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Essential oils of aromatic plants with antifungal, antibacterial, antiviral, and cytotoxic properties – an overview

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Abstract
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 here by the most important results published in the last decade.

Keywords: Essential oils, Medicinal plants, Antimicrobial effects, Cytotoxic properties.

Introduction
The indiscriminate use of antimicrobial agents has resulted in the emergence of a number of drug-resistant fungi, bacteria, 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. (Cowan, 1999) [5]. Essential oils derived from aromatic medicinal plants (e.g. fennel (*Foeniculum vulgare*), peppermint (*Mentha piperita*), and 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 the 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 (Reichling, 1999) [15]. Whilst it is beyond the scope of the present survey to review this expanding scientific field extensively, its progress has been documented by the most important results published in the last decade.

Medicinal Plants with Antifungal and Antibacterial 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

Table 1: A list of aromatic plants with antimicrobial active essential oil

Origin of essential oil	Bacteria Gram (+)	Bacteria Gram (–)	Yeasts, (y)	Fungi(f)	MIC(µg/ml)
Allium sativum			y	f	64
Artemisia douglasiana	+	–	y		156–625
Commiphora mukul	+	–			0.31–5% of oil
Cryptomeria japonica			y	f	EC50: 39–110
Foeniculum vulgare	+	–			0.25–2.0% of oil
Juniperus communis	+	–			1.0–2.0% of oil
Lavandula angustifolia			y		0.69–1.8% of oil
Melaleuca alternifolia			y		0.03–0.125
Mentha arvensis	+	–		f	400–800
Mentha spicata	+	–			400–800
Nigella sativa	+		y	f	2500
Peumus boldus	+	–	y		0.9–58.0
Pimpinella anisum			y	f	0.78–1.56% of oil
Salvia sclarea				f	EC50: 493–584 µl/
Tagetes patula			y	f	1.25–10.0 µl/ml
Thymbra capitata			y	f	0.08–0.32 µl/ml
Thymus pulegioides			y	f	0.16–0.64 µl/ml
Ziziphora clinopodioides	+	–			3,750

*Note: 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%.

microdilution/macrodilution method. 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 (Ohno *et.al*. 2003; Tzakou *et.al*. 2003) [14-23]. 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 (Bergonzelli *et al*. 2003) [2].

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 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. pneumonia* 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 (Harkenthal *et al.* 2000; Furneri *et al*. 2006) [8-6]. 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. All *Mycoplama* 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 on the susceptibility of these bacteria to essential oils. 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 (Inouye *et al.* 2001) [9].

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. Takaisi-Kikuni *et al.* (1996) [22] 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.* (2004) [7] 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 cytoplasmatic 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 (Reichling *et al.* 2006; Sikkema *et al.* 1994) [17-18].

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 (Jassim *et al.* 2003) [10]. 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 important 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 phyto-antiviral 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 oil (*Origanum vulgare*) and clove oil (*Syzygium aromaticum*) 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 herpes viruses have been increasingly isolated, particularly from immune compromised hosts, such as patients with AIDS or malignancy and recipients of bone marrow or organ transplantation (Bacon *et al.* 2003 ; Morfin *et al*. 2003) [1-13].

The antiviral activity of the essential oils tested were demonstrated for enveloped and non-enveloped DNA and RNA viruses results showed that, 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 (Schnitzler *et al*. (2007) [20].

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, 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, viricidal 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, Schnitzler *et al.* (2001) [19] investigated exemplarily the antiviral activity of anise oil (*Pimpinella anisum)*, hyssop oil (*Hyssopus officinalis)*, thyme oil (*T. vulgaris)*, dwarf-pine oil (*Pinus mugo)*, citrus oil (*Citrus limon)*, manuka oil (*Leptospermum scoparium)*, ginger oil (*Zingiber officinale)*, chamomile oil (*Matricaria recutita)*, and sandalwood oil (*Santalum album)* against HSV-1 and HSV-2 in vitro. 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.

Inhibition of HSV replication was measured by a plaque reduction assay. 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 non-cytotoxic concentrations of the essential oils tested were used.

Results showed that, 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.

Out of the oils tested, only dwarf-pine oil (*P. mugo*) and citrus oil (*C. limon*) reduced plaque formation of about 80% for HSV-1 and HSV-2 when added during adsorption of virus to host cells (Koch *et al.* 2008; Reichling *et al.* (2005) [11-16]. In contrast, when essential oils were added to the overlay medium after penetration of viruses into the host cells, only manuka oil (*L. scoparium*) significantly reduced plaque formation of HSV-1 of about 40%. Saddi *et al.* (2007) [21] recently demonstrated the virucidal effect of *Artemisia arborescens* essential oil against HSV-1 and HSV-2. The 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 oil (*O. vulgare*) and clove oil (*S. aromaticum*) (Tragoolpua *et al.* (2007) [24]. 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.

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. 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 depolarization and to reduce the activity of membrane-associated enzymes (Carnesecchi *et al.* 2001) [3]. In addition, an interaction with cellular metabolism and an induction of apoptosis have been demonstrated for essential oils and oil components (Cavalieri *et al.* 2004; Kumar *et al.* 2008) [4-12]

Conclusion

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

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