Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, antiinflammatory and other related pharmacological activities.

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Abstract

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This review describes the potential uses of essential oils from temperate aromatic plants. The constituents of the oils are mainly monoterpene and sesquiterpene hydrocarbons with the general formulae $(C_5H_8)_n$. Oxygenated compounds derived from these hydrocarbons include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. It is estimated that there are more than 1000 monoterpene and 3000 sesquiterpene structures. The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way. The main plant species and microorganisms investigated are covered in comprehensive tables and references. The brine shrimp bioassay used for the oil toxicity testing is described in detail.

Essential oils; Biological activities; Enantiomeric composition; Artemia salina bioassay

Introduction

Volatile oils are very complex mixtures of compounds. The constituents of the oils are mainly monoterpenes and sesquiterpines which are hydrocarbons with the general formula $(C_5H_8)_n$. Oxygenated compounds derived from these hydrocarbons include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. It is estimated that there are more than 1000 monoterpene and 3000 sesquiterpene structures. Other compounds include phenylpropenes and specific compounds containing sulphur or nitrogen. Hundreds of new natural substances are being isolated and identified every year, but data concerning their biological activities are known for only some. In certain plants, one main constituent may predominate. In basil, for example, methyl chavicol makes up 75% of the oil. In other species, there is no single component which predominates. Instead, there is a balance of various components, as for example in the oil of sweet marjoram where the individual chemicals are represented by 0.1 – 10% of total oil volume. The presence of trace components, even those as yet unidentified, can influence the odour, flavour and possibly also the biological activity of the oil to a significant extent.

Is there a future for naturally occurring compounds

Various industries are now looking into sources of alternative, more natural and environmentally friendly antimicrobials, antibiotics, antioxidants and crop protection agents. The possibility of utilising volatile oils is now being investigated as, although their biological activity has been known for centuries, their mode of action was not fully understood. The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way (Colgate, 1993; Svoboda et al., 1998; Svoboda and Deans 1995; Baratta et al., 1998a,b). Generally, their action is the result of the combined effect of both their active and inactive compounds. These inactive compounds might influence resorption, rate of reactions and bioavailability of the active compounds. Several active components might have a synergistic effect. To add to the complexity of volatile oils, there is evidence that the time of harvest influences the oil composition and consequently the potency of their biological activity (Deans and Svoboda, 1988; Lis-Balchin et al., 1992; Galambosi et al., 1993; Marotti et al., 1994a, 1994b). Other factors such as genotype, chemotype, geographical origin and environmental and agronomic conditions, can all influence the composition of the final natural product (Svoboda et al., 1992, 1995; Collins et al., 1994; Galambosi et al., 1999). In addition, enantiomeric composition of various monoterpenes in different species (Tables 1, 2 and 3) can further complicate the biological activity of a given oil (Ravid et al., 1987; 1992a, b; 1994a – e, 1996). This complexity of the final product explains why pharmaceutical firms prefer synthetic or semisynthetic compounds. They are mainly interested in the discovery of active chemical structures from which they can develop and prepare synthetic analogues. These are more controllable from the point of reproducibility, patentability, safety, and are more economically viable. Consequently, individual components of essential oils are at present under specific investigation to elucidate their particular activity (Table 4). For example, carvacrol, citronellol, eugenol, geraniol and limonene were tested as efficient food preservatives, without showing any mutagenicity.

Mode of action of essential oils

An excellent survey on the uses of fragrances and essential oils as medicaments was published by Buchbauer and Jirovetz (1994). It has been suggested that volatile oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes, and as a result, modify the activity of the calcium ion channels. At certain levels of dosage, the volatile oils saturate the membranes and show effects similar to those of local anaesthetics. They can interact with the cell membranes by means of their physiochemical properties and molecular shapes, and can influence their enzymes, carriers, ion channels and receptors. The authors describe various studies concerning the physiological effects on humans. These include brain stimulation, anxiety-relieving sedation and antidepressant activities, as well as increasing the cerebral blood flow. The studies also describe the effects of odours on cognition, memory, and mood. The fragrance compounds are absorbed by inhalation and are able to cross the blood-brain barrier and interact with receptors in the central nervous system. Bioassays used for the description and explanation of volatile oil action, are usually carried out on mice, rats and toads e.g. the influence of peppermint oil on intestinal transport (Beesley et al., 1996); the effect of volatile oils on the skin penetration (Abdullah et al., 1996); the effect on skeletal muscle fibres (Fogaca et al., 1997); the screening for analgesic properties (Aydin et al., 1996). Increasing numbers of aromatherapists and physiotherapists are using essential oils both in private practice and

within NHS hospitals and hospices, and their reports in all the main aromatherapy journals stress the positive effects of oils (The Aromatherapist, The Aromatherapist Journal and Aromatherapy).

Potential suggested uses of naturally occuring compounds as human medicants

The most important suggested areas of essential oil use are in urology, dermatology, sleep and nervous disorders, laxatives, erosive gastritis, cardiac and vascular systems, immunomodulating drugs, colds and coughs. Various plant species are being investigated in detail and are being thoroughly tested for their pharmacological properties. The oils are tested in behavioural pharmacological experiments, their effects being monitored for use over a wide range of conditions as well as for any signs of toxicity. These conditions include stress-related illness, formation of stomach ulcers and tumor growth. Their effects on blood circulation, nerve growth, nucleic acid, liver, protein, lipid, carbohydrate and cholesterol metabolisms are also monitored, as well as any effects on the activity of the adrenal gland and the body's immune system. Antiviral activity of the volatile oil from Houttuynia cordata was tested against herpes simplex virus, influenza and HIV-1 (Hayashi et al., 1995). It was suggested that the antiviral activity of the oil may be due to interference with the virus envelope. In another experiment (Tkachenko et al., 1995) essential oil from several species of the genus Heracleum showed promising activity against influenza virus. Further experiments are required to substantiate claims of antiviral activities and to elucidate the mode of action. Only 3 references were found from the last 5 years concerning detailed investigation of anticancer activities (Aruna and Sivaramakrishnan, 1996; Hailat et al., 1995 and Saenz et al., 1997), where the authors used enzymes and cellular polypeptides assays to assess various cancer cell lines. Nigella sativa, Cuminum cyminum, Papaver somniferum and Ocimum sanctum volatile oils can be considered as protective agents against carcinogenesis.

The importance of essential oils as potential antioxidants

Plant essential oils as antioxidants were researched in detail with the view to investigating their protective role for highly unsaturated lipids in animal tissues (Deans et al., 1993). The oils have shown their action as those of hepatoprotective agents in ageing mammals and these studies described the beneficial impact of volatile oils upon the PUFA's, in particular the long chain C_{20} and C_{22} acids. In addition, volatile oils also demonstrated a positive effect upon docosahexaenoic acid (DHA) levels in ageing rodent retinas. The reason that antioxidants are important to human physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen and hydroxyl radicals, collectively known as active oxygen. These molecules are formed in living cells by various metabolic pathways. Specific molecules, pollution from tobacco smoke and burning of fossil fuels, together with UV radiation and pollutants such as ozone, nitrogen oxide and sulphur dioxide, add to the formation of free radicals. Superoxide is converted by an enzyme, superoxide dismutase, into H_2O_2. H_2O_2 is able to cross all biological membranes. Much of the damage done by these compounds is thought to be due to their conversion to highly reactive oxidants such as the hydroxyl radical. Formation of OH from O_2 requires traces of catalytic metal ions, mainly iron and copper. The ability of the copper ion / H_2O_2 system to do severe damage to proteins and DNA is well established. The hydroxyl radical is very

reactive as it combines with almost all molecules found in living cells. Proteins, lipids, carbohydrates and DNA in living cells represent oxidizable substrates. The secondary events include changes in membrane structure, permeability and fluidity, lysosomal destabilization and stimulation of apoptosis. Lipid peroxidation finally leads to loss of membrane function and integrity leading to cell necrosis and death. Hydroxyl radicals can also react with bases in the DNA and cause mutations. Oxygen free radicals appear to be an important factor in chronic inflammatory joint disease such as rheumatoid arthritis. There are several known reactions of oxygen centred free radicals which are relevant to tissue injury in inflamed joints. Single oxygen can also be generated in the lens of the eye and contribute to the development of cataracts. Superoxide and hydrogen peroxide can stimulate growth in a variety of malignant mammalian cell types. They may have an important role as extracellular messengers for cell growth and viability.

Several substances have been proposed to act as antioxidants in vivo. They include beta carotene, albumin, uric acid, oestrogens, polyamines, flavonoids, ascorbic acid, plant phenolics, vitamin E and some drugs such as non-steroidal anti-inflammatories. They can stabilise the membranes by decreasing their permeability and they also have an ability to bind free fatty acids. It has been suggested that volatile oils could act as such agents. It has been found that certain volatile oils and their components are cytostatic to tumour cell lines and can offer potential as novel antiproliferative agents. Dorman et al. (1995) screened Pelargonium sp., Monarda citriodora var. citriodora, Myristica fragrans, Origanum vulgare ssp. hirtum and Thymus vulgaris for their antioxidative effect using a thiobarbituric acid (TBA) assay. The oils showed active antioxidant capacities at extremely low levels of dilution. Rosemary has long been recognised as having antioxidant molecules and these have been identified as carnosic acid, carnasol, carsolic acid, rosmaridiphenol and rosmarinic acid, found in ethanol-soluble fraction. Antioxidant properties are also found in the volatile oil fraction. However, it is very important to realise that in certain cases, antioxidants can be pro-oxidant and can stimulate free radical reactions.

A simple method for testing of toxicity of essential oils

The brine shrimp (Artemia salina) bioassay was used to test the toxicity of 5 volatile oils (Svoboda et al., 1998a). The brine shrimp is a crustacean found in saline water world-wide. The availability of eggs, the ease of hatching them into larvae, the rapid growth of nauplii and the relative ease of maintaining a population under laboratory conditions, make the brine shrimp a simple and effective test animal in bioassays and toxicology studies. All stages in their life cycle have been used, and the hatching rate of the eggs after the exposure to petroleum oil, pesticides, carcinogens and other contaminants has been used as a criterion for toxicity (Doganca et al., 1997; Meyer et al., 1982; Mongelli et al., 1996). The commonest stage used is the one 24-48 hours after hatching. Identification of the lethal concentration for 50% mortality after 6 hours of exposure (the acute LD_{50}) makes the test rapid and simple. Five oils were used in the tests: Roman chamomile (Anthemis nobilis), German chamomile (Matricaria chamomilla), thyme (Thymus vulgaris), lemon balm (Melissa officinalis) and fennel (Foeniculum vulgaris), in concentrations of 30, 60, 100, 150, 200, 240 and 480 ppm. These concentrations were chosen after estimates of the LD_{50} were obtained from another set of experiments using concentrations of 33, 100, 500 and 1000 ppm. The standard statistical method of analysing the relationship between a quantal response (alive/dead) and a stimulus (concentration of oil) is probit analysis. This method is available as a procedure in the statistical package GENSTAT (copyright 1997, Lawes Agricultural Trust, Rothamsted

Experimental Station). The model parameters are given in Table 5. Although there are no references available regarding volatile oils, terpinen-4-ol, carvone, camphor, limonene, menthone and citral have all been tested and showed relatively low toxicity if used between 500-1800ppm. The significance of the test lies mainly as an indicator of the possible antitumour activity of compounds (ideal LD_{50} being less than 40 ppm), or for use as an insecticide (ideal LD_{50} being around 1ppm). All five oils had LD_{50} between 107ppm (fennel) and 279 ppm (German chamomile) which indicates a comparatively high bioactivity. Further tests are required to assess specific activites of the oils and their individual components.

Volatile oils as plant protection agents and their antimicrobial activity

Volatile oils of many plants are known to have antimicrobial activity (Deans et al., 1992; Piccaglia et al., 1993). This activity could act as chemical defence against plant pathogenic diseases. Pathogens can readily penetrate at wound sites caused, for example, by herbivores. Wounding of leaves which are covered with volatile oil glands results in the rupture of glands causing the oil to flow over the wound. The existence, therefore, of antimicrobial activity in the oil, would be of considerable benefit to the plant. Indeed, a good majority of aromatic and medicinal plants do not succumb to many of the commonest diseases. It is also suggested that a complex oil presents a greater barrier to pathogen adaptation than would a more simple mixture of monoterpenes. This theory is well documented in the detailed study of Myrica gal*e* volatile oil and its inhibitory properties against a broad spectrum of fungal species (Carlton et al., 1992; Svoboda et al., 1998b). The complicated mixtures of monoterpenes and sesquiterpenes in the whole oil represented the strongest barrier to fungal infection.
Deans and Ritchie (1987) examined 50 plant volatile oils for their antibacterial properties against 25 genera of bacteria, using an agar diffusion technique. The main plant species and microorganisms investigated by other researchers over subsequent years using this same technique, with or without modifications, are listed in Tables 6 and 7. Volatile oils exhibited various reductions in growth of microorganisms, depending on the oil concentration and chemical composition. Food microorganisms (e.g. Salmonella enteritidis and Listeria monocytogenes) are of particular interest (Fyfe et al., 1998; Tassou et al., 1995).

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Table 1. The enantiomeric composition (%) and relative quantity of essential oil components in selected plant species (after Ravid *et al*, 1987; 1992a,b; 1994 a,b,c,d,e; 1996).

Species	Origin	Relative quantity of total oil	(4S)-(+) - piperitone	(4R)-(-)- piperitone
Mentha longifolia (L.)	Yizre'el Valley	2.4	99	1
Mentha longifolia (L.)	Negev	49.7	6.5	93.5
Mentha piperita (L.) Peppermint Black	Iden Croft Herbs	0.7	96	4
Mentha piperita (L.)	Romania	1.0	98	2
Mentha piperita (L.)	USA	0.1	87	13
Mentha piperita (L.)	USA	0.1	>99	tr
Mentha arvensis (L.)	Brazil	1.2	79	21
Salvia officinalis (L.)	Igalo, Dalmatia	3.9	11	89
Salvia officinalis (L.)	Trebinje, Dalmatia	0.9	16	84
Salvia fruticosa (Mill.)	NA	0.4	11	89
Lavandin, essence	France	3.0	97	3.0
Lavandin, oil	Israel	2.1	96	4.0
Lavender, essence	France	0.3	70	30
Lavender, oil	Switzerland	0.3	71	29
Coridothymus capitatus (L.)	NA	1.3	tr	>99
Origanum vulgare (L.)	Greece	0.5	2.0	98
Origanum syriacum (L.)	NA	0.5	5	95
Mentha longifolia (L.)	Negev	2.2	6.9	4.0
Artemisia arborescens (L.)	NA	0.2	24	76
Achillea fragrantissima (Forsk)	Negev	0.2	49	51
Chrysanthemum parthenium (L.)	NA	0.4	2	98
Tanacetum vulgare (L.)	NA	0.4	60	40
Rosmarinus officinalis (L.)	Israel	8.4	55	45
Rosmarinus officinalis (L.)	Greece	6.2	43	57
Rosmarinus officinalis (L.) var. Majorca	Iden Croft Herbs	0.8	4	96
Rosmarinus officinalis (L.) var. Tuscan Blue	NA	11.2	5	95
Rosmarinus officinalis (L.) var. Frimley Blue	NA	1.3	83	17
Rosmarinus officinalis (L.) var. Prostrate	NA	6.1	72	27

Table 1. cont. The enantiomeric composition (%) and relative quantity of essential oil components in selected plant species (after Ravid *et al*, 1987; 1992a,b; 1994 a,b,c,d,e; 1996).

Species	Origin	Relative quantity of total oil	(IR)-(+)-borneol	(IS)-(-)borneol
Salvia officinalis (L.)	Dubrovnik, Dalmatia	2.6	61	39

Species	Origin	Relative quantity of total oil	R-(-)- linalyl acetate
Salvia sclarea	Various	42.7-77.7	tr
Mentha citrata Ehrh	England	22.5	tr
Lavender	various	NA	tr
Lavandin	various	NA	tr

Species	Origin	Relative quantity of total oil	(S)-(+)-carvone	R-(-)-carvone
Carum carvi seeds	Newe Ya'ar	51.1-82.4	99	tr
Anethum graveolens seed	Newe Ya'ar	89.5	99	tr
Mentha spicata	USA	86.9	Tr	99
Mentha spicata	England	54.2	Tr	99

Table 2. List of aromatic plant species investigated for the presence of enantiomeric components (after Ravid et al., 1987; 1992a,b; 1994 a,b,c,d,e; 1996).

Andropogon jwarancusa Jones	Lavandin
A. jwarancusa Schult	Lippia adoensis
Abies concolor	Mentha arvensis var. piperescens
Andropogon fragrans	M. citrata Ehrh
Anethum graveolens L.	M. longifolia
Artemisia herba-alba	M. piperita L.
Asarum canadense	M. pulegium
Barosma pulchella	M. pulegium var. hirsute
Blumea balsamifera	M. spicata L.
Boronia citriodora	M. sylvestris
Calamintha incana (Sm) Heldr	M. tumija
C. nepeta	Micromeria abissinica
Carum carvi L.	M. biflora
Chrysanthemum parthenium	M. fruticosa L.
Citronella sp.	Myrtus communis
Citrus aurantium L.	Nepeta japonica
Coriandrum sativum	Ocimum canum
Coridothymus capitatus	Origanum vulgare
Cymbopogon senarensis Choiv	Orthodon perforatum Ohwi
C. winterianus	Pelargonium capitatum
Dryobalanops spp.	P. odoratissimum
Eucalyptus dives	P. tomentosum
E. radiata	Pinus palustris
Eucalyptus citriodora	P. sylvestris
Lavandula sp.	Salvia dominica L.

Table 3. Enantiomeres in essential oils (after Ravid *et al.*, 1987; 1992a,b; 1994 a,b,c,d,e; 1996).

Alpha terpineol	Linalol
(1R)-(+) - borneol	R -(-) - linalyl acetate
(1S)-(-) - borneol	Menthol
Camphor	(1S,4R)-(+) - menthone
(S)-(+) - carvone	(1R,4S) - (-) - menthone
(R)-(-) - carvone	Methyl acetate
(-) - citronellol	(4S)-(+) - piperitone
(+) - citronellol	(4R)-(-) - piperitone
Citronellyl acetate	(1R)-(+) - pulegone
Fenchone	(1S)-(-) - pulegone
Iso-menthol	Terpinen - 4 - ol
(1R,4R)-(+) - isomenthone	Verbenone
(1S,4S)-(-)- isomenthone	

Table 4. Individual components of essential oils from temperate regions investigated for their bioactivity.

Component	Plant species	Bioactivity	Reference
1,8-cineole	Hyssopus officinalis	Antimicrobial	Mazzanti et al., 1998
Anethol/estragole	Croton zehntneri	Antispasmodic Blocks neuromuscular transmission Increases myoplasmic calcium	Albuquerque et al., 1995 Coelho-de Souza et al., 1997
Anisaldehyde	Pimpinella anisum	Inhibition of oxidation of L-DOPA	Kubo et al., 1998
Beta asarone	Acorus calamus	Sedative Hypothermic	Zanoli et al., 1997
Carvacrol, thymol	Origanum sp.	Antimicrobial	Sivropoulou et al., 1996
Linalol	Hyssopus officinalis	Antimicrobial	Mazzanti et al., 1998
Linalol	Aeollanthus suaveolens	Sedative Hypnotic Hypothermic Anticonvulsant	Elisabetsky et al., 1995
Sabinyl acetate	Juniperus sabina	Anti-implantation effect	Pages et al., *1996*

Table 5. Toxicity study of five volatile oils using the brine shrimp bioassay.

	Estimates of	Model Parameters	LD_{50} Transformed Scale		LD_{50} - Original Scale
Volatile oil	LD_{50}	Slope	95% confidence interval	Point estimate	95% confidence interval
German chamomile	2.445 ± .043	6.923 ± 1.12	2.358 - 2.532	279	228 - 340
Fennel	2.029 ± .043	8.715 ± 2.17	1.942 - 2.116	107	88 - 131
Thyme	2.093 ± .043	6.923 ± 1.12	2.006 - 2.180	124	101 - 151
Roman chamomile	2.284 ± 0.50	6.923 ± 1.12	2.184 - 2.384	192	153 - 242
Lemon balm	2.216 ± .043	6.923 ± 1.12	2.129 - 2.303	164	135 - 201

Table 6. List of bacteria and fungi used in antimicrobial assays (Aromatic and Medicinal Plant Abstracts CAB 1995 - 1999).

Bacteria

Acinetobacter	Beneckea	Epicoccum	Listeria	Ramularia
Aeromonas	Brevibacterium	Escherichia	Micrococcus	Salmonella
Alcaligenes	Brocothrix	Erwinia	Moraxella	Septoria
Alternaria	Citrobacter	Flavobacterium	Mycobacterium	Serratia
Apiospora	Campylobacter	Klebsiella	Ovularia	Staphylococcus
Azotobacter	Clostridium	Lactobacillus	Proteus	Vibrio
Bacillus	Enterobacter	Leuconostoc	Pseudomonas	Yersinia

Fungi

Alternaria	Helminthosporium	Saccharomyces
Aspergillus	Macrophoma	Sclerotinium
Botrytis	Microsporum	Sporotrichium
Candida	Mucor	Trichoderma
Cryptococcus	Penicillium	Trychophytum
Fusarium	Rhizopus	

Table 7. List of aromatic plants from temperate regions used in experiments to demonstrate bioactivity (Aromatic and Medicinal Plant Abstracts, CAB 1995 - 1999).

Abies sp.	Helichrysum sp.	Ocimum sp.
Achillea sp.	Heracleum sp.	Origanum sp.
Acorus calamus	Houttuynia cordata	Pelargonium sp.
Allium cepa	Hyssopus sp.	Petroselinum crispum
Anethum graveolens	Inula helenium	Pimpinella anisum
Angelica archangelica	Lamium garganicum	Pinus sp.
Artemisia herba-alba	Lavandula sp.	Rosmarinus sp.
Cedrus deodora	Lippia multiflora	Salvia sp.
Chamaecyparis obtuse	Melissa officinalis	Satureja sp.
Cryptomeria japonica	Minthostachys verticillata	Sideritis sp.
Cypressus sempervirens	Mentha sp.	Tagetes erecta
Daucus carota	Monarda citriodora var citriodora	Thymus sp.
Foeniculum vulgare	Myrica gale	Verbena sp.