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Chemistry and Bioactivity of Essential Oils

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9.1 Introduction

Essential oils are natural, volatile, complex plant compounds, oily or lipid-like in nature and frequently characterized by a strong fragrance [1, 2]. They have a low solubility in water but are soluble in fats, alcohol, organic solvents and other hydrophobic substances and are generally liquid at room temperature. They are stored in specialized plant cells, usually oil cells or ducts, resin ducts, glands or trichomes (glandular hairs) [3, 4] and may be extracted from the leaves, flowers, buds, seeds, fruits, roots, wood or bark of plants by a variety of methods, including solvent and supercritical fluid extraction, expression under pressure, fermentation or enfleurage, but either low- or high-pressure [1] steam or hydro-distillation are used predominantly for commercial production [2, 5]. Essential oils make up only a small proportion of the wet weight of plant material, usually approximately 1% or less [3, 6]. The presence, yield and composition of essential oils may be influenced by many factors, including climate, plant nutrition and stress [7]. In commercial production settings, selection and breeding programmes are often instigated to improve yields and foster desired compositions [8].

Essential oils are also called ethereal oils, volatile oils, plant oils or aetheroleum but the term 'essential oil' will be used throughout this chapter. The 'essential' part of the term 'essential oil' is thought to be derived from a phrase attributed to Phillippus Aureolus Theophrastus Bombastus von Hohenheim (1491–1541), or Paracelsus as he became known, a Swiss physician who named the active component of a drug preparation 'quinta essentia' [2, 9, 10]. The term 'essential oil' groups together a wide range of chemical compounds on the basis of their historic use and method of extraction, usually steam distillation, and belies the variety and complexity of compounds found within them [11].

Some plant families are particularly well known for their oil-bearing species. These include Apiaceae (also known as Umbelliferae), Asteraceae (also known as Compositae), Cupressaceae, Hypericaceae (sometimes included as a subfamily of the Guttiferae/Clusiaceae), Lamiaceae (previously known as Labiatae), Lauraceae, Fabaceae (also known as Leguminosae), Liliaceae, Myrtaceae, Pinaceae, Piperaceae, Rosaceae, Rutaceae, Santalaceae, Zingiberaceae and Zygophyllaceae [4, 8].

Essential oils are often described as secondary plant metabolites. Traditionally, secondary plant metabolites have been all those compounds synthesized by the plant which do not appear to be essential for plant growth and development and/or those compounds without an obvious function [12]. They are also not universally synthesized in all plants. In contrast, primary metabolites are produced by all plants, usually have an obvious function and are part of the essential metabolic processes of respiration and photosynthesis [13]. This artificial and rather simplistic division is also naïve because the natural functions of many secondary

plant metabolites are unknown simply because they have never been investigated; this lack of evidence or knowledge is then interpreted as a lack of function [14]. Greater interest in and investigation of secondary metabolites in recent years has led to the discovery that they have roles in defence, signalling and as intermediates in secondary metabolism [15–17].

This chapter will focus on the chemistry of the compounds found in essential oils and the biological activity that they possess. To better illustrate the advances in our comprehension of this biological activity and the mechanisms by which they are exerted, the biological activity will focus on the antimicrobial and anticancer activities of essential oils and components, two areas in which research efforts have been especially focussed. A brief discussion of the applications of essential oils in pharmaceuticals and foods follows.

9.2 Chemistry of Essential Oils

Essential oils are not simple compounds or even simple mixtures of several individual compounds. They may contain up to approximately 100 components, although many contain about 20 to 60 [3, 6, 18, 19]. The compounds found in essential oils are from a variety of chemical classes, predominantly terpenes, but phenylpropanoids and other compounds also occur although at lesser frequency and often, but not always, in smaller proportions [20]. They are all hydrocarbons and their oxygenated derivatives, and they may also contain nitrogen or sulfur. They are generally low-molecular-weight compounds with limited solubility in water [21, 22].

The classification and nomenclature of essential oil compounds is complicated by the fact that many were isolated and studied before the instigation of systematic chemical nomenclature. Consequently, many are known by nonsystematic or trivial or common names [11]. These are sometimes but not always based on their source, such as eucalyptol, limonene, pinene and thymol, names which hint at historical botanical origins of these compounds. In terms of shedding light on their chemistry, the long history and widespread use of these nonsystematic names further obfuscates the chemical nature and characteristics of essential oils and their components.

9.2.1 Terpenes

Terpenes, also known as isoprenes, or terpenoids or isoprenoids when they contain oxygen, are the largest group of natural compounds, with over 30 000 known structures [4, 13, 23, 24]. The name ‘terpene’ comes from the fact that the first described members of this class were isolated from turpentine, the monoterpene-rich liquid obtained from the resin of various *Pinus* spp. [23].

Traditionally, terpenes have been regarded as polymers of isoprene (C_5H_8) joined together in a repetitive head-to-tail manner. This is largely a legacy of work by the German chemist Otto Wallach, who was the first to recognize that many terpene compounds could be hypothetically constructed in this fashion [12]. This concept, known as the isoprene rule, was the first step in rationalizing the enormous variety of terpenes, since it accounted for the structure of many, but not all, terpenes. However, head-to-head combinations also occur, as do tail-to-tail and head-to-middle combinations [12, 25, 26]. This variation in initial arrangement of the isoprene units coupled with the numerous rearrangements and

substitutions that can occur afterwards mean that the isoprene origins of the final compound are often obscured, or at the very least not obvious to the nonchemist. Furthermore, although terpenes may be viewed as polymers of isoprene, the biosynthesis of terpenes does not occur by the successive addition of single isoprene units. Leopold Ruzicka, recipient of the 1939 Nobel Prize in Chemistry, addressed many of the limitations of Wallach's isoprene rule when he proposed the biogenetic isoprene rule [4, 7, 27]. His concept was revolutionary since it emphasized the single biochemical origin of terpenes rather than the ultimate structure [12, 28] and this approach has proved the most practical.

Terpenes are classified by the number of isoprene units from which they were biogenetically derived [12], even though loss or addition of carbon atoms may have subsequently occurred [29]. Therefore, hemi-, mono-, sesqui- and diterpenes contain 1, 2, 3 and 4 isoprene units, respectively. Triterpenes and tetraterpenes contain 6 and 8 isoprene units, respectively. Monoterpenes are the most common terpenes found in essential oils, followed by sesquiterpenes. Many essential oils are composed mainly of monoterpenes and sesquiterpenes and their oxygenated derivatives [18, 19, 30–33].

9.2.1.1 Biosynthesis of Terpenes

The synthesis of terpenes in plants occurs via two distinct, mostly compartmentally separated, biological pathways [24]. The mevalonic-acid pathway was the first described and takes place mainly in the cytoplasm, endoplasmic reticulum and mitochondria, producing sesquiterpenes, sterols and ubiquinones predominantly [12, 24, 34]. Remarkably, the existence and role of the second pathway was confirmed and characterized relatively recently. Known as the nonmevalonic-acid or methyl–erythritol phosphate pathway, this pathway takes place in the plastids of plant cells and is largely responsible for the synthesis of hemi-, mono- and diterpenes, in addition to other higher terpenes not found in essential oils such as carotenoids and the phytols of chlorophyll [24, 34]. Separation of the two pathways for the purposes of terpene synthesis is not absolute; metabolites formed in one pathway and plant-cell compartment may cross over to the other pathway and compartment [24]. Both pathways produce isopentenyl diphosphate (IPP) and its isomer dimethyl-allyl diphosphate (DMAPP) [12, 35], the basic building blocks of terpenes. When combined in different ratios these precursors yield geranyl diphosphate (DMAPP + IPP), farnesyl diphosphate (DMAPP + 2 IPP) and geranylgeranyl diphosphate (DMAPP + 3 IPP), the main precursors of mono-, sesqui-/tri- and di-/tetraterpenes, respectively [12, 26, 34, 35]. Exceptions to this occur in the Apiaceae, Asteraceae and Lamiaceae families, where irregular monoterpenes, sesquiterpenes and diterpenes are derived from the head-to-head coupling of 2 DMAPPs, of DMAPP and GPP, and of 2 GPPs, respectively [26].

9.2.1.2 Monoterpenes

Monoterpenes are formed when two C_5 isoprene units are joined, yielding a skeleton with the molecular formula $C_{10}H_{16}$ [3]. Despite this initial simplicity, subsequent substitutions, cyclizations and/or isomerizations result in a remarkable number of monoterpenoid structures. Approximately 1500 monoterpenoids have been described [23], although not all occur in essential oils. Monoterpenes may be cyclic (that is, ring-forming) or acyclic (also known as linear), regular or irregular, and their derivatives include alcohols, esters, phenols, ketones, lactones, aldehydes and oxides [35].

Cyclic monoterpenes include the monocyclic, bicyclic and even tricyclic compounds. The rings are produced in a multistep process called cyclization by enzymes called monoterpene cyclases via the universal intermediate, α-terpinyl cation [4, 36, 37]. Cyclic monoterpenes that contain a benzene ring such as *p*-cymene are known as aromatic monoterpenes and are common components of many essential oils. In this context, the term 'aromatic' refers to the benzene ring, consisting of a ring of delocalized electrons. In many instances the benzene ring makes a significant contribution to the biological activity of the component and to the whole essential oil, especially when a hydroxyl group is attached to the ring, forming a phenol [38]. Use of the term 'aromatic' in this fashion should not be confused with the terms 'aromatic plants' or 'aromatic oils', which are often also used in discussions about medicinal plants and essential oils and refer to the aroma or fragrance of plants and oils.

Acyclic monterpenes found in essential oils may be regular, linear structures in which the head-to-tail arrangement of isoprene units is readily observed, such as the hydrocarbons β-myrcene or the *(E)* and *(Z)* isomers of β-ocimene. Note that the *(E)* and *(Z)* notation for stereoisomers supercedes the *cis–trans* notation for stereoisomers. The obsolete term 'geometric isomer' is strongly discouraged by the International Union of Pure and Applied Chemistry [39]. Other examples of acyclic monoterpenes commonly found in essential oils include geraniol, linalool and citronellol.

Monocyclic monoterpenes include the largest group of naturally occurring monoterpenes [4], those that arise from the *p*-menthane skeleton by cyclization of a regular acyclic monoterpenoid. Important monoterpenes in this group include limonene, α-terpinene, β-terpinene, γ-terpinene and terpinolene, as well as the aromatic hydrocarbon *p*-cymene and its hydroxylated derivatives thymol and carvacrol, both noted for their antimicrobial activity. Other notable compounds in this group are the carbonyls piperitone and pulegone.

The biogenesis of bicyclic monoterpenes occurs by the further cyclization of monocyclic monoterpenes. They may be further categorized on the basis of the skeleton from which they are derived, including bornane, carane, camphane, fenchane, pinane and thujane [7]. α-pinene and β-pinene are common important constituents of essential oils, particularly pine oils, and are bicyclic monoterpenes formed by intramolecular rearrangement of the universal intermediate α-terpinyl cation, producing the bicyclic structure. Alternative cyclizations of the terpinyl cation yield the bicyclic skeletons for the bornane-, camphane- and fenchane-type monoterpenes. Thujane monoterpenes come from either the terpinen-4-yl cation or from the sabinyl cation and include α-thujene, sabinene and α- and β-thujone. δ-3-carene, a carane-type bicyclic monoterpene, is a common component of various essential oils including those from *Pistacia lentiscus* L. and *Juniperus* spp. [40, 41]. Other important members of this group include the cyclic ethers 1,8-cineole, 1,4-cineole and ascaridol.

Tricyclic monoterpenes occur infrequently in essential oils compared to monocyclic and bicyclic monoterpenes. However, pinene oxide and tricyclene are two important examples found in essential oils.

Irregular monoterpenes also occur and fall into two categories. The first is the troponoids or substituted cycloheptane monoterpenes. These are thought to be formed by ring expansion of the *p*-menthane skeleton (forming a seven-membered ring structure) and oxygenation of the side chain(s) [42]. Many are found in the heartwood of trees in the Cupressaceae family of evergreen shrubs and trees [42, 43]. Examples include the thujaplicins (α-, β-and γ-isoforms) and nezukone. β -thujaplicin is also known as hinokitiol.

The second category of irregular monoterpenes is formed by joining isoprene units in the less common non-head-to-tail arrangements [12]. Compounds in this category include artemisia ketone, chrysanthemol and lavandulol. Many irregular monoterpenes are found in the genus *Artemisia* (Asteraceae) [44–46].

9.2.1.3 Sesquiterpenes

In terms of their frequency in essential oils, sesquiterpenes are the second most common, after the dominant monoterpenes. They are formed from the combination of three isoprene units, giving them the molecular formula $C_{15}H_{24}$. They are a structurally diverse group, all deriving from farnesyl pyrophosphate by various cyclization processes often followed by skeletal rearrangement [12]. Of the terpenoids found in essential oils, they are the most structurally diverse, with over 120 different skeletal types [4, 11]. Sesquiterpenes may be linear, branched or cyclic.

Acyclic sesquiterpenes feature in many essential oils and include the isomers nerolidol and farnesol and the α- and β- structural isomers of farnesene. *(E)* isomers occur more commonly in nature than *(Z)* isomers and *(E)*-nerolidol is also found in many commercially important essential oils, such as neroli oil from the flowers of *Citrus aurantium* [47]. Essential oils containing more than 90% *(E)*-nerolidol have been identified [48]. Farnesol is an important component of the commercially important rose flower essential oil [4] and of Australian sandalwood oil, *Santalum spicatum* [49]. Irregular acyclic sesquiterpenes have been identified in *Santolina* spp. (Asteraceae) [50, 51].

Cyclic sesquiterpenes may be mono-, bi- or tricyclic. Monocyclic sesquiterpenes include abscisic acid, α-bisabolene and its oxygenated derivatives, α- and β-bisabolol, both present at high levels in chamomile (*Matricaria chamomilla*) oils [52]. Bicyclic sesquiterpenes include eudesmol, widdrol, guaiol and the group known as azulenes. Azulenes are responsible for the blue colour of some essential oils, such as chamazulene in chamomile oil [52] and *Artemisia aborescens* oil [53]. The bicyclic caryophyllene is present in many essential oils, β-caryophyllene being the most common form, which may also occur as a major component [54, 55]. Cedrene and santalol are examples of tricyclic sesquiterpenes. Cedrene occurs in many essential oils, including various cedarwood oils derived from *Juniperus* spp., *Cupressus* spp. and *Cedrus* spp. [56, 57], while santalols are important constituents of sandalwood (*Santalum album*) oil [58].

9.2.1.4 Diterpenes

Most diterpenes in essential oils are formed by the head-to-tail combinations of four isoprene units followed by rearrangement and/or substitutions. They are very common and important components of plant resins [59] but are also found in small quantities in many essential oils. They have the general molecular formula $C_{20}H_{32}$ and so are much heavier than their mono- and sesquiterpenoid counterparts. Their heavier molecular mass relative to the mono- and sesquiterpenes means they require a greater amount of energy to be liberated from plant parts by steam distillation. Their recovery and the concentration obtained from essential oils increases with increasing steam-distillation times [4] and can be influenced by the extraction method. For example, supercritical CO_2 extraction of essential oils has been shown to increase the concentration of diterpenes recovered from essential oils [60]. As with monoterpenes and sesquiterpenes, they may be acyclic or cyclic.

Acyclic diterpenes include phytol. Phytol forms the hydrophobic side chain of chlorophyll and so is found in the leaves of all green plants [11,12]. It occurs in many essential oils [61–64]. Another important linear diterpene is plaunotol, the main component of the Thai medicinal plant *Croton stellatopilosus* (Euphorbiaceae; formerly known as *C. sublyratus*) [23,65].

A notable cyclic diterpene in essential oils is the monocyclic camphorene (also known as dimyrcene), a component of camphor oil from the tree *Cinnamonum camphora* (Lauraceae), more commonly known as the camphor laurel. Several isomers of camphorene are found in the essential oils distilled from the leaves and twigs of *P. lentiscus* [66] and from mastic gum derived from the same plant [67]. Bicyclic and tricyclic diterpenes also occur in essential oils. Bicyclic diterpenes found in essential oils fall largely into two structural groups, the labdanes and the clerodanes. Labdane representatives include manool and manoyl oxide while sclareol typifies the clerodane class of bicyclic diterpenes. These components can be found in essential oils such as those from *Salvia* spp. including *Salvia sclarea* or clarysage [68–70]. Tricyclic diterpenes that occur in essential oils include phyllocladene and 16-kaurene [4]. Phyllocladene constitutes a significant portion of essential oils from *Araucaria* spp. (up to 61%) from the Araucariaceae family and 16-kaurene constitutes 60% of the essential oil from the ancient Wollemi pine, *Wollemia nobilis*, from the same family [71].

Tetracyclic and pentacyclic diterpenes also occur in essential oils, although they are minor components [72,73].

9.2.1.5 Norterpenes

Carotenoids (C_{40}) are a class of higher terpenes based on eight isoprene units and are important in plants for several reasons, including their role in photosynthesis [74]. They do not occur in essential oils. However, they are relevant to oils because when their carbon backbone is cleaved, usually oxidatively, they yield a range of smaller compounds known as apocarotenoids [75,76]. The most common and widespread group of apocarotenoids occur when carotenoids are cleaved at the 9–10 position, yielding C_{13} products known as norterpenoids or norisoprenoids. These are important minor components of some essential oils, contributing particularly to aroma and flavour [75,77]. Examples include β-ionone, the violet-like aroma found naturally in *Boronia megastigma* [78,79], and β-damascone from *Rosa damascena* [80].

9.2.2 Phenylpropanoids

9.2.2.1 Biosynthesis of Phenylpropanoids

Continuing the biogenetic theme seen with the terpenoids, phenylpropanoids are grouped together on the basis of their common biosynthetic origin from the shikimic acid pathway. This pathway occurs only in microorganisms and plants, never in animals. This adds weight to the possibility that compounds affecting this pathway will have the selective toxicity and safety profile that is advantageous for their use in humans and other animals. The shikimic acid pathway is responsible for the synthesis of many of the phenolic compounds in plants and, beginning with glucose in plants, produces the aromatic amino acids phenylalanine, tyrosine and tryptophan [81]. Shikimic acid is one of the pathway intermediates and lends its name to the whole pathway. Phenylpropanoids arise from the aromatic amino acids

phenylalanine and to a minor extent tyrosine [12, 58, 82, 83]. They have a C_6C_3 skeleton composed of a six carbon aromatic ring with a three-carbon side chain. The aromatic ring is also known as a benzene ring. When the three-carbon side chain attached to the phenyl ring is shortened by two carbons, benzenoids are formed [84]. The term is often used to include phenylpropanoids [67, 85, 86].

9.2.2.2 Phenylpropanoids in Essential Oils

Only approximately 50 phenylpropanoids have been described. Phenylpropanoids occur in essential oils less frequently and usually less abundantly than terpenoids [1, 20, 58, 87]. However, some of the oils in which phenylpropanoids do occur contain significant proportions of them, such as the eugenol in clove oil, present at 70 to 90% of the oil [88], or the methyleugenol-rich chemotype of the root essential oil of *Anemopsis californica*, or yerba mansa, containing 59% methyleugenol [89]. Plant families in which phenylpropanoids occur more frequently include Apiaceae (Umbelliferae), Lamiaceae, Myrtaceae [90], Piperaceae [91] and Rutaceae [79].

Important phenylpropanoids include the hydroxycinnamic acids, anethole, chavicol, eugenol, and their methylated derivatives, estragol (methyl chavicol) and methyl eugenol, as well as the widely distributed cinnamaldehyde. Myristicin and dillapiole are two other phenylpropanoids that occur commonly in essential oils when phenylpropanoids are present [87, 91–94].

As seen with the terpenoids, the extraction method used to produce essential oils may influence their phenylpropanoid content [95, 96].

9.2.3 Sulfur and Nitrogen Compounds of Essential Oils

More rarely, a few compounds found in essential oils contain one or more sulfur or nitrogen molecules. The presence of sulfur in particular confers an often strong, characteristic odour [3, 97, 98]. Sulfur- and nitrogen-containing compounds occur mainly as aglycones or glucosinolates, or their breakdown products, which include isothiocyanates. Aglycones are the nonsugar portion of a glycoside, a compound made up of a sugar group, termed the glycone, joined to another group. Glucosinolates, historically known as mustard oil glucosides, are sulfur- or nitrogen-containing compounds formed from glucose and one of eight amino acids [99]. When endogenous plant enzymes called myrosinases act on glucosinolates to cleave the glucose group they leave an unstable aglycone which then rearranges to form various breakdown products, including isothiocyanates, thiocyanates and nitriles [99, 100]. It is these breakdown products which may be major constituents of essential oils, such as phenylacetonitrile which makes up 85.9% of the oil from *Lepidium meyenii* (Walp.) [101] and various isothiocyanates, which are the major constituents of mustard (*Brassica rapa*) seed essential oil [102–104]. The Brassicaceae family, with over 350 genera and 3000 species, is an important source of glucosinolates and isothiocyanates [100]. This includes the cruciferous vegetables, such as broccoli, cauliflower, brussel sprouts and various cabbages.

In addition to the isothiocyanates, cyanates and nitrile compounds, other nitrogen-containing compounds occasionally occur in essential oils. The seed oil of *Azadirachta indica* (neem) contains the nitrogen compounds 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine, 2,6-diethylpyridine, 1H-pyrazole, 1H-benzotriazole and dodecanamide. These

compounds were also detected in *A. excelsa* oil, although at lower levels. Notably, the first compound made up 11.7% of the *A. indica* oil [105], illustrating that although nitrogen compounds are usually present at very low concentrations, they may occasionally be major components of oils.

Methyl anthranilate is found in a variety of citrus oils, including lemon and mandarin oils [106], jasmine oil [107], and in the essential oil derived from the flowers of *Murraya exotica* L. (Rutaceae) [108]. Methyl *N*-methyl anthranilate is found in mandarin oil [106, 109] as well as oils from ylang-ylang [110] and the seeds of *Nigella damascena* and *N. sativa* [111]. It is the main component of mandarin petitgrain oil [112, 113]. The nitrogen-containing pyridines and pyrazines have been detected in oils including vetiver and black pepper [114, 115].

Oils from plants in the Alliaceae family are also particularly well known for sulfur-containing compounds; these include plants such as *Allium cepa* L. (onion), *Allium porrum* L. (leek) and *Allium sativum* L. (garlic), in which the sulfur compounds are responsible for the characteristic aroma and taste [116, 117]. Cysteine sulfoxides including alliin predominate in mature, intact *Allium* spp., along with γ-glutamyl cysteines [118]. Upon rupture, such as when chopped or pressed, the action of a class of enzymes known as alliinases catalyses the conversion of cysteine sulfoxides into the volatile thiosulfinates [101, 116] including allicin. Typically, allicin makes up 70 to 80% of the thiosulfinates. Allicin and other thiosulfinates quickly decompose to other compounds, including diallyl sulfide, diallyl disulfide, diallyl trisulfide, dithiins and ajoene, while the γ-glutamyl cysteines are converted to S-allylcysteine through a nonalliin/allicin pathway [118]. It is these end products rather than their precursors that may be found in essential oils.

Other sulfur-containing compounds have been detected, frequently at trace levels, in many essential oils. Mint sulfide has been identified in many oils important in the perfume industry, including peppermint, spearmint, pepper, ylang-ylang, narcissus, geranium, chamomile, davana [98] and rose oil [119], as well as cumin oil [120].

9.3 Biological Activity of Essential Oils

9.3.1 General Overview

Despite their history of being regarded as secondary, non-essential plant metabolites, it is becoming clear that essential oils and their components have specific biological functions [14,17,121], many of which lend themselves to commercial exploitation. Given the range and complexity of the compounds present in essential oils it is hardly surprising that they have the capacity to affect many biological systems. The biological activities of greatest interest centre around applications in health, agriculture and the cosmetic and food industries. In the arena of health and medicine the diverse array of biological properties now being characterized includes antimicrobial, anticancer, analgesic, antioxidant, antiinflammatory, other immunomodulatory and antiplatelet, and antithrombotic [122–126] activities. Along with fragrance and solvent properties, several of these activities also find application in the cosmetic and food industries. Of greatest interest in agriculture is the antimicrobial and insecticidal potential of essential oils and their components.

9.3.2 Antimicrobial Activity

Microorganisms such as bacteria, fungi, viruses and protozoa are the aetiological agents of many infectious diseases, and compounds with specific activity against these microorganisms, that is antimicrobial activity, are our best weapon for treating these diseases. Even before the role of microorganisms in disease pathogenesis was appreciated or understood, attempts at treating such illnesses often utilized plant-based medicines that contained compounds with antimicrobial activity. These plant-based medicines included essential oils. Interest in their use for the treatment of bacterial infections only seemed to wane significantly with the advent of first the antibacterial sulfur drugs in the early twentieth century and then β-lactam antibiotics and others beginning in the 1940s. Given that modern antibiotics had the advantage of selective toxicity and the capacity to be administered systemically and that many important pathogenic bacteria were exquisitely susceptible to them, it is no wonder that they soon became the primary means of treating bacterial infections and that the use of essential oils and other plant-based medicines diminished. Renewed recent interest in their use has been attributed to several factors, including a general renaissance in the appeal of 'natural' products, the desire for antimicrobial compounds with even better safety and toxicity profiles, and more importantly, the need for alternative agents due to the reduced susceptibility to conventional antimicrobial agents shown by many important pathogens.

Whatever the reasons for the apparently renewed interest, there are now hundreds of reports of the *in vitro* antimicrobial activity of essential oils in the scientific and medical literature, including reviews of the medicinal properties of some of the more popular oils such as clove [90], lavender [127], *Lippia* spp. [128] and tea tree [30]. This antimicrobial activity includes activity against bacteria, fungi, viruses and protozoa. Whereas typically these reports used to describe the activity of a single compositionally unspecified essential oil against one or two isolates of the microorganism of interest using nonstandard or *ad hoc* methods, increasingly they report on the activity of well-characterized essential-oil samples [129] or individual components against a wider range of genera and species [130], often testing larger number of isolates [131–133] using widely-applied or standardized methods. Reports of antibacterial and antifungal activity seem to dominate these reports, perhaps because of greater access to and simplicity of these methods. However, data on the activity of essential oils against viruses and protozoa are becoming more available.

The antimicrobial activity of essential oils can be attributed largely to the major groups of compounds found in them: monoterpenes, sesquiterpenes and nonterpenaceous components such as phenylpropanoids. Where they are present in significant proportions, sulfur compounds such as those found in *Allium* spp. are often the main antimicrobial compounds.

9.3.2.1 Antibacterial Activity

Both major groups of bacteria, Gram-positive and Gram-negative, have demonstrated susceptibility *in vitro* to essential oils and components. The methods used are usually disc-diffusion methods or agar- or broth-dilution methods [2, 5, 134–136]. In disc-diffusion methods, a paper disc impregnated with essential oil is laid on an inoculated agar medium and after incubation the diameter of the area around the disc in which bacteria were unable to grow is measured. Although disc-diffusion methods are popular the data they offer, in the form of zones of inhibition, are less useful than data from agar- and broth-dilution

methods. Furthermore, the diffusion of essential oils through agar, a fundamental aspect of disc-diffusion tests, is greatly compromised by the hydrophobic nature and limited aqueous solubility of essential oils. Agar- or broth-dilution methods, in which serial dilutions of the test oil in agar or broth media are inoculated with a known concentration of test organism, allow minimum inhibitory concentrations (MICs) to be determined. The MIC is generally defined as the lowest concentration of essential oil that inhibits growth of the test organism. Although solubilization of essential oils in these systems is still problematic, adequate solubilization or dispersion may be achieved through the use of low concentrations of surfactants or solvents. MICs are more useful than zones of growth inhibition since they can help establish safe and effective final concentrations in formulated products.

Most essential oils possess at least some degree of antibacterial activity. However, those attracting the most attention are the ones which inhibit or kill bacteria *in vitro* at concentrations below 1% vol/vol (10 000 ppm). Oregano (*Origanum* spp.), tea-tree (*Melaleuca alternifolia*), lemongrass (*Cymbopogon citratus*), lemon-myrtle (*Backhousia citriodora*) and clove (*Syzigium aromaticum*) oils are examples of essential oils that have activity against a wide range of Gram-positive and Gram-negative bacteria (with the exception of *Pseudomonas aeruginosa*) with MICs of less than 1% or approximately 10 mg/ml. It is worth noting here that this level of activity, while still potentially useful, is about 1000-fold lower than the activity of conventional antibiotics, for which MICs of susceptible bacteria are expressed in μg/ml quantities.

The *in vitro* susceptibility to essential oils of a wide range of bacteria has been tested, but those bacteria important in human health care [2,30,134] and the food industry [2,135,137] have been the focus of most investigations. In the human health-care field there has been particular interest in the susceptibility to essential oils of multidrug-resistant bacteria, such as the Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci, which cause serious infections. Table 9.1 summarizes the antimicrobial activity determined by broth- or agar-dilution methods of essential oils against MRSA. The number of publications and the range of essential oils tested typify the interest in the susceptibility of multidrug-resistant bacteria and bacteria in general. Although many essential oils may not be taken internally for the treatment of frank infections due to their systemic toxicity at the doses required to be antimicrobially effective, there is still interest in using them topically to prevent handborne transmission of pathogenic bacteria in skin antisepsis products.

Many investigations of the antibacterial activity of essential oils report greater antibacterial activity against one or other of the two major divisions of bacteria, namely Gram-positive and Gram-negative. In most cases greater essential oil activity against Gram-positive bacteria is claimed [138–144] and this has led to the notion that in general essential oils have greater activity against Gram-positive bacteria. Such generalizations are without sound basis [145] and compelling evidence for this apparent bias in activity is lacking since in many cases the differences in activity between the groups are insufficient to support such claims. In addition, the sample of bacterial genera tested often skews the results. For example, many investigations include in their data for Gram-negative bacteria the susceptibility of the Gram-negative bacterium *P. aeruginosa*. This bacterium is widely acknowledged as being highly resistant to many antimicrobial agents and its inclusion unfairly skews data in favour of the greater susceptibility of Gram-positive bacteria. Studies testing a larger number of essential oils against a wider variety of bacteria tend to identify no such pattern

Table 9.1 *The minimum inhibitory concentrations (MICs) of essential oils against methicillin-resistant* Staphylococcus aureus *(MRSA).*

Plant source	Essential oil (common name)	No. isolates	Method	MIC range	Reference
Allium odorum L.	Chinese leek	60	Broth microdilution	48 mg/l	[132]
Allium sativum L.	Garlic	60	Broth microdilution	32 mg/l	[132]
Backhousia citriodora	Lemon myrtle	1	Agar dilution	0.2%	[146]
Centaurea aladagensis	—	1	Broth microdilution	0.22 mg/ml	[63]
	—	3	Broth microdilution	8 μl/ml	[19]
Juniperus communis	Juniper berry	15	Broth microdilution	>2%	[147]
		1	Agar dilution	1.2 μl/ml	[148]
Lavandula angustifolia	Lavender	15	Broth microdilution	0.5%	[147]
Melaleuca alternifolia	Tea tree	64	Broth microdilution	0.25–0.312%	[149]
		100	Broth microdilution	0.32%, median	[131]
		28	Broth microdilution	0.25–0.5%	[150]
		15	Broth microdilution	0.25%	[147]
		30	Broth microdilution	0.25–2%	[151]
		1	Agar dilution	0.3%	[146]
		30	Broth microdilution	0.125–1%	[152]
		98	Broth dilution	512–2048 mg/l	[133]
		1	Broth dilution	0.125%	[153]
		4	Agar dilution	1.05 ± 0.29%	[143]
		4	Broth microdilution	1%	[154]

[57, 130, 155]. While the phenomenon may occur, there are only sufficient, convincing data in very few cases, such as manuka oil from the New Zealand native *Leptospermum scoparium* (Myrtaceae), which has activity against Gram-negative bacteria that is 32- to 64-fold lower than that against Gram-positive bacteria [156].

The antimicrobial activity of the complex chemical mixtures that constitute essential oils has led to attempts to identify and isolate the antimicrobially active components of these oils. In many cases, the component(s) or fraction(s) responsible for the antibacterial activity or for a large part of it have been identified, such as terpinen-4-ol in *M. alternifolia* (tea-tree) oil [157], carvacrol and thymol in oregano oil [158], and carvacrol and eugenol in *Eugenia caryophyllata* (clove) oil [90].

Most essential oils possess at least limited antibacterial activity, with some oils and components exhibiting a much greater degree of activity. Surveys of the antibacterial activity of essential-oil constituents have consistently indicated that aldehydes and phenolics tend to

exhibit greater antibacterial activity [159] than other types of constituents, often followed by the nonphenolic alcohols, with oxides and hydrocarbons having the least antibacterial activity [140, 143, 160–162]. More comprehensive analyses of structure–activity relationships have confirmed and expanded our knowledge of this trend. Griffin *et al*. [22] found that terpene acetates and hydrocarbons tended to exhibit the lowest levels of antimicrobial activity and were able to relate this to their limited hydrogen-bonding capacity and lower water-solubility. Higher levels of antimicrobial activity were associated with hydrogen-bonding parameters and, in the case of Gram-negative bacteria, smaller molecular size [22]. Subgroups such as the phenolics have also been subjected to such analyses [163], with hydrophobic factors being identified as the main determinant of antibacterial activity at least for the bacteria investigated [38].

9.3.2.2 Antifungal Activity

Essential oils and components also exhibit activity against fungi, activity that is becoming increasingly well described. A wide range of human, animal and agricultural fungal pathogens have been shown *in vitro* to be inhibited and/or killed by essential oils, heightening interest in their therapeutic or industrial application. Amongst the human and animal pathogens of interest, yeasts in the genus *Candida* and the dermatophytes *Epidermophyton*, *Microsporum* and *Trichophyton* have attracted the greatest interest, perhaps because the limited range and effectiveness of conventional antifungal agents fuels the search for novel therapies. In contrast to the pattern seen with bacteria, in which minimum inhibitory and cidal concentrations of oils are frequently the same or only one or two serial dilutions different [31, 130, 164, 165], the oil concentrations necessary to kill fungi are often much higher than those required to merely inhibit their growth [166].

There has been particular interest in the activity of essential oils and their components against food-spoilage fungi and essential oils and their components have been shown to inhibit the growth of many of them, including species of *Aspergillus*, *Microsproum*, *Mucor*, *Penicillium*, *Eurotium*, *Debaryomyces*, *Pichia*, *Zygosaccharomyces* and *Candida* [41, 135, 167–178]. However, one of the key issues with agents intended to preserve food is maintenance of the aroma, taste, colour and texture of the food. An undesirable effect of using essential oils or their components as food-preservation agents is that these organoleptic properties may be compromised at the concentrations required to inhibit microbial growth [135, 179–183]. More highly flavoured foods lend themselves better to preservation in this manner [2]. Alternatively, the use of lower concentrations of essential oils or components may be possible if multiple food-preservation strategies that result in additive or synergistic effects on antimicrobial activity are involved [184–186]. This approach fits well with the concepts of hurdle technology, in which multiple simultaneous preservation strategies are applied [187].

One application of the antibacterial and antifungal activity of essential oils and components in food preservation that has received particular interest is active packaging, in which oils or components are incorporated into the packaging. They may be included in the plastic or paper-based packaging itself or in the atmosphere contained within it. When incorporated into the atmosphere around bread, mustard oil and its primary antimicrobial component, allyl isothiocyanate, have been shown to effectively inhibit the growth of several bread-spoilage fungi, including *Aspergillus flavus*, *Endomyces fibuliger*, *Penicillium*

spp. and *Pichia anomala* [188, 189]. Mould and yeast counts on sweet cherries were significantly reduced in modified atmospheres containing eugenol, thymol or menthol but not eucalyptol [190].

9.3.2.3 Mechanisms of Antibacterial and Antifungal Action

While the spectrum and scale of the antimicrobial activity of essential oils are becoming better characterized, much less is known about the means by which they exert their activity. For many years, the precise mechanisms by which microorganisms were inhibited and/or killed remained unclear and were attributed largely to unspecified effects on microbial membranes or envelopes. Over the last decade or two, a deeper understanding has been gained of the precise effects of essential oils and their components on microorganisms. As long believed, many of the described effects involve interactions with biological membranes. However, the specificity and subtlety of many of these interactions is only beginning to be fully appreciated. Where the mechanisms of antimicrobial action of essential oils have been investigated, many varied and specific effects have been described. For example, in bacteria, carvacrol has been shown to cause collapse of the proton-motive force and depletion of the ATP pool, leading to death [159, 191–193], while tea-tree oil (*M. alternifolia*) and its major component terpinen-4-ol increase membrane permeability to potassium ions [194] and 260 nm-absorbing materials presumed to be nucleotides [157], and the phenylpropanoid cinnamaldehyde has been shown to interfere with the crucial bacterial division protein FtsZ, thereby preventing cell division [195]. Carvacrol has also been shown to inhibit the synthesis of flagellin, the protein that makes up flagella used for bacterial motility, in the important foodborne pathogen *Escherichia coli* O157 : H7 [196]. Specific effects on bacterial virulence factors, that is the products by which bacteria establish infection and produce disease, have also been identified. Examples include that cinnamaldehyde interferes with quorum sensing communication processes mediated by two different types of signalling compounds, acyl homoserine lactones and a group known collectively as autoinducer-2 (AI-2) [197, 198]; *Ocimum gratissimum* essential oil inhibits extracellular protease activity and cell-wall lipopolysaccharide expression [199]; eugenol inhibits listeriolysin O production [200]; and mint (*Mentha piperita*) essential oil reduces levels of staphylococcal enterotoxin B [201]. In fungi too, specific effects have been identified that compromise cell integrity [202], viability or virulence [203, 204]. These examples serve to illustrate that where specific effects of essential oils and components have been investigated, many have been identified, greatly enhancing our understanding of their mechanisms of action and undermining previous assumptions of generic 'cytotoxic effects'.

9.3.2.4 Antiviral Activity

For many years data on the antiviral properties of essential oils and their constituents lagged behind those for other microorganisms with respect to the range of oils and viruses tested and characterization of the mechanisms of action. This was reflected in the relatively few publications covering the subject. More recently, numerous publications have described the *in vitro* activity of a wide range of essential oils. The majority of *in vitro* studies have been conducted using the enveloped influenza or herpes simplex viruses 1 or 2 (HSV-1 or -2). Essential oils from *Artemisia glabella* [205], *Cynanchum stauntonii* [206], *Houttuynia cordata* [207], *Oenanthe crocata* [208], *Origanum acutidens* [176], *Salvia limbata*

and *S. sclarea* [209] and the component cinnamaldehyde [210] have been tested against influenza viruses. Oils from tea tree and eucalyptus [211], anise (*Illicium verum*), hyssop (*Hyssopus officinalis*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), chamomile (*Matricaria recutita*) and sandalwood (*S. album*) [212], *A. aborescens* [53,213], *H. cordata* [207], *L. scoparium* [214], *Melaleuca ericifolia*, *M. leucadendron* and *M. armillaris* [215], *Melissa officinalis* [216,217], *M. piperita* [218], *O. crocata* [208], *Salvia fructicosa* [219], *S. limbata* and *S. sclarea* [209], *S. album* [220], *Santolina insularis* [221, 222], a range of South American plants including *Aloysia*, *Artemisia* and *Lippia* spp. [223, 224], and the components eugenol [225, 226] and isoborneol [227] have been tested against HSV-1 and/or -2. Minami *et al.* [228] tested oils from *Cupressus sempervirens* (cypress), *Juniperus communis* (juniper), *M. alternifolia* (tea tree), *Ocimum basilicum album* (tropical basil), *M. piperita* (peppermint), *Origanum majorana* (marjoram), *Eucalyptus globulus* (eucalyptus), *Ravensara aromatica* (ravensara), *Lavandula latifolia* (lavender), *Citrus limonum* (lemon), *Rosmarinus officinalis* (rosemary) and *Cymbopogon citrates* (lemongrass) against HSV-1. Most of these essential oils have been evaluated by measuring the inhibition of plaque formation in tissue cultures of appropriate host cells, a widely used method of measuring antiviral activity. In general, the concentration of oils or components that reduced plaque formation by 50% ranged from 0.000 06 to 1%. Often the concentrations of oil that inhibit plaque formation are only marginally lower than the concentrations that prove cytotoxic to the tissue-culture cells, resulting in a comparatively low therapeutic index. In some instances, no antiviral activity is seen at concentrations that are noncytotoxic to the host cell line, such as when *Juniperus oxycedrus* ssp. *badia* oil was tested against two strains of human immunodeficiency virus (HIV) [229]. Nevertheless, interest in the potential of oils as antiviral agents persists, particularly for topical use such as hand and skin antisepsis.

Apart from the more widely tested influenza virus and HSV, dengue virus type 2 and Junin virus [223, 224], adenovirus and mumps virus [230], human respiratory syncytial virus [231], HIV [232], Newcastle disease virus [205], poliovirus [208], tobacco mosaic virus [233], two bacteriophage [234], yellow fever virus [235] and the viral aetiological agent of severe acute respiratory syndrome, a novel coronavirus [236], have also been tested against a range of oils and components with similar results.

With regard to the mechanism of antiviral action, in most cases where antiviral effects have been assessed before and after host-cell adsorption, the antiviral effect has occurred largely upon treatment of virus particles with oil prior to their adsorption or addition to cell monolayers. This suggests a direct effect of oil on free virus particles rather than an intracellular antiviral effect [211, 217, 218, 221, 228]. The site of action has not been identified but most of the viruses tested have been enveloped viruses, with the exception of adenovirus, poliovirus and tobacco mosaic virus. Viral envelopes are typically derived from the membrane of the host cell and have a phospholipid bilayer structure. Since many essential oils have the capacity to disrupt biological membranes, it follows that viral envelopes may also be disrupted by essential oils, a contention supported by electron micrographs of HSV-1 after treatment with oregano or clove oils showing envelope disruption [237].

In contrast to the growing body of *in vitro* data, there are very few reports of *in vivo* activity. Black-seed oil (*Nigella sativa*) was tested against murine cytomegalovirus [238] and *C. stauntonii* oil [206], *Heracleum* spp. oils [239] and cinnamaldehyde [210] were tested against influenza in mouse models. Studies in humans are also very limited. Tea-tree oil has been evaluated for the treatment of herpes labialis in a small pilot study with

promising results, suggesting a reduction in the time to complete healing with the use of tea-tree-oil ointment compared to control [240], and *B. citriodora* essential oil was trialled in the treatment of molluscum contagiosum, a cutaneous viral infection caused by the virus of the same name, in which lesions were resolved completely in 5 of 16 participants, reduced in number by more than 90% in 4 of 16 and reduced in number less than 90% in 6 of 16. One participant was lost to follow-up. In the vehicle placebo group 0 of 15 met the criteria of a 90% reduction in lesion number while 12 of 15 had no change or an increase in lesion number [241].

9.3.2.5 Antiprotozoal Activity

As with studies investigating the antiviral properties of essential oils and their components, data on the activity of essential oils against parasites such as protozoa have become increasingly available in the last decade. Protozoa are single-celled eukaryotic microorganisms and many oils have now been evaluated as antiprotozoal agents [242] with a view to applications in human and animal health care. The more complex life cycle of protozoa compared to bacteria and fungi complicates the determination of their susceptibility and most methods assess the susceptibility of one or two life cycle stages, such as the promastigotes and amastigotes of trypanosomal protozoa.

Leishmania spp. are the causative agents of leishmaniasis, a disease that manifests itself in a variety of presentations, and their susceptibility to several essential oils has been investigated, including *O. gratissimum* oil [243], *T. vulgaris* oil [244] and the components limonene [245], linalool [246], nerolidol [245] and terpinen-4-ol [244]. *Leishmania amazonensis* proved susceptible to the linalool-rich essential oil of *Croton cajucara* and to linalool with MICs of 85 and 22 pg/ml, respectively [246], and to *Chenopodium ambrosioides* oil with a promastigote MIC of 27.82 μg/ml [247]. The MIC of *C. ambrosioides* oil for *Leishmania donovani* promastigotes was 25 μg/ml [248].

Lemongrass (*C. citratus*) oil showed *in vitro* antitrypanosomal activity against *Crithidia deanei* [249], as did *O. gratissimum* oil against *Herpetomonas samuelpessoai* [250]. While both test organisms are considered nonpathogenic in vertebrates, they have proved to be useful models of trypanosomal infections important in humans. *Trypanosoma cruzi* were inhibited by lemongrass oil and citral [251], *Origanum vulgare* and *T. vulgaris* oils, and thymol [252] and *Achillea millefolium*, *Syzygium aromaticum* and *O. basilicum* oils, as well as eugenol and linalool [253]. Essential oil from the leaves of *Strychnos spinosa* inhibited *Trypanosoma brucei brucei*, but when tested alone two of its components, (*E*)-nerolidol and linalool, showed more potent and selective activity against the test organism [62].

The susceptibility of *Plasmodium* spp. has also been evaluated. A selection of oils from the Cameroonian medicinal plants *Xylopia phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, *Xylopia aethiopica* and *Hexalobus crispiflorus* inhibited the growth of *Plasmodium falciparum* [254] and farnesol, nerolidol, limonene and linalool inhibited parasite development and isoprenoid synthesis in *P. falciparum* [255]. Two *Lavandula* essential oils, including *L. angustifolia*, inhibited the human protozoan pathogens *Giardia duodenalis* and *Trichomonas vaginalis* and the fish pathogen *Hexamita inflata* [256]. *O. basilicum* oil, as well as linalool and to a lesser extent eugenol, two of its major components, demonstrated cidal activity against *Giardia lamblia* [257]. The susceptibility of *Histomonas meleagridis*, *Tetratrichomonas gallinarum* and *Blastocystis* sp.

to carvacrol, *Cassia* oil and an essential oil mixture containing thyme and rosemary oil was evaluated with minimal lethal concentrations of 0.1 to 0.75 μl/ml [258]. The minimum lethal concentrations of oils from *Cinnamomum aromaticum*, *Citrus limon* and *A. sativum* were determined for *T. gallinarum* and *H. meleagridis* and ranged from 0.125 to 1 μl/ml [259].

The limited *in vivo* work includes the assessment of *C. citratus* and *O. gratissimum* oils in a murine model of *Plasmodium berghei* infection in which both oils significantly suppressed parasitaemia [260]. In another mouse study, the intraperitoneal and oral routes of administration of *C. ambrosioides* oil prevented lesion development and retarded the course of infection with *L. amazonensis* compared to untreated mice [261]. Poultry experimentally infected with *Eimeria tenella*, the aetiological agent of caecal coccidiosis, whose diet was supplemented with oregano oil fared no differently from uninfected control group [262]. Human studies are rare but in one 14 adults with positive stool tests for one or more of the enteric parasites *Blastocystis hominis*, *Entamoeba hartmanni* and *Endolimax nana* received oregano oil daily for six weeks, eliminating the organisms in eight, four and one cases, respectively [263].

The mechanisms of antiprotozoal action are believed to be twofold. Direct effects of the essential oil on protozoa have been described, such as the disruption of flagellar membranes, mitochondrial swelling, and gross alterations in the organization of the nuclear and kinetoplast chromatins seen by electron microscopy after *L. amazonensis* promastigotes were treated with *C. cajucara* oil [246]. Monoterpenoids and sesquiterpenoids have been shown to exert antiprotozoal activity by interfering with the isoprenoid pathway present in protozoa, providing another rationale for their antiprotozoal effects [255].

Indirect effects of essential oils on the host immune system have also been described. *C. cajucara* oil can increase nitric oxide production by infected peritoneal macrophages, an important mechanism of intracellular parasite killing, but alone the major component of this oil, linalool (approximately 40% of the oil) [264], cannot [246]. Immunomodulatory effects that seem disadvantageous to the intracellular killing of parasites, such as the reduction of nitric oxide production by infected peritoneal macrophages, have also been described but have been shown to induce a cascade of events that results in parasite death [242].

9.3.3 Anticancer Activity

The sheer complexity of the processes involved in carcinogenesis not only confounds their elucidation and hampers attempts to find effective preventive or therapeutic interventions, but also provides a myriad of potential targets for chemopreventive or chemotherapeutic action. Key amongst these potential targets is the mevalonic-acid pathway in mammalian cells [265], the functioning of which is known to be modified by many isoprenoids found in essential oils. This is reflected in the diverse ways in which essential oils and their components have been demonstrated to possess anticancer properties. For example, one function of the mevalonic-acid pathway in mammalian cells is cholesterol synthesis. Cancer cells synthesize and accumulate cholesterol faster than normal cells and isoprenoid influences on the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase early in this pathway can prevent or inhibit tumour growth [266, 267]. Monoterpenes such as cineole, farnesol, geraniol, d-limonene, menthol and perillyl alcohol have been shown to

significantly reduce the activity [268, 269], synthesis [270] and degradation of HMG-CoA reductase [271, 272].

In vitro, many essential oils and their components have been shown to inhibit the proliferation of numerous cell lines representative of different cancers. Oils from *Casaeria sylvestris* and *Zanthoxylum rhoifolium* have been tested against cervical-, colon- and lung-cancer cell lines [273, 274], *Comptonia peregrina* against colon- and lung-cancer cell lines [275], *Curcuma wenyujin* against liver-cancer cells [276], *Cyperus rotundus* against a leukaemia cell line [277], *Eugenia zuchowskiae* against breast- and melanoma-cancer cell lines [278], *Juniperus excelsa* against breast-, colon-, epidermal-, lung- and prostate-cancer cell lines [279], *Photinia serrulata* against cervical-, lung- and liver-cancer cell lines [280], *Salvia libanotica* against colon-cancer cell lines [281], *Schefflera heptaphylla* against breast-, liver- and melanoma-cancer cell lines [282], *Schinus molle* against breast-cancer and leukaemia cell lines [283], *Tetraclinis articulata* against human melanoma-, breast- and ovarian-cancer cell lines [284], *Thymus broussonettii* against ovarian-cancer cell lines [285] and *Photinia serrula* against cervical-, liver- and lung-cancer cell lines [280].

Many isoprenoids that occur in essential oils have been shown to suppress the proliferation of cancer cell lines *in vitro*, including carvacrol, citral, *p*-cymene, farnesol, geraniol, *d*-limonene, nerolidol, perillyl alcohol, α-pinene, α-terpineol, thymol, verbenone and α- and β-ionone [267, 286–288]. Mechanistic studies of the effects of essential-oil compounds highlight the numerous ways in which these compounds have chemotherapeutic potential. For instance, the cyclic monoterpene perillyl alcohol, one of the most studied and most promising anticancer terpenoids, has been shown to arrest cell proliferation in the G1 phase at numerous different points [289–294], induce apoptosis (the programmed cell death considered desirable in cancer chemotherapeutic agents) [293, 295–299], suppress prenyl transferase activities [300] (preventing protein prenylation and in turn the signalling and oncogenic activities of proteins such as Ras that play a role in cell-growth-promoting signal transduction) [301, 302] and inhibit synthesis and activity of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme in mevalonate biosynthesis in mammalian cells [270], which is thought to suppress tumour cell growth and induce apoptotic cell death [267, 286]. Perillyl alcohol has also been demonstrated to suppress the statin-mediated upregulation of Ras protein and other G proteins [303–305], suppress the synthesis of G proteins [306] and affect signal transduction [307].

Isothiocyanates [100], thiosulfinates and other oil components derived from *Allium* spp. [308, 309] and to a lesser extent phenylpropanoids such as myristicin [310] or oils rich in them [89, 92] have also been investigated for their anticancer properties [311]. The anticancer properties of thiosulfinates are thought to be mediated by a number of mechanisms, including stimulation of the important hepatic detoxifying glutathione transferase enzymes, downregulation of cancer-promoting enzymes [312, 313], inhibition of proliferation [308, 314] and induction of apoptosis [315, 316].

Amongst the phenylpropanoids, eugenol has been identified as having anticancer effects [317–320], along with oils from *Pimpinella* spp. [321]. Cinnamaldehyde has received considerable attention, showing apoptotic activity mediated by the generation of reactive oxygen species and a caspase-dependent mechanism [322] and the ability to inhibit the development of mutagen-induced lung carcinogenesis in mice [323]. More recently, the activation of pro-apoptotic proteins and the family of mitogen-activated protein

kinases that play a role in cell signalling, particularly pathways involved in regulating cell survival, differentiation and apoptosis, have been implicated in the apoptotic activity of cinnalmaldehyde [324, 325]. The release of proteins from the mitochondrial membrane bilayer is known to play a crucial role in apoptosis, and permeabilization of the outer membrane can influence this [326]. Cinnamaldehyde has been shown to adversely influence several mitochondrial membrane functions [327] and may also exert its apoptotic effects in this manner.

Apart from their direct effects on the proliferation of cells, essential oils and their components may also exert anticancer effects through their antimutagenic, antiangiogenic, antiinflammatory [321, 328] or antioxidant properties. These properties may be effected by multiple mechanisms, once again highlighting the complexity of unravelling the effects of essential oils and components *in vitro* and *in vivo*. Antimutagenic properties can be due to altered cell permeability which prevents or inhibits mutagen penetration into cells, extracellular interaction between the antimutagen and the mutagen that results in physical, chemical or enzymatically-catalysed changes in mutagenicity, alteration of cellular mechanisms that results in mutagenicity, or effects on DNA repair [1, 329]. The phenylpropanoid cinnamaldehyde has been shown to possess modest antimutagenic activity through its influence on DNA repair processes [330]. It has also been shown to inhibit chemical and physical mutagenesis in bacterial and mammalian models and to reduce chromosomal aberrations in Chinese hamster ovary cells exposed to UV light and X-rays, possibly through the activation of recombinatorial repair [331, 332].

The formation of new blood vessels, or angiogenesis, is crucial for the progression of solid tumours, and inhibitors of this process can have potent chemotherapeutic effects in the treatment of cancer [333, 334]. In addition to other effects, perillyl alcohol has also been shown to be antiangiogenic in that it inhibited the growth of new vessels in the chorioallantoic membrane assay, inhibited endothelial cell proliferation and their organization into tube-like structures, and altered the production of angiogenic growth factors in a manner that encouraged vessel regression [335]. Inhibition of angiogenesis has also been reported for *d*-limonene [336], perillyl alcohol, farnesol, and geraniol [298] and mastic (*P. lentiscus* var. *chia*) oils [337].

Despite all the promise of *in vitro* work and *in vivo* animal models, early trials in humans using compounds such as perillyl alcohol to treat breast [338], colorectal [339], ovarian [340] and prostate [341] cancer have been disappointing. However, results from the treatment of some malignancies such as gliomas are worth pursuing [342] and interest in the therapeutic potential of this and other essential oil compounds remains, as does the chemopreventive possibilities for such compounds [343].

9.4 Uses of Essential Oils

9.4.1 Pharmaceutical Products

Combined with their established historical use as medicines, the range of biological activities demonstrated by essential oils and their components has naturally aroused great interest in their use as medicinal products. Consequently, essential oils are ingredients in medicinal products sold for a wide range of therapeutic applications. The range of products

into which essential oils are formulated, their indications and the claims made for these products vary considerably throughout the world, as do the laws governing their sale. In the United States herbal products designed for ingestion, including those containing essential oils, are classified as dietary supplements. As such they are usually exempt from the stricter regulatory requirements applicable to drugs and foods. In Australia, medicinal products containing essential oils for which therapeutic claims are made are regarded as pharmaceutical products. Under the Australian pharmaceutical regulatory framework, they may be classified as registered or listed products. Listed products must meet less stringent criteria than those in the registered products category and may make concomitantly smaller therapeutic claims [344, 345]. In the European Union, different regulatory approaches are taken in different member states, although the goal is harmonization across member states [345, 346]. It is hoped that once harmonized, essential oils formulated into therapeutic products may be approved for sale under a simplified procedure, without needing to fulfil the criteria for a full product license [346].

Over-the-counter (OTC) medicines in most Western countries are designed for the treatment of minor, self-limiting conditions and their symptoms. Essential oils are active ingredients or excipients in many OTC medicines. For example, eucalyptus oil is found in more than 100 OTC products [347] intended mainly for use in the treatment or management of symptoms of upper respiratory tract infections. Since these infections are usually mild and self-limiting, the therapeutic claims made for these OTC medicines are generally more modest than those that can be made for prescription products.

Worldwide, few if any pharmaceutical agents containing essential oils as the active ingredient have been fully licensed as drugs by fulfilling the requirements for safety and efficacy data that must be met by new, conventional pharmaceutical agents. The reasons for this are likely to be manifold, including limitations on the resources available to adequately research and document the safety and efficacy of such products. In most cases the intellectual property arising from such efforts could not be protected sufficiently to warrant the investment by private enterprise. Publicly funded research seems the most likely mechanism by which some essential-oil therapeutic products might eventually be evaluated and registered in the same manner as drugs.

9.4.2 Foods and Beverages

Essential oils and their constituents are widely used in many foods and beverages, primarily as flavouring agents [348, 349]. Citrus-peel essential oils are amongst the most important of these, including orange, lemon, mandarin, tangerine and grapefruit oils [350, 351]. Peppermint, cornmint, eucalyptus and citronella oils are other leading oils in terms of volume [351]. Amongst single constituents, one of the most important to the flavour industry is menthol [352].

The concentrations used in foods and beverages are generally low; in beverages levels are typically at or below 0.1% [349]. In foods in Europe, for example, eucalyptus oil is approved for use as a flavouring agent at 5 mg/kg or less and in confectionery at 15 mg/kg [353]. As discussed previously, the levels of essential oils that are used in foods are governed largely by their effect on the organoleptic properties of the food. Their presence in food may also contribute to preservation of the products, depending on the concentrations used and the interaction they have with other ingredients and preservation factors in the product [135].

9.5 Conclusions

The diversity of compounds that make up essential oils is becoming increasingly well characterized. Similarly, the spectrum of biological activity of essential oils and their components is beginning to be fully appreciated and understood. The challenge remains to further explore the range of biological effects of essential oils and their potential applications.

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