

Essential Oils of Aromatic Plants with Antibacterial, Antifungal, Antiviral, and Cytotoxic Properties – an Overview

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Key Words

Essential oils · Medicinal plants · Antimicrobial effects · Cytotoxic properties

Summary

The abundant use of anti-infective agents resulted in the emergence of drug-resistant bacteria, fungi, and viruses. To overcome the increasing resistance of pathogenic microbes, a variety of medicinal plants have been screened worldwide for their antimicrobial properties. The aim is to find new, effective antimicrobial agents with novel modes of actions. Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses. The progress of this expanding scientific field will be documented by the most important results published in the last decade.

Introduction

The indiscriminate use of antimicrobial agents has resulted in the emergence of a number of drug-resistant bacteria, fungi, and viruses. To overcome the increasing resistance of pathogenic microbes, more effective antimicrobial agents with novel modes of action must be developed. Medicinal plants used in traditional medicines to treat infectious diseases seem to be an abundant source of new bioactive secondary metabolites. Therefore, in the last few years, a variety of medicinal plants and plant extracts have been screened for their antimicrobial activity [1, 2]. Essential oils, derived from aromatic medicinal

Schlüsselwörter

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Zusammenfassung

Der übermäßige Gebrauch von Antiinfektiva führte zum Auftreten von resistenten Bakterien, Pilzen und Viren. Um die vermehrte Resistenz von Mikroben zu überwinden, sind weltweit verschiedene Arzneipflanzen auf ihre antimikrobiellen Eigenschaften untersucht worden. Das Ziel ist es, neue, wirksame Antiinfektiva mit neuartigen Wirkmechanismen zu finden. Ätherische Öle bestimmter Arzneipflanzen sind dafür bekannt, dass sie eine besonders gute Wirkung gegen Bakterien, Pilze und Viren aufweisen. Der Fortschritt auf diesem wachsenden Forschungsgebiet soll durch die wichtigsten Ergebnisse, die im letzten Jahrzehnt publiziert worden sind, dokumentiert werden.

plants (e.g. fennel (*Foeniculum vulgare*), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*)), have been reported to be active against Gram-positive and Gram-negative bacteria as well as against yeasts, fungi, and viruses. They are mixtures of different lipophilic and volatile substances, such as monoterpenes, sesquiterpenes, and/or phenylpropanoids, and have a pleasant odor. Furthermore, they are considered to be part of the preformed defense system of higher plants [3].

Whilst it is beyond the scope of the present survey to review this expanding scientific field extensively, its progress will be documented by the most important results published in the last decade.

Table 1. A selection of aromatic plants with antimicrobial active essential oils

Origin of essential oil	Bacteria Gram (+)	Bacteria Gram (-)	Yeasts, y	Fungi, f	MIC, µg/ml	References
<i>Allium sativum</i>			y	f	64.0	[4]
<i>Artemisia douglasiana</i>	+	-	y		156–625	[5]
<i>Commiphora mukul</i>	+	-			0.31–5% of oil	[6]
<i>Cryptomeria japonica</i>			y	f	EC50: 39–110	[7]
<i>Foeniculum vulgare</i>	+	-			0.25–2.0% of oil	[8]
<i>Juniperus communis</i>	+	-			1.0–2.0% of oil	[8]
<i>Lavandula angustifolia</i>			y		0.69–1.8% of oil	[9]
<i>Melaleuca alternifolia</i>			y		0.03–0.125	[10]
<i>Mentha arvensis</i>	+	-		f	400–800	[11]
<i>Mentha spicata</i>	+	-			400–800	[11]
<i>Nigella sativa</i>	+		y	f	2500	[12]
<i>Peumus boldus</i>	+	-	y		0.9–58.0	[13]
<i>Pimpinella anisum</i>			y	f	0.78–1.56% of oil	[14]
<i>Salvia sclarea</i>				f	EC50: 493–584 µl/l	[15]
<i>Tagetes patula</i>			y	f	1.25–10.0 µl/ml	[16]
<i>Thymbra capitata</i>			y	f	0.08–0.32 µl/ml	[17]
<i>Thymus pulegioides</i>			y	f	0.16–0.64 µl/ml	[18]
<i>Ziziphora clinopodioides</i>	+	-			3,750	[19]

MIC = Minimum inhibitory concentration; Gram(+) = Gram-positive; Gram(-) = Gram-negative; EC₅₀ = effective concentration of the test compound which inhibit the growth of fungus by 50%.

Medicinal Plants with Antibacterial and Antifungal Essential Oils

During the last decade, a variety of essential oils have been screened to assess their antimicrobial activity (table 1). The antimicrobial activity of plant-derived essential oils formed the basis of many applications, especially in food preservation, aromatherapy, and complementary medicine.

Essential Oils with Anti-Helicobacter Activity

Helicobacter pylori is a Gram-negative bacterium that colonizes the epithelial surface of gastric mucosa. Nowadays, there is no doubt that *H. pylori* is a major etiological agent of acute and chronic gastritis. The role of the bacterium in the pathogenesis of peptic ulcer as well as in the development of adenocarcinoma of the distal stomach has been well-established. To cure a *H. pylori* infection, a combined treatment of proton pump inhibitor with two antibiotics has shown to be successful. Since antibiotic resistance has developed, it is also necessary to find new agents against this type of bacterium as alternatives to existent antibiotics or as adjuvant agents in combination with established and still effective antibiotics.

Recently, isolated plant substances (e.g. alkaloids, flavonoids, polysaccharides) as well as plant extracts have been shown to be effective against *H. pylori*. In the last decade, several research groups have investigated essential oils from different plant origin for their anti-*Helicobacter* activity using a broth microdilution/macrodilution method (table 2).

All essential oils tested exhibited a high anti-*Helicobacter* activity in vitro with MIC/MBC values of 20.0–589.4 µg/ml. Of all essential oils tested, carrot (*Daucus carota*) seed oil was the

Table 2. Essential oils with antibacterial activity against *Helicobacter pylori* (values in italics indicate MBC values)

Origin of essential oil	MIC/MBC, µg/ml	References
<i>Daucus carota</i>	20.0	[20]
<i>Cinnamomum zeylanicum</i>	40.0	[20]
<i>Satureja montana</i>	40.0	[20]
<i>Matricaria recutita</i>	35.7–70.4	[21]
<i>Nepeta argolica</i>	64.0	[22]
<i>Citrus aurantium</i>	65.1	[21]
<i>Mentha spicata</i>	50.0–100.0	[11]
<i>Zingiber officinale</i>	65.4–130.9	[21]
<i>Eugenia caryophyllus</i>	100.0	[20]
<i>Mentha arvensis</i>	100.0	[11]
<i>Nepeta camphorata</i>	128.0	[22]
<i>Melissa officinalis</i>	135.7	[21]
<i>Mentha piperita</i>	135.7	[21]
<i>Salvia officinalis</i>	137.6	[21]
<i>Rosmarinus officinalis</i>	137.0	[21]
<i>Leptospermum scoparium</i>	140.0	[21]
<i>Elettaria cardamomum</i>	130.0–278.0	[21]
<i>Thymus vulgaris</i>	275.2	[21]
<i>Coriandrum sativum</i>	259.3	[21]
<i>Foeniculum vulgare</i>	288.3	[21]
<i>Carum carvi</i>	273.1	[21]
<i>Ocimum basilicum</i>	286.7–573.4	[21]
<i>Illicium verum</i>	294.7–589.4	[21]
<i>Melaleuca alternifolia</i>	539.0	[21]

MIC = Minimum inhibitory concentration; MBC = minimum bactericidal concentration.

most active one with an MBC value of 20.0 µg/ml. Moreover, recent studies reported the in vivo (e.g. mice and rats) efficiency of different essential oils against antibiotic-susceptible and

-resistant *H. pylori* strains. It was also of interest that the bactericidal activities of the essential oils tested were enhanced at acidic pH values [20, 23, 24]. Some scientists speculate that the anti-*Helicobacter* activities of several essential oils are relevant if one intends to use them as food supplement to complement standard therapy [20].

Tea Tree (*Melaleuca alternifolia*) Oil (TTO) with Anti-*Mycoplasma pneumoniae* Activity

Mycoplasmas are bacteria without a rigid cell wall. Their physiological habitats are plants and animals but in various circumstances they may become pathogenic for humans, too. *Mycoplasma pneumoniae* is spread all over the world. It frequently causes atypical courses of pneumonia, particularly in children between 5 and 15 years and adults between 30 and 35 years. As a result of lung inflammations, myocarditis, arthritis, polyneuritis, and other chronic diseases may appear. Tetracyclines and macrolides are the preferred antibiotics in the treatment of mycoplasmal infections. However, in recent years bacterial strains emerged with a resistance to macrolide antibiotics.

The most common morphological shape of *M. pneumoniae* is the typical 'pear shape' with a tip structure at one end of the cell. There are specific protein filaments inside the tip structure which form the cytoskeleton [25]. When *M. pneumoniae* was treated with 0.006% TTO in ethanol (1%) for 12 h, the cells lost their typical 'pear-shaped' appearance and became rounded. The rounded shape resembles mutants which have lost their virulence as a result of this morphological change and the loss of their attachment site. TTO seems to affect the intracellular cytoskeletal structure in a way that *M. pneumoniae* cells become rounded and lose their virulence. On the

other hand, the integrity of the cell membrane was not impaired by TTO [25].

In a recent in vitro experiment, Furneri et al. [26] exposed 25 clinically isolated strains from vagina, urethra, cervix, 1 reference strain of *Mycoplasma hominis*, 1 clinically isolated strain, 1 reference strain of *M. pneumoniae*, 4 clinically isolated strains (from vagina), and 2 reference strains of *Mycoplasma fermentans* to TTO. The MIC values were determined by a broth microdilution assay (table 3).

All *Mycoplasma* species tested revealed, independently of their origin, a high susceptibility against TTO in vitro.

Antibacterial Activity against Bacteria from the Respiratory Tract

Essential oils are traditionally used for the treatment of respiratory tract infections due to their secretolytic and secretomotoric properties. Therefore, essential oils are either inhaled by steam, applied by inunction to the chest, or administered orally.

Bacterial respiratory tract infections develop in many cases from viral infections as common colds and include tonsillitis, sinusitis, bronchitis, and pneumonia. The bacteria most frequently isolated from the respiratory tract are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes*. Therefore, it is of interest to focus

Table 3. Susceptibility of different *Mycoplasma* species against tea tree (*Melaleuca alternifolia*) oil

Bacteria	MIC, %
<i>Mycoplasma hominis</i> (26 isolates)	0.06–0.12
<i>Mycoplasma fermentans</i> (6 isolates)	0.01–0.06
<i>Mycoplasma pneumoniae</i> (2 isolates)	0.01

Table 4. Anti-microbial activity of various essential oils against bacteria of the respiratory tract (MIC values in µg/ml)

Origin of essential oil	<i>S. pneumoniae</i>	<i>S. pyogenes</i>	<i>H. influenzae</i>	<i>M. catarrhalis</i>	References
<i>Thymus vulgaris</i>	800	200	200	–	[27, 28]
	3.13*	6.25*	3.13*	–	
<i>Cinnamomum verum</i>	6250	12500	12500	–	[29]
	400	200	200	–	[27, 28]
	3.13*	6.25*	3.13*	–	[27, 28]
<i>Melissa officinalis</i>	6250	6250	6250	–	[29]
	139	557	278	139	[30, 31]
<i>Nepeta cataria</i>	332	1329	664	332	
<i>Cymbopogon citratus</i>	800	400	800	–	[27, 28]
	6.25*	6.25*	1.56*	–	[27, 28]
<i>Mentha piperita</i>	3200	1600	800	–	[27, 28]
	25*	25*	12.5*	–	[27, 28]
<i>Melaleuca alternifolia</i>	3200	3200	1600	–	[27, 28]
	25*	50*	50*	–	[27, 28]
<i>Eucalyptus radiata</i>	3200	>3200	–	–	[27, 28]
	25*	50*	50*	–	[27, 28]
<i>Syzygium aromaticum</i>	12500	12500	12500	–	[29]

*MIC-values in gaseous phase.

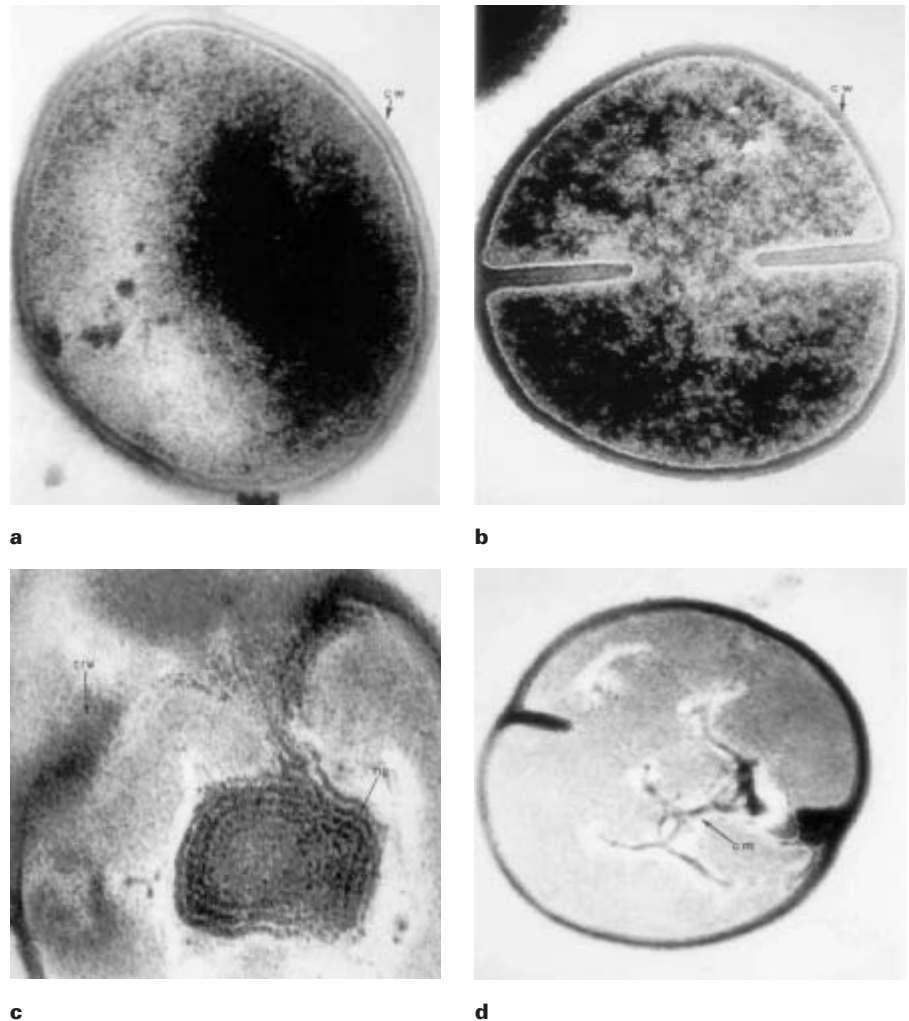


Fig. 1. **a** Normal coccoidal *S. aureus* cell; cw = cell wall, **b** Dividing *S. aureus* cell with cross wall formation; cw = cell wall, **c** TTO(0.12%)-treated *S. aureus* cell with a lamella-like membrane (= mesosome), **d** TTO(0.25%)-treated *S. aureus* cell with condensed material (= cm).

on the susceptibility of these bacteria to essential oils. Table 4 gives an overview on the MIC values of different oils tested. Especially *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were susceptible in vitro to lemon balm (*Melissa officinalis*) oil, thyme (*T. vulgaris*) oil, cinnamon bark (*Cinnamomum verum*) oil, and lemon grass (*Cymbopogon citratus*) oil. The oils of peppermint (*M. piperita*) and eucalyptus (*Eucalyptus globulus*) frequently used for the treatment of colds displayed lower activity. Interestingly, in gaseous phase, concentrations of 1.56–6.25 µg/ml of the most active oils were sufficient to inhibit bacterial growth, so that an antibacterial effect on inhalation might be plausible [27, 28].

Investigations on the antibacterial activity of essential oil components displayed similar results: most active groups of constituents were monoterpene alcohols and aldehydes as well as phenols and cinnamaldehyde with MIC-values of 160–300 µg/ml to both *S. pneumoniae* and *H. influenzae* [27, 28, 32].

Tee Tree Oil Activity in Staphylococcus aureus Cells – an Electron Microscopic Study

Staphylococcus aureus is one of the most important Gram-positive bacteria in humans, causing localized or generalized

septic infections. Methicillin-resistant strains (MRSA) usually colonize the anterior nares of hospital patients and healthy individuals and cause epidemics in hospitals.

TTO is successfully used worldwide in nursing, in skin care cosmetics, and to treat certain local bacterial and fungal infections [33–35]. TTO could be shown to reveal a high antibacterial activity against *S. aureus* in vitro and in vivo [36, 37]. To learn more about the mode of action, we studied the biological effect of TTO on cell ultrastructures, such as cytoplasm, cytoplasmic membrane, and cell wall using electron microscopy [38].

S. aureus belongs to the micrococcus family forming round to oval cells (fig. 1a) which are usually arranged in clusters. Untreated bacteria displayed normally dividing staphylococci cells (fig. 1b) and a sharp delineation between cell wall, cytoplasmic membrane, and the cytoplasm. In addition, cytoplasm revealed an evenly granular distribution. After 12 h exposure to a subminimum inhibitory concentration (sub-MIC) of 0.12% TTO, neither cell shape nor cell wall and cytoplasmic membrane revealed any irregularities or alterations. In contrast, cell division of the bacterial cells seemed to be interrupted, and, in the cytoplasm, lamellar-like membrane rods were seen (fig. 1c). The formation of lamellar-like membrane

Table 5. Cell targets and physiological effects of selected essential oils

Targets	Bacteria/Fungi	Substances	References
<i>Cell morphology</i>			
Forming elongated filamentous forms after treatment with essential oil; normal cells: 3–5 µm in length; elongated cells: 10–25 µm in length	<i>E. coli</i>	palmarosa oil; peppermint oil	[44]
Alteration of cell shape: cells of wild type exhibit a flask-shaped morphology, whereas TTO-treated strains form ovoid or round cells.	<i>M. pneumoniae</i>	TTO	[25]
<i>Cytoplasmic membrane (alteration of integrity and permeability)</i>			
Inhibition of cell respiration	<i>E. coli</i> ; <i>S. aureus</i> ; <i>C. albicans</i>	TTO	[35, 40, 41]
Inhibition of oxygen uptake, respiratory electron flow and oxidative phosphorylation	<i>R. sphaeroides</i>	thymol, carvacrol and other monoterpene alcohols	[45]
K ⁺ leakage	<i>E. coli</i> ; <i>S. aureus</i>	TTO; farnesol, nerolidol	[40, 41, 46, 47]
Depletion of intracellular ATP concentration	<i>E. coli</i> , <i>L. monocytogenes</i> ; <i>E. coli</i>	oregano oil, cinnamon oil, savory oil; carvacrol, thymol	[48, 49]
Formation of multilamellar, mesosome-like structures	<i>S. aureus</i>	TTO; terpinen-4-ol	[38, 50]
Changes in membrane permeability	<i>Candida albicans</i> , <i>C. glabrata</i> , <i>Saccharomyces cerevisiae</i>	TTO; terpinen-4-ol; α-terpineol; 1.8-cineol; γ-terpinene; α-terpinene	[34]
Changes in membrane fluidity	<i>Candida albicans</i> ; <i>C. glabrata</i> ; <i>S. cerevisiae</i>	TTO; 1.8-cineol; terpinen-4-ol; α-terpinene	[34]
Lesion of cytoplasmic membrane; reduction of ergosterol content in the cell membrane	<i>C. albicans</i> , <i>Aspergillus fumigatus</i>	<i>Thymus pulegioides</i> oil	[18]
<i>Cell wall</i>			
Formation of extracellular blebs	<i>E. coli</i>	TTO; lemongrass oil	[51, 52]
Disintegration of outer membrane (OM) and OM-associated LPS release	<i>E. coli</i>	thymol, carvacrol	[48]
Cell lysis	<i>S. pneumoniae</i> ; <i>E. coli</i> , <i>B. subtilis</i>	oregano oil, thyme oil; oregano oil, clove oil	[33, 53]
<i>Cell division</i>			
Total inhibition of cell division	<i>S. aureus</i>	TTO	[38]
<i>Anti-R-plasmid activity</i>			
Elimination of R-plasmids	<i>E. coli</i>	peppermint oil, rosemary oil, eucalyptus oil; menthol	[54]
<i>Cell cytoplasm/cytosol</i>			
Formation of condensed, filamentous, electron-dense material in the cytoplasm/cytosol	<i>S. aureus</i>	TTO	[38]
Bacteria: <i>Bacillus subtilis</i> (<i>B. subtilis</i>), <i>Escherichia coli</i> (<i>E. coli</i>), <i>Staphylococcus aureus</i> (<i>S. aureus</i>), <i>Streptococcus pneumoniae</i> (<i>S. pneumoniae</i>), <i>Rhodospseudomonas sphaeroides</i> (<i>R. sphaeroides</i>), <i>Listeria monocytogenes</i> (<i>L. monocytogenes</i>), <i>Mycoplasma pneumoniae</i> (<i>M. pneumoniae</i>).			
Fungi: <i>Candida albicans</i> (<i>C. albicans</i>).			
Essential oil: Palmarosa oil: <i>Cymbopogon martini</i> oil; peppermint oil: <i>Mentha piperita</i> oil; TTO: <i>Melaleuca alternifolia</i> oil; oregano oil: <i>Origanum vulgare</i> oil; cinnamon oil: <i>Cinnamomum verum</i> oil; savory oil: <i>Satureja montana</i> oil; lemon grass oil: <i>Cymbopogon citratus</i> oil; clove oil: <i>Syzygium aromaticum</i> oil; rosemary oil: <i>Rosmarinus officinalis</i> oil; eucalyptus oil: <i>Eucalyptus globulus</i> oil.			

or so-called mesosome-like membrane structures seems to be an expression of general cell damage. This observation indicates that there is a lasting effect of sub-MIC of 0.12% TTO on cell physiology.

These findings correspond very well to experiences with antibiotics. Ultrastructural changes in bacterial cells produced

by antibiotics at MIC levels differ clearly from those obtained by antibiotics at sub-MIC [39]. After 12 h of incubation of the bacterial cells with the MIC of 0.25% TTO, dramatic cellular alterations became visible on electron microscopic image: Cell division was inhibited completely, in cytoplasm a clear segregation of previously distributed cell components was found,

new fibrous electron-dense structures were seen, and lamellar-like membrane rods were no longer visible (fig. 1d).

Interestingly, there was no shrinkage or busting of bacterial cells in the MIC dose range; furthermore, neither the cell walls nor the cytoplasmic membranes seemed to be destroyed by TTO.

In a similar electron microscopic study, *Escherichia coli* cells cultivated in the presence of TTO (MIC: 0.25%) showed a loss of electron-dense material, coagulation of cell cytoplasm as well as the formation of extracellular blebs. In addition, Cox et al. [40, 41] could demonstrate that TTO concentrations that inhibit the growth of *E. coli* and *S. aureus* also inhibited cell respiration, stimulated the leakage of intracellular K⁺ ions, and altered the permeability of the bacterial cytoplasmic membrane.

Mode of Antimicrobial Action

While essential oils were extensively tested against a broad spectrum of bacteria, yeasts, and fungi, the interaction between essential oils and microbes which ultimately induces the antimicrobial activity is not well understood. Hitherto, different target sites and modes of action are discussed (table 5). Previously, Takaisi-Kikuni et al. [42] studied the effect of various amounts of the essential oil of *Cymbopogon densiflorus* on the metabolic activity, growth, and morphology of *S. aureus*. Relatively high concentrations of the oil impaired staphylococcal growth in a bacteriostatic manner (chloramphenicol-type), and in low doses metabolism became ineffective due to energy losses in the form of heat. Ultrastructural data revealed morphological changes characteristic of the induction of bacteriolysis by bactericidal antibiotics (penicillin-type). Hammer et al. [34] investigated the antifungal effects of tea tree (*M. alternifolia*) oil and several of its components on *Candida albicans*, *Candida glabrata*, and *Saccharomyces cerevisiae*. TTO and its components were reported to alter both permeability and membrane fluidity of the yeasts tested. Based on these results, it was assumed that the essential oils may have antimicrobial activity by influencing bacterial and fungal targets involved in cytoplasmic and cell wall metabolism. It is stated by several researchers that especially monoterpenes will increase cytoplasmic membrane fluidity and permeability, disturb the order of membrane embedded proteins, inhibit cell respiration, and alter ion transport processes [32, 43].

Essential Oils with Antiviral Properties

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new antiviral drugs, since the chemical diversity provides unmatched availability [55]. Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases. Infectious viral diseases remain an im-

portant worldwide problem, since many viruses have resisted prophylaxis or therapy longer than other microorganisms. At the moment, only few effective antiviral drugs are available for the treatment of viral diseases. There is a need to find new substances with not only intracellular but also extracellular antiviral properties. The methods commonly used for the evaluation of in vitro antiviral activities of synthetic and natural substances are based mainly on the inhibition of cytopathic effects, the reduction or inhibition of plaque formation, and the reduction in the virus yield, but also on other viral functions in selected host cell cultures.

Inhibition Activity against Different Human Viruses

There is considerable evidence emerging from in vitro studies and controlled trials of the potential of plant-derived phytoantiviral agents for the treatment of human viral infections. Many essential oils were investigated towards their antiviral activity. Most of them were tested against enveloped RNA and DNA viruses, such as herpes simplex virus type 1 and type 2 (DNA viruses), dengue virus type 2 (RNA virus), Junin virus (RNA virus), and influenza virus (RNA virus), whereas only few essential oils, e.g. oregano (*Origanum vulgare*) oil and clove (*Syzygium aromaticum*) oil, were also tested against non-enveloped RNA and DNA viruses, such as adenovirus type 3 (DNA virus), poliovirus (RNA virus), and coxsackievirus B1 (RNA virus).

Herpes simplex virus type 1 (HSV-1) causes some of the most common viral infections in humans, such as mucocutaneous herpes infections, herpetic keratitis, herpetic encephalitis, and neonatal herpes. Following primary infection, the particles of HSV-1 are carried by retrograde transport via sensory nerve endings to the ganglia, where the virions remain in a latent state until the development of reactivation by different stimuli. Acyclovir, a nucleoside analogue and selective anti-herpetic agent which has been widely used for therapy, inhibits the viral DNA replication through viral thymidine kinase, resulting in a potent inhibition of viral DNA synthesis. However, acyclovir-resistant herpesviruses have been increasingly isolated, particularly from immunocompromised hosts, such as patients with AIDS or malignancy, and recipients of bone marrow or organ transplantation [56, 57].

The antiviral activity of the essential oils tested could be clearly demonstrated for enveloped DNA and RNA viruses (table 6). In contrast, the non-enveloped viruses were not affected by essential oils. A high antiviral effect of several essential oils against acyclovir-resistant clinical isolates of herpes simplex virus has been demonstrated recently [58].

Mode and Mechanism of Antiviral Action

The best candidates as clinically useful antiviral drugs are substances which act on specific steps of viral biosynthesis. They inhibit specific processes in the viral replication cycle,

Table 6. Antiviral activity of essential oils against different human viruses

Origin of essential oil	Virus	IC ₅₀ , %; ppm	References
<i>Aloysia gratissima</i>	HSV-1	65 ppm	[59]
<i>Artemisia arborescens</i>	HSV-1/HSV-2	2.4/4.1 µg/ml	[60]
<i>Artemisia douglasiana</i>	HSV-1	83 ppm	[59]
<i>Cinnamomum verum</i>	HSV-1	0.008%	[61]
<i>Citrus limon</i>	HSV-1	0.0015%	[62]
<i>Cymbopogon citratus</i>	HSV-1	0.1%	[63]
<i>Eucalyptus globulus</i>	HSV-1/HSV-2	0.009/0.008%	[64]
<i>Eupatorium patens</i>	HSV-1	125 ppm	[59]
<i>Hyssopus officinalis</i>	HSV-1/HSV-2	0.0001/0.0006%	[62, 65]
<i>Illicium verum</i>	HSV-1/HSV-2	0.004/0.003%	[62, 66]
<i>Juniperus oxycedrus</i>	HSV-1	0.02%	[67]
<i>Lavandula latifolia</i>	HSV-1	1%	[63]
<i>Leptospermum scoparium</i>	HSV-1/HSV-2	0.0001/0.00006%	[68]
<i>Matricaria recutita</i>	HSV-1/HSV-2	0.00003/0.00015%	[62, 66]
<i>Melaleuca alternifolia</i>	HSV-1/HSV-2	0.0009/0.0008%	[64]
<i>Mentha piperita</i>	HSV-1/HSV-2	0.002/0.0008%	[69]
<i>Origanum majorana</i>	HSV-1	1%	[63]
<i>Pinus mugo</i>	HSV-1/HSV-2	0.0007%	[62, 66]
<i>Rosmarinus officinalis</i>	HSV-1	1%	[63]
<i>Santalum album</i>	HSV-1/HSV-2	0.0002/0.0005%	[62, 65]
<i>Santolina insularis</i>	HSV-1/HSV-2	0.0001%	[70]
<i>Tessaria absinthioides</i>	HSV-1	105 ppm	[59]
<i>Thymus vulgaris</i>	HSV-1/HSV-2	0.001/0.0007%	[62, 65]
<i>Zingiber officinale</i>	HSV-1/HSV-2	0.0002/0.0001%	[62, 65]
<i>Artemisia douglasiana</i>	DEN-2	60 ppm	[59]
<i>Eupatorium patens</i>	DEN-2	150 ppm	[59]
<i>Origanum vulgare</i>	NDV	0.025%	[71]
<i>Laurus nobilis</i>	SARS-CoV	0.012%	[67]
<i>Lippia junelliana</i>	Junin virus	20 ppm	[59]
<i>Lippia turbinata</i>	Junin virus	14 ppm	[59]

IC₅₀ = 50% inhibitory concentration; HSV = herpes simplex virus (DNA virus); DEN = dengue virus (RNA virus); NDV = Newcastle disease virus (DNA virus); SARS = severe acute respiratory syndrome; SARS-CoV = SARS-associated coronavirus (RNA virus); Junin virus (RNA virus).

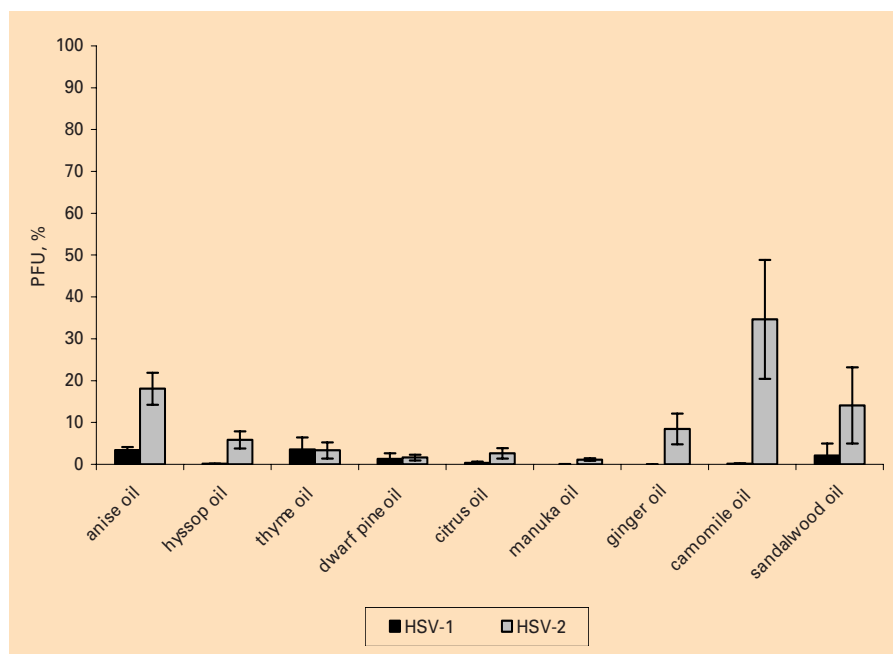
so that little or no viral progeny is produced. These antiviral drugs should act at low concentrations and should not influence the host cell machinery, prevent the spread of viruses, and ultimately cure infected cells. On the other hand, virucidal drugs denature viral structural proteins or glycoproteins, thus, infectivity of virus particles is completely lost. To learn more about the antiviral mechanism of essential oils on enveloped viruses, we investigated exemplarily the antiviral activity of anise (*Pimpinella anisum*) oil, hyssop (*Hyssopus officinalis*) oil, thyme (*T. vulgaris*) oil, dwarf-pine (*Pinus mugo*) oil, citrus (*Citrus limon*) oil, manuka (*Leptospermum scoparium*) oil, ginger (*Zingiber officinale*) oil, camomile (*Matricaria recutita*) oil, and sandalwood (*Santalum album*) oil against HSV-1 and HSV-2 in vitro. The replication cycle of herpes simplex virus is characterized by a complex sequence of different steps which offers opportunities to antiviral agents to intervene. In order to determine the mode of action, essential oils were added to host cells (African green monkey kidney cells) and viruses at different times during

viral infection to identify the stage and target site at which infection might be inhibited,

- (i) host cells (African green monkey kidney cells) were pre-treated for 1 h with essential oils prior to inoculation with herpesviruses (pretreatment cells),
- (ii) herpesviruses were incubated with essential oils for 1 h prior to infection of host cells (pretreatment viruses),
- (iii) herpesviruses were mixed with essential oils and added to the host cells immediately (adsorption),
- (iv) host cells were incubated with essential oils after penetration of herpesviruses into the cells (intracellular replication).

Inhibition of HSV replication was measured by a plaque reduction assay as described previously [64]. In this assay, the number of plaques (pfu; plaque forming units) of drug-treated viruses were expressed in percent of the untreated control (number of plaques formed by viruses in the absence of essential oil). In all assays the maximum noncytotoxic concentrations of the essential oils tested were used.

Fig. 2. Effect of different essential oils on herpes simplex virus type 1 and type 2 infectivity after pretreatment of viruses for 1 h with maximum noncytotoxic concentrations of essential oils. Untreated controls were arbitrarily set to 100%. Data represent remaining decreased viral infectivity compared to untreated herpesvirus. PFU = Plaque forming unit. Anise oil: *Pimpinella anisum* oil; hyssop oil: *Hyssopus officinalis* oil; thyme oil: *Thymus vulgaris* oil; dwarf pine oil: *Pinus mugo* oil; citrus oil: *Citrus limon* oil; manuka oil: *Leptospermum scoparium* oil; ginger oil: *Zingiber officinale* oil; camomile oil: *Matricaria recutita* oil; sandalwood oil: *Santalum album* oil.



According to our findings, pretreatment of cells with essential oils for 1 h prior to virus infection did not reduce the virus plaque formation, indicating that essential oils did not affect the adsorption of viruses to cell surface, and did not interfere with virus binding by blocking cellular receptors. On the other hand, pretreatment of viruses with essential oils for 1 h prior to cell infection caused a significant reduction of plaques of 95–99% for HSV-1 and of 70–98% for HSV-2, respectively (fig. 2). Out of the oils tested, only dwarf-pine (*P. mugo*) oil and citrus (*C. limon*) oil reduced plaque formation of about 80% for HSV-1 and HSV-2 when added during adsorption of virus to host cells [62, 65, 68].

In contrast, when essential oils were added to the overlay medium after penetration of viruses into the host cells, only manuka (*L. scoparium*) oil significantly reduced plaque formation of HSV-1 of about 40%. Only few reports demonstrated a virucidal activity of essential oils against herpesviruses. Saddi et al. [60] recently demonstrated the virucidal effect of *Artemisia arborescens* essential oil against HSV-1 and HSV-2.

In conclusion, our results indicate that in particular free viruses are very sensitive to essential oils. Both types of herpes simplex virus are affected before adsorption or during adsorption to cell surface but not after penetration into cells, the typical mode of action of nucleoside analogues like acyclovir. These findings suggest that essential oils interfere with the virus envelope or by masking viral components which are necessary for adsorption or entry into host cells. An electron microscopic examination demonstrated that the envelope of HSV-1 was disrupted when treated with oregano (*O. vulgare*) oil and clove (*S. aromaticum*) oil [71]. Furthermore, eugenol (4-hydroxy-3-methoxy-allyl-benzene), the main component of clove oil, was shown to be a very effective agent against

HSV-1 and HSV-2 in vitro [72]. All these findings are in accordance with the data of investigations on the antiviral activity of essential oils against enveloped DNA and RNA viruses mentioned above.

Clinical Trials

A randomized, placebo-controlled, investigator-blinded protocol was used to evaluate the efficacy of tea tree essential oil (6% TTO gel) in the treatment of recurrent herpes labialis [73]. The median time to re-epithelization after treatment with this essential oil was 9 days compared to 12.5 days after placebo, indicating some benefit from essential oil treatment. TTO might be a potentially useful, cheaper alternative for other topical therapies, which poses little threat of inducing resistance to antiviral agents. Besides essential oils, many other herbal preparations with antiviral activity were identified, clinical trials have been performed, and most of them described benefits for the treated patients [74]. There remains a need for larger, stringently designed, randomized clinical trials with essential oils to provide conclusive evidence of their efficacy.

Summary

In summary, there is considerable evidence emerging from in vitro studies and controlled clinical trials of the potential of plant-derived substances as leads for the development of antiviral drugs against viral infections. In particular, the antiviral properties of essential oils from several plant extracts, responsible for their characteristic odor, have been described in recent years. Various viruses, including the human pathogen herpes simplex virus, were found to be very susceptible to the inhibitory action of essential oils. These results support the potential use of essential oils in toto from medicinal plants

Table 7. Cytotoxicity of essential oils in vitro

Origin of essential oil	Cell line	Incubation time, h	CC ₅₀ , µg/ml	References
<i>Backhousia citriodora</i> (lemon myrtle)	F1-73	4	140	[75]
	dermal epithelial cells	24	100	
	dermal fibroblasts	4	120	
		24	70	
	HepG2 (liver carcinoma)	4	80	
		24	40	
<i>Commiphora molmol</i> (myrrh)	gingival fibroblasts	24	≥25	[76]
		48	5–10	
	gingival epithelial cells	24	≥25	
		48	5–10	
<i>Lavandula angustifolia</i> (lavender)	HMEC-1 (microvascular endothelial cells)	1	1950	[77]
	HNDF (dermal fibroblasts)	1	1840	
	153BR (fibroblasts)	1	1690	
<i>Lavandula stoechas</i>	KB (epidermoid carcinoma)	n.a.	>20	[78]
	KB-V (drug resistant KB)		>20	
	BC-1 (mamma carcinoma)		>20	
	Lu-1 (lung carcinoma)		>20	
	Col-2 (colon carcinoma)		9.8	
	LNCaP (prostate carcinoma)		17.6	
	P388 (murine leukemia)		>5	
3T6 (fibroblasts)		286.8		
<i>Syzygium aromaticum</i> (clove)	HMEC-1 (see above)	1	180	[79]
	HNDF	1	250	
	153BR	1	170	
<i>Melissa officinalis</i> (lemon balm)	HEp-2 (epidermoid carcinoma)	48	>100	[80]
<i>Melissa officinalis</i>	A549 (lung carcinoma)	48	20–100	[81]
	Caco-2 (colon carcinoma)			
	MCF-7 (mamma carcinoma)			
	HL-60 (leukemia)			
	K562 (leukemia)			
	B16F10 (murine)			
<i>Melissa officinalis</i>	HaCaT (keratinocytes)	48	15.3	[30]
	BEAS-2B (bronchial epithelial cells)	48	10.6	
<i>Nepeta cataria</i> (catnip)	HaCaT (keratinocytes)	48	165.7	
	BEAS-2B (bronchial epithelial cells)	48	161	
<i>Santalum album</i>	RC-37 (monkey kidney)	96	15	[62]
<i>Matricaria recutita</i>			30	
<i>Leptospermum scoparium</i>			35	
<i>Pinus mugo</i>			40	
<i>Zingiber officinale</i>			40	
<i>Citrus limon</i>			45	
<i>Melaleuca alternifolia</i>	RC-37	96	60	[64]
<i>Thymus vulgaris</i>	RC-37	96	70	[62]
<i>Hyssopus officinalis</i>			75	
<i>Mentha piperita</i>	RC-37	96	140	[69]
<i>Illicium verum</i>	RC-37	96	160	[62]
<i>Eucalyptus globulus</i>	RC-37	96	300	[64]

CC = Cytotoxic concentration; CC₅₀ = effective concentration of the test compound which kills 50% of the cells tested; n.a. = not applicable.

as agents for the treatment of viral infections and suggests the application of this type of natural products as disinfectants or topical antiviral drugs.

Cytotoxicity and Its Consequences on the Antibacterial and Antiviral Properties of Essential Oils

Cytotoxicity of Essential Oils in vitro

The pharmaceutical market offers a wide range of drug products for topical application that contain essential oils. The use of essential oils as antimicrobial agents is not only limited by their effective concentrations in vitro but also by the concentrations that can be obtained at the site of action. These depend on the one hand on resorption and transport of the active constituents but on the other hand on the maximum dosage that can be administered without toxic side effects.

Regarding essential oils a number of investigations in cell culture systems have been carried out in order to predict their toxicity to mammalian cells in vivo (table 7). Most of the in vitro tests were carried out using human fibroblasts or dermal epithelial cells, but also tumor cell lines were used. Others use monkey kidney cells as they are also suitable for antiviral tests. Essential oils exert cytotoxic activity in vitro at CC_{50} values from 5.0–1,950 $\mu\text{g/ml}$ depending on incubation time (1–96 h). In comparison, to achieve an antibacterial effect in vitro, essential oil concentrations of about 20–20,000 $\mu\text{g/ml}$ are required. That means that essential oils may exert cytotoxic effects to tissue cells at concentrations which do not yet show an antibacterial effect. On the other hand, essential oils exhibit antiviral activity even at IC_{50} values of 1–200 $\mu\text{g/ml}$.

The cytotoxic activity of essential oils is based on their individual components. As in bacterial cells, the cell membrane is one of the sites of action where essential oils and essential oil components were shown to cause permeabilization and depolarisation and to reduce the activity of membrane-associated enzymes [82]. In addition, an interaction with cellular metabolism [82, 83] and an induction of apoptosis have been demonstrated for essential oils and oil components [84–86].

Implications for Therapeutic Use of Essential Oils

In vitro cytotoxicity data may overestimate the toxicity of a substance in vivo, as neither tissue structures nor biotransformation and transport processes may be simulated in cell culture. Most cell culture models use a cell monolayer which is brought in direct contact with the test substance in culture medium and incubated for up to 96 h – these test conditions display the ‘worst case’, which is unlikely to occur in vivo. Correlations of in vitro and in vivo toxicity data have been carried out in order to develop models that allow a prediction of systemic toxicity in vivo from cell culture data. One example is the system of Halle and Göres [87] (table 8).

According to this classification, the expected systemic toxicity of most essential oils can be rated as moderate to low. Clinical studies about the topical use of essential oils (e.g. TTO, eucalyptus oil, and pine (*Pinus sylvestris*) needle oil) demonstrate that they are tolerated well both when used inhalatively or when applied to the skin in topical formulation. Adverse effects that were reported are local irritation on skin and mucous membranes as well as allergic reactions including contact dermatitis [35–37, 88, 89]. However, the ingestion of a few milliliters of essential oils may cause severe symptoms of intoxication like vomiting, respirational failure, and unconsciousness and may lead to death especially when infants are concerned [89]. From in vitro cytotoxicity data and the reports about toxicity and irritation potential in vivo, it is recommended strictly not to exceed the maximum daily dosage, when administered orally. In addition, the undiluted oils or preparations with high concentrations of essential oils should not be applied to mucous membranes or damaged skin. For inhalational use, the oils should be dosed in a way that they are barely detectable by odor as for many oils these concentrations were shown to facilitate secretolysis in an animal model most effectively in comparison to higher doses [90]. In addition, it was shown on ciliated nasal epithelium that low concentration of essential oil (0.2%) stimulated the ciliary beat frequency more effectively than high concentrations (2%) [91]. This is in accordance to the findings of Riechelmann and co-workers [92], who reported a decrease of ciliary beat frequency in human ciliated

Table 8. In vitro classification of cytotoxicity and prediction of in vivo toxicity of substances according to Halle and Göres [87]

Classification	CC_{50} , mmol/l	CC_{50} , $\mu\text{g/ml}$; for MW = 200	LD_{50} (oral, rat/mouse), mg/kg
1 Highest level of toxicity	<0.0001	<0.02	>5
2 Extremely toxic	0.0001–0.001	0.02–0.2	5–50
3 Highly toxic	0.001–0.01	0.2–2.0	50–500
4 Moderately toxic	0.01–0.1	2.0–20	500–5000
5 Little toxic	0.1–1.0	20–200	5000–15000
6 Very little toxic	1.0–10	200–2000	>15000
7 Non-toxic	>10	>2000	–

CC_{50} = Effective concentration of the test compound which kills 50% of the cell lines; LD_{50} = lethal dose; LD_{50} is the amount of a compound/material which causes the death of 50% of test animals; MW = molecular weight.

respiratory cells when exposed to air concentrations of more than 5 g/m³ of menthol, eucalyptus oil, or pine needle oil.

In order to evaluate benefits and risks of application of essential oils, it has to be taken into account that they are not primarily used as antibacterial/antiviral agents. When used at concentrations below their MIC, they may as well exert rubefacient, local anaesthetic, spasmolytic, antiphlogistic, secretolytic,

or secretomotoric effects, which altogether contribute to their therapeutic efficacy.

Conflict of Interest

The authors declared no conflict of interest.

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