995). “Constituents of the plants of the Zingiberaceae family and their.” Ceska a Slovenska Farmacie 44(6): 305-307. The compounds contained in various plants of the Zingiberaceae family, e.g. zingeron, gingerols and curcumin, as well as their naturally occurring and synthetic analogues showed interesting anti-inflammatory properties. The present paper briefly deals with the structures and activities of these compounds.

Schuhbaum, H., J. Burgermeister, et al. (2000). “Anti-inflammatory activity of Zingiber officinale extracts.” Pharmaceutical and Pharmacological Letters 10(2): 82-85. The anti-inflammatory effect of ginger root extracts could be substantiated with an acetonic extract containing essential oil components and the ginger specific compounds such as gingerols, shogaols and minor compounds like gingerenone A, [6]-gingerdiol, hexahydrocurcumin and zingerone. The total extract, in the HET-CAM-test showed a pronounced dose dependent anti-inflammatory overall effect. However, testing the purified individual components of the acetonic extract, a close relationship with a single substance or a series of homologues could not be established. It is obvious that the optimal anti-inflammatory effect is correlated with the genuine extract composition where individual components might have synergistic effects. Comparable results were obtained in the Griess-assay, where the ability to reduce the liberation of NO by the iNOS is examined. The total extract showed a dose dependent effect in this in vitro assay. Individual purified components however could not be closely correlated to the NO-interfering activity. In conclusion, the acetonic ginger extract is an interesting substrate for formulations of the appropriate anti-inflammatory remedies.

Suri, K. A., R. P. Sood, et al. (1981). “Synthesis of potential anti-inflammatory compounds.” Indian Journal of Pharmaceutical Sciences 43(6): 226-228.

Ziment, I. (2002). “Herbal antitussives.” Pulmonary Pharmacology and Therapeutics 15(3): 327-333. The mechanisms of actions of cough medicines are not always known. The problem is exacerbated for herbal medicines, where the effectiveness of the plant or its phytochemicals have rarely been carefully evaluated. Moreover, the most active phytomedicinal constituent is difficult to identify, and the expense and difficulty of such studies discourages sponsors who may not be able to benefit by subsequent exclusive

marketing of the herbal remedy. Most popular herbs used as cough medicines appear to be demulcents whose action is confined to the oropharynx. It is probable that the vast majority of allegedly effective herbal cough medicines act as non-specific emetic-expectorants. The proof of activity of even marketed herbal derivatives such as guaifenesin and codeine is difficult to obtain. It is therefore likely that herbal cough medications will never be shown to be more active than placebos. Nevertheless, these plant products will continue to be popular remedies for patients and their health care advisors.

(2000). "Ginger." American Journal of Health-System Pharmacy 57(10): 945-947.

Afzal, M., D. Al-Hadidi, et al. (2001). "Ginger: An ethnomedical, chemical and pharmacological review." Drug Metabolism and Drug Interactions 18(3-4): 159-190. Powerful medicinal properties have been recorded for Zingiber officinale, commonly known as ginger. All of these medicinal activities have been compiled with 99 references to the present status of the plant in the literature. Volatile components and the presence of trace metals are included. In addition, details of individual medicinal activities are given and the molecular structures of identified organic metabolites and their synthesis are described.

Cyong, J. and Y. Otsuka (1982). "A pharmacological study of the anti-inflammatory activity of Chinese." Acupuncture and Electro-Therapeutics Research 7(2-3): 173-202.

Endoh, M. (2002). "Mechanisms of action of novel cardiotonic agents." Journal of Cardiovascular Pharmacology 40(3): 323-338. Regulation of myocardial contractility by cardiotonic agents is achieved by an increase in intracellular CaSUP2+ mobilization (upstream mechanism), an increase in CaSUP2+ binding affinity to troponin C (central mechanism), or facilitation of the process subsequent to CaSUP2+ binding to troponin C (downstream mechanism). cAMP mediates the regulation induced by CaSUP2+ mobilizers such as beta-adrenoceptor agonists and selective phosphodiesterase III inhibitors acting through the upstream mechanism. These agents act likewise on the central mechanism to decrease CaSUP2+ sensitivity of troponin C in association with the cAMP-mediated phosphorylation of troponin I. In addition to such a well-known action of cAMP, recent experimental findings have revealed that CaSUP2+ sensitizers, such as levosimendan, OR-1896, and UD-CG 212 Cl, require the cAMP-mediated signaling for induction of CaSUP2+ sensitizing effect. These agents shift the [CaSUP2+]SUBi-force relationship to the left, but their positive inotropic effect (PIE) is inhibited by carbachol, which suppresses selectively the cAMP-mediated PIE. These findings imply that cAMP may play a crucial role in increasing the myofilament CaSUP2+ sensitivity by cross-talk with the action of individual cardiotonic agents. No clinically available cardiotonic agents act primarily via CaSUP2+ sensitization, but the PIE of pimobendan and levosimendan is partly mediated by an increase in myofilament CaSUP2+ sensitivity. Evidence is accumulating that cardiotonic agents with CaSUP2+ sensitizing action are more effective than agents that act purely via the upstream mechanism in clinical settings. Further clinical trials are required to establish the effectiveness of CaSUP2+ sensitizers in long-term therapy for congestive heart failure patients.

Jae Youl, C., J. Park, et al. (1999). "Inhibitory principles from Magnolia officinalis on tumor necrosis." Natural Product Sciences 5(2): 70-74. In the course of a search for tumor necrosis factor (TNF)-alpha inhibitory compounds from medicinal plants, we identified neolignans, honokiol and magnolol, from the alcoholic extract of Magnolia

officinalis as active inhibitory principles. These compounds dose-dependently inhibited TNF-alpha production without displaying cytotoxicity and their inhibitory activities measured by ICinf 5inf 0 values were 53.7 and 61.4 muM, respectively.

Kondo, K., N. Hattori, et al. (2000). “Interspecific and local variations of magnolol, honokiol, and beta.” Natural Medicines 54(2): 61-69. To clarify the interspecific and local variations of magnolol, honokiol, and beta -eudesmol contents in the magnolia barks, their contents and morphological characteristics of Magnolia obovata, M. officinalis, and M. officinalis var. biloba were studied. The results were as follows. Magnolia officinalis examined had the significantly higher magnolol and honokiol contents than M. obovata and M. officinalis var. biloba. Magnolia obovata had a significantly higher beta -eudesmol content than M. officinalis and M. officinalis var. biloba. Additionally, in M. officinalis, a comparatively close correlation was recognized between the magnolol content and the honokiol content, and between the magnolol content and the thickness of bark. Therefore, we propose the ways to choose the Chinese magnolia barks similar to the Japanese magnolia barks in the magnolol content based on the thickness of bark.

Kuroyanagi, M., K. Yoshida, et al. (2000). “Bicyclo[3.2.1]octane and 6-oxabicyclo[3.2.2]nonane type neolignans from.” Chemical and Pharmaceutical Bulletin 48(6): 832-837. From the ethyl acetate soluble fraction of twigs of Magnolia denudata (Magnoliaceae), seven new neolignam derivatives, 1-7, were isolated along with eighteen known lignan and neolignan derivatives, 8-25. The structures of the new neolignans were elucidated by means of spectral methods, especially by sup 1H-NMR and sup 1sup 3C-NMR spectra, and two dimensional NMR methods such as sup 1H-detected heteronuculear multiple bond connectivity (HMBC), sup 1H-detected multiple quantum coherence (HMQC) and sup 1H-sup 1H-correlation spectroscopy (COSY). Compounds 1-4 have novel structures possessing a 6- oxabicyclo[3.2.2]nonane skeleton and compounds 5-8 also have novel structures possessing a bicyclo[3.2.1]octane skeleton. The anti-platelet-activating factor (PAF) activity of these compounds was tested by measurement of inhibition activity against acetyl transferase to lyso-PAF.

Langner, E., S. Greifenberg, et al. (1998). “Ginger: History and use.” Advances in Therapy 15(1): 25-44. Ginger is well known in the form of ginger sticks or ginger ale. If these are consumed during travel, the traveler imbibes, albeit subconsciously, a healing plant for motion sickness. The efficacy of ginger rhizome for the prevention of nausea, dizziness, and vomiting as symptoms of motion sickness (kinetosis), as well as for postoperative vomiting and vomiting of pregnancy, has been well documented and proved beyond doubt in numerous high-quality clinical studies. The use of this ancient medicine for gastrointestinal problems (stimulation of digestion) has been given scientific approval. Today, medicinal ginger is used mainly for prevention of the symptoms of travel sickness.

Lohman, P. H. M., J. M. Gentile, et al. (2001). “Antimutagenesis/anticarcinogenesis 2001: Screening, methods and.” Mutation Research - Genetic Toxicology and Environmental Mutagenesis 496(1-2): 1-4.

Mori, H., Y. Yamada, et al. (2002). “Chemoprevention of large bowel carcinogenesis; The role of control of.” European Journal of Cancer Prevention 11(SUPPL. 2): S71-S75. Control of cell proliferation is important for cancer prevention since cell proliferation has essential roles in carcinogenesis in the processes of both initiation and promotion. In large bowel carcinogenesis, carcinogens produce hyperproliferation of cells in the target sites and the cell proliferation persists even after the cessation of carcinogen exposure. Chemopreventive agents principally control the increased cell proliferation when given in the initiation as well as post-initiation phases. Aberrant crypt foci (ACF) which appear soon after carcinogen exposure in large bowel carcinogenesis in rodents have been used as a reliable biomarker for screening of potential chemopreventive agents. Recently, our group demonstrated the presence of probable premalignant lesions with frequent beta-catenin gene mutations and accumulation of the corresponding protein in the colonic epithelium of rats given a large bowel carcinogen. Such early-appearing lesions lack the morphological appearance of ACF. Expression of these beta-catenin-accumulated crypts (BCAC) is markedly suppressed by a chemopreventive cyclooxygenase-2 inhibitor, celecoxib. BCAC are suggested to be more reliable biomarkers than ACF for screening effective chemopreventive agents for colorectal cancer and for investigating the mode of action of the agents. (c) 2002 Lippincott Williams & Wilkins.

Mustafa, T., K. C. Srivastava, et al. (1993). “Pharmacology of ginger, Zingiber officinale.” Journal of Drug Development 6(1): 25-39. In traditional medicine the rhizome of ginger was held to possess medicinal properties. The scientific investigations relating to consumption of fresh or powdered rhizome by humans and in vitro effects of aqueous and organic extracts and of volatile oils are reviewed. Pungent components of ginger inhibit cyclooxygenase and lipoxygenase activity in the arachidonic acid metabolic pathway and thereby probably reduce inflammation and relieve pain in rheumatic disorders and migraine headache. Consumption of ginger reduces plasma thromboxane Binf 2 (TXBinf 2) levels in humans. Ginger is reported to reduce nausea vertigo and vomiting for which the mechanism of action is however not yet understood. Effects on the gastrointestinal system include increase in bile secretion and anti-emetic action. An acetone extract of ginger and (6)-shogaol given orally, accelerate gastroinstestinal movement in mice while given i.v. (6)-shogaol inhibits such movement. Galanolactone antagonises 5-HTinf 3 receptors which may explain the anti-emetic and gastrointestinal movement enhancing effects. Zingiberone and(6)-gingerol are reported to protect against gastric mucosal lesions. (6)-Shogaol is known to reduce blood pressure by both a central and a peripheral action. (8)-Gingerol has a cardiotonic action via enhancement of the Ca-ATPase in the sarcoplasmic reticulum. Ginger contains mutagenic (gingerol and shogaol) and anti-mutagenic (zingiberone) compounds. Ginger extract exhibits cytotoxic effects in cultured plant cells but it is not known whether ginger can suppress tumour growth in experimental animals or humans. Some of the chemical compounds from ginger may prove to have anti-inflammatory, anti-emetic, cardiotonic and gastroprotective properties in humans without side effects.

Opletalova, V. (1995). “Constituents of the plants of the Zingiberaceae family and their.” Ceska a Slovenska Farmacie 44(6): 305-307. The compounds contained in various plants

of the Zingiberaceae family, e.g. zingeron, gingerols and curcumin, as well as their naturally occurring and synthetic analogues showed interesting anti-inflammatory properties. The present paper briefly deals with the structures and activities of these compounds.

Parka, K. K., K. S. Chun, et al. (1998). “Inhibitory effects of [6]-gingerol, a major pungent principle of.” Cancer Letters 129(2): 139-144. A wide array of phytochemicals have been shown to possess potential cancer chemopreventive properties. Ginger contains pungent phenolic substances with pronounced antioxidative and antiinflammatory activities. In the present study, we have determined the antitumor promotional activity of [6]-gingerol, a major pungent principle of ginger, using a two-stage mouse skin carcinogenesis model. Topical application of [6]-gingerol onto shaven backs of female ICR mice prior to each topical dose of 12-O-tetradecanoylphorbol-13-acetate (TPA) significantly inhibited 7,12-dimethylbenz[a]anthracene-induced skin papillomagenesis. The compound also suppressed TPA-induced epidermal ornithine decarboxylase activity and inflammation.

Ruedi, P. and M. Juch (1999). “Chemistry and biological activities of long-chain alkyloxy-catechols of.” Current Organic Chemistry 3(6): 623-646. The current state of research on the biologically active phenolic constituents of Zingiberaceae and Labiatae with oxygenated alkyl chains is reviewed. The report covers structure, reactivity, pharmacology and synthesis of the title compounds as well as the most recent significant advances in the chemistry of the structurally related diarylheptanoids. Particular emphasis is placed on stereochemical implications.

Schuhbaum, H. and G. Franz (2000). “Ginger: Spice and versatile medicinal plant.” Zeitschrift fur Phytotherapie 21(4): 203-209. Ginger, Zingiber officinale, is grown in many parts of the world, mainly in subtropical regions. The rhizome contains 1-2% volatile oil, 5-8% resinous matter, starch and other polysaccharides. The essential oil of ginger contains a complex mixture of monaterpenes, sesquiterpene hydrocarbons and sesquiterpene alcohol. The pungency is mostly due to the gingerols and their derivatives. A considerable number of pharmacological studies involving digestive, central nervous and cardiovascular system have been reported for extracts and isolated constituents of ginger. A potent inhibitory action of gingerols against prostaglandin systhesis corresponding to the anti-inflammatory activities has been described. Shogaol produces enhanced gastro intestinal activity with effects on bile secretion. The sesquiterpene hydrocarbons have been associated with the anti-ulcer activity of the drug. Many data support the findings that, specific ginger constituents have inhibitory activity on malignant cells.

Schuhbaum, H., J. Burgermeister, et al. (2000). “Anti-inflammatory activity of Zingiber officinale extracts.” Pharmaceutical and Pharmacological Letters 10(2): 82-85. The anti-inflammatory effect of ginger root extracts could be substantiated with an acetonic extract containing essential oil components and the ginger specific compounds such as gingerols, shogaols and minor compounds like gingerenone A, [6]-gingerdiol, hexahydrocurcumin and zingerone. The total extract, in the HET-CAM-test showed a pronounced dose

dependent anti-inflammatory overall effect. However, testing the purified individual components of the acetonic extract, a close relationship with a single substance or a series of homologues could not be established. It is obvious that the optimal anti-inflammatory effect is correlated with the genuine extract composition where individual components might have synergistic effects. Comparable results were obtained in the Griess-assay, where the ability to reduce the liberation of NO by the iNOS is examined. The total extract showed a dose dependent effect in this in vitro assay. Individual purified components however could not be closely correlated to the NO-interfering activity. In conclusion, the acetonic ginger extract is an interesting substrate for formulations of the appropriate anti-inflammatory remedies.

Sekiya, K., S. Kadota, et al. (1997). “Study on baths with crude drug. III. The effect of Ligustici Chuanxiong.” Biological and Pharmaceutical Bulletin 20(9): 983-987. To investigate the permeability of natural compounds through hairless mouse skin, compounds having a range of lipophilicity, i.e., ginsenoside-Re (1), baicalin (2), glycyrrhizin (3), baicalein (4), wogonin (5), honokiol (6), magnolol (7) bergapten (8), shikonin (9) and sinomenine (10) were used. These compounds permeated through the skin a little, however, they were generally accumulated trite the skin. The uptake amount into the skin of each compound related to their lipophilicities in the in vitro experiment. Furthermore, Ligustici Chuanxiong Rhizoma (Senkyu) ether extract (SEE) enhanced their permeability into the skin; especially, it exhibited an effect on the skin permeability of moderately lipophilic compounds such as 4, 8. The effect of SEE in vivo was similar to that obtained in the in vitro experiment. From these results, it was clarified that natural compounds having high lipophilicity sufficiently permeated into the hairless mouse skin owing to their accumulative property, and SEE enhanced the permeability of the moderately lipophilic compounds into the skin.

Surh, Y. J. (1999). “Molecular mechanisms of chemopreventive effects of selected dietary and.” Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 428(1-2): 305-327. Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. A wide array of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of these naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of hot chili pepper, protects against experimentally-induced mutagenesis and tumorigenesis. It also induces apoptosis in various immortalized or malignant cell lines. Plants of ginger family (Zingiberaceae) have been frequently and widely used as spices and also, in traditional oriental medicine. Curcumin, a yellow ingredient from turmeric (Curcuma longa L., Zingiberaceae), has been extensively investigated for its cancer chemopreventive potential. Yakuchinone A [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanone] and yakuchinone B [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenylhept-1-en-3-one] present in Alpinia oxyphylla Miquel (Zingiberaceae) have inhibitory effects on phorbol ester-induced inflammation and skin carcinogenesis in mice, and oxidative stress in vitro.

These diarylheptanoids suppress phorbol ester-induced activation of ornithine decarboxylase and production of tumor necrosis factor-alpha or interleukin-1alpha and their mRNA expression. They also nullified the phorbol ester-stimulated induction of activator protein 1 (AP-1) in cultured human promyelocytic leukemia (HL-60) cells. In addition, both yakuchinone A and B induced apoptotic death in HL-60 cells. Ginger (Zingiber officinale Roscoe, Zingiberaceae) contains such pungent ingredients as [6]-gingerol and [6]-paradol, which also have anti-tumor promotional and antiproliferative effects. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin found in grapes and other dietary and medicinal plants, and (-)-epigallocatechin gallate, a major antioxidative green tea polyphenol, exert striking inhibitory effects on diverse cellular events associated with multi-stage carcinogenesis. In addition, these compounds have ability to suppress proliferation of human cancer cells via induction of apoptosis. Copyright (C) 1999 Elsevier Science B.V.

Surh, Y. J. (2002). “Anti-tumor promoting potential of selected spice ingredients with.” Food and Chemical Toxicology 40(8): 1091-1097. A wide variety of phenolic substances derived from spice possess potent antimutagenic and anticarcinogenic activities. Examples are curcumin, a yellow colouring agent, contained in turmeric (Curcuma longa L., Zingiberaceae), [6]-gingerol, a pungent ingredient present in ginger (Zingiber officinale Roscoe, Zingiberaceae) and capsaicin, a principal pungent principle of hot chili pepper (Capsicum annuum L, Solanaceae). The chemopreventive effects exerted by these phytochemicals are often associated with their antioxidative and anti-inflammatory activities. Cyclo-oxygenase-2 (COX-2) has been recognized as a molecular target of many chemopreventive as well as anti-inflammatory agents. Recent studies have shown that COX-2 is regulated by the eukaryotic transcription factor NF-kappaB. This short review summarizes the molecular mechanisms underlying chemopreventive effects of the aforementioned spice ingredients in terms of their effects on intracellular signaling cascades, particularly those involving NF-kappaB and mitogen-activated protein kinases. (c) 2002 Elsevier Science Ltd. All rights reserved.

Surh, Y. J., K. K. Park, et al. (1999). “Anti-tumor-promoting activities of selected pungent phenolic substances.” Journal of Environmental Pathology, Toxicology and Oncology 18(2): 131-139. Ginger (Zingiber officinale Roscoe, Zingiberaceae) has been widely used as a dietary spice, as well as in traditional oriental medicine. The rhizome of ginger contains pungent vanillyl ketones, including [6]-gingerol and [6]paradol, and has been reported to possess a strong anti-inflammatory activity. These pungent substances have a vanilloid structure found in other chemopreventive phytochemicals, including curcumin. In our study, we found anti-tumor-promoting properties of [6]-gingerol and [6]-paradol. Thus, topical application of [6]-gingerol or [6]-paradol 30 min prior to 12-O-tetradecanoylphorbol-13-acetate (TPA) attenuated the skin papillomagenesis initiated by 7,12-dimethylbenz[a]anthracene in female ICR mice. These substances also significantly inhibited the tumor-promoter-stimulated inflammation, TNF-alpha production, and activation of epidermal ornithine decarboxylase in mice. In another study, [6]-gingerol and [6]-paradol suppressed the superoxide production stimulated by TPA in differentiated HL- 60 cells. Taken together, these findings suggest that pungent vanilloids found in ginger possess potential chemopreventive activities.

Wagner, H. (1989). “Search for new plant constituents with potential antiphlogistic and.” Planta Medica 55(3): 235-241.

Yamahara, J., Q. Huang, et al. (1990). “Gastrointestinal motility enhancing effect of ginger and its active.” Chemical and Pharmaceutical Bulletin 38(2): 430-431.