Research Signpost
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Ethnomedicine: A Source of Complementary Therapeutics, 2010: 179-201 ISBN: 978-81-308-0390-6
Editor: Debprasad Chattopadhyay

6. Recent advances in research of antimicrobial effects of essential oils and plant derived compounds on bacteria

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Abstract. Antibiotic resistance is a great burden from medicinal and economic point of view, which stems from the overprescription and misuse of anti-infective drugs. It is further aggravated by the horizontal spread of resistance genes between bacterial species and genera. The increasing rate of antibiotic resistance of bacteria urges new attempts to overcome the problem. Antimicrobial agents with new mechanisms resistance modifiers of synthetic or natural origin would serve an alternative way of antimicrobial chemotherapy targeting the inhibition of bacterial growth and the spread of antibiotic resistance.

This review reports the biological properties of essential oils and other plant derived compounds with special regard to their anti-infective features and resistance modifier activity, including antibacterial (gram-positive, gram-negative bacteria and Mycobacteria), antiplasmid activities both *in vitro* and *in vivo*.

Introduction

Medicinal plants have been used for thousands of years; in the ancient times, plant remedies provided the medicinal armamentarium in the treatment

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of diverse ailments. There is ample archaeological evidence that people of prehistoric time regularly employed medicinal plants, therefore the application of herbal drugs can be considered universal from the very early ages. Some of the earliest known written records deal with the subject of healing with medicinal substances. The ancient Egyptians of 3000 to 6000 years ago are credited with developing an elaborate and effective pharmacological collection of numerous curing materials obtained from natural resources. In the ancient Greece, Dioscorides is noted for assembling 24 detailed books on over 600 curative plants and their proper uses under the title *De Materia Medica*, the earliest known designation of that terminology [1]. The system of herbal medicine is built on the rich experience in use of herbs, and this knowledge has been accumulated since the birth of Chinese culture more than 2000 years ago. Thus, plants have an advantage that their application is based on long-term use by humans, often hundreds or thousands of years. The number of studies and surveys of which results can give sufficient information about the adverse effects and toxicity of legendary herbal remedies is increasing, due to the extended awareness and interest in complementary and alternative medicine. This trend can be attributed to several factors: dissatisfaction of patients with the high costs and potentially hazardous side effects of factory-made pharmaceuticals, wide availability of complementary medicinal products, which are often used in self-reported health problems without consultation with a health professional, the misconception about herbal remedies in terms of side effects and safely use. Nearly 1 in 5 people in the US population report using a herb for treatment of health conditions and/or health promotion. More than half did not disclose this information to their physician according to the 2002 National Health Interview Survey in the USA [2]. Based on the presence or absence of scientific evidence, there are two groups of how herbal products may be used: traditional use with limited documentation and established use, which is supported by scientific evidences [3]. Since most of the herbal drugs are Over The Counter (OTC) drugs and are obtainable at herbal drug suppliers, the established use would be the proper approach, therefore the elucidation of safety, toxicity, proper dosage, contamination, potential interactions with synthetic and other natural drugs and possible hazards would be essential [4,5].

Medicinal plants have always provided a stable source for medicines. Not only the herbs themselves but certain plant-derived compounds have served as lead molecules for further chemical modulation and natural products still continue to play a highly significant role in drug discovery and development process [6]. Herbal drugs have the advantage that they were used on a regular basis in the past and those products are still available in the same drug formulation (teas, lotions, powders, ointments, emollients, oils,

dressings, cleansers) and plants, especially those with ethnopharmacological uses, have been the primary sources of medicines for early drug discovery [7,4]. It was proved by a study that 122 compounds of defined structure, obtained from only 94 species of plants, that are used globally as drugs and demonstrated that 80% of these had an ethnomedical use identical or related to the current use of the active element of the plant. Because these compounds are derived from only 94 species of plants, and a conservative estimate of the number of flowering plants occurring on the planet is 250,000, there should be an abundance of drugs, remaining to be discovered in these plants, which are hidden in undiscovered rain forests and in the oceans [8]. Several plants are part of our diet, which may exert medicinal properties as well. In addition, there is a sharp increase in the use of dietary supplements and great deals of them have a plant origin, however this field is understudied and there are not efficient food regulations. Therefore, the growing concern of food safety also necessitates the broader establishment of the biological activities and toxicity of herbs and plant derived purified compounds. Furthermore, there are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combination and multiple target approaches, and for these attempt substances of natural origin are proved appropriate resource [9].

Essential oils are broadly studied of their anti-infective properties, of which importance is supported by their availability. Since essential oils are used in external and internal medicinal products, and are widely used in aromatherapies, in cosmetics as fragrances, there is a demand on a profound knowledge of their activities. *In vitro* experiments showed that certain essential oils and purified components are able to act against resistance mechanisms in bacteria and tumor cells via plasmid curing and inhibition of efflux processes. The available data in this respect are limited; therefore it would be worthwhile to establish a broader research in this field. The increasingly growing rate of antibiotic resistance of microorganisms necessitates the development and research of new antimicrobial agents or resistance modifiers. The kingdom of plants still provides a wide source for new drugs, therefore substances of herbal origin with antimicrobial properties may be potential candidates for the development of new anti-infective agents. Reversal of multidrug resistance may be another attempt to mitigate the spread of resistance. One approach is to interfere with the genetic material of bacteria, i.e. bacterial plasmids, which may encode the antibiotic resistance mechanisms. Terpenes of essential oils extracted from different herbs are proved to have antimicrobial activity, and some of them may act as resistance modifiers.

The purpose of the present article is to review previously published and original data on the antimicrobial activities of the most common essential oils

and purified components of these plant secondary metabolites, in order to sum up the versatile profile of their effectivity.

Antimicrobial effects of plant extracts and plant derived compounds

Several plants exert antimicrobial activity, however this effect is much more pronounced when its crude extracts or purified components are studied. Most available data about the antimicrobial effect of a herb or plant extract are based on *in vitro* studies, but several herbs are used due to its antimicrobial effects in the complementary- or ethnomedicine based on empirical application *in vivo* [10]. Several Ayurvedic drugs have been investigated by Gautam *et al* and it was revealed that the ethnomedical use of herbal remedies for *Mycobacterium* related infection strongly correlated with their *in vitro* antibacterial activity against *M. tuberculosis* [11].

The main disadvantage of the results of *in vitro* studies that it is difficult to compare to each other because of the different test methods, different methods of extraction, test assays, and variation in chemical phytoconstituents in plants due to different agroclimatic conditions and plant phenotype.

Shan *et al* investigated a series of dietary spices and herbal medicines in order to establish their antibacterial effects against bacteria, of which infections are related to food poisoning. Results emphasized that phenolic compounds significantly contributed to the antibacterial activity of the studied herbs [12]. This activity may be attributed to the enzyme inhibition by the more oxydized phenolic compounds possibly through reaction with sulfhydryl compounds or through more non-specific interactions with the protein. Cinnamic acid, caffeic acid, catechol and pyrogallol were shown to be toxic to microorganisms [13].

A great deal of flavonoids are synthesised by plants to fight against bacterial infections, therefore it is no surprise that they exert *in vitro* antimicrobial activity. Their effectivity is possibly due to their ability to form complex with extracellular soluble proteins and with bacterial cell walls [14,15]. Catechins deserve special mention, since they considerably contribute to the beneficial effects of oolong green teas. Beside their versatile activities, catechins exert antibacterial effects as well via DNA gyrase inhibition. Specific binding of selected catechins were proved to bind to the N-terminal fragment of gyrase B [16]. Furthermore, catechins are able to restore the susceptibility of resistance bacteria to different antibiotics e.g. tetracycline and beta-lactams, beta-lactamase inhibitors [17,18,19]. Tannins are polymeric water-soluble phenols that are common in higher herbaceous

and woody plants. Tannins are known of their free radical scavenging activity and have antibacterial effects [20]. Hydrolysable tannins have antibacterial potential against *Helicobacter pylori* and it seems promising in the eradication of the bacterium without affecting intestinal bacterial flora [21]. Oligomeric proanthocyanidines are mainly used in vascular diseases and it is based on their ability to trap lipid peroxides and free radicals and that they non-competitively inhibit xanthine oxidase, which is a major generator of free radicals [22,23].

Among plant peptides, thionines have toxic effects on different gram-positive and gram-negative pathogenic bacteria. Fabatins, which are recently identified peptide molecules from fava beans appeared to inhibit the growth of *E. coli* and *P. aeruginosa* [24]. The tea and petroleum extract of St John's wort (*Hypericum perforatum*) were proved to have antibacterial effect against methicillin resistant *Staphylococcus aureus* strains, which are among the most common and most problematic resistant strains to eradicate. Most probably, hyperforin may be responsible for the beneficial effects, which is one of the active agents responsible for the antidepressant activity [25].

The fruit of *Malpighia emarginata* DC (Malpighiaceae) is called barbados cherry or acerola fruit. This healthy foodstuff is nutritionally attractive since it contains high amounts of vitamin C, beta-carotene, minerals, plant fibre and small amount of vitamin B. The extracts of the fruit has been used as folk medicines and shows biological effects including antioxidant activity for the prevention of age-related diseases. Motohashi *et al* examined the antibacterial and antifungal activity of the previously successively extracted fruit and found that the components extracted with acetone showed higher activity against *S. aureus*, while buthanol extracts had more pronounced antimicrobial effect against *Candida albicans*. The hexane extracts were effective against *Helicobacter pylori* with minimal inhibitory concentrations (MICs) between 17-27 μg/mL [26]. Similarly, to acerola fruit, the hypophasic and epiphasic extracts of the ripe fruit of spice paprika, *Capsicum annuum* and Valencia orange and Golden delicious apples were investigated. These extracts displayed potent anti-*H. pylori* activity with MICs comparable with metronidazole. In an *in vitro* study, the extracts of a special variant of *Capsicum annuum* was examined: the fruit of Russian Black sweet pepper was successively extracted and fractionated, and beside the anti-tumor activities, its effects were established against *H. pylori*. Those extracts and fractions eluted by less polar eluents, and which contain apolar components i.e. carotenoids, showed comparable antibacterial activities with metronidazole [27]. These fruits have a high carotenoid content, which compounds are well known of their antioxidant properties [28]. A high intake of carotenoids has been shown to prevent the development of *H. pylori*-

associated diseases [29]. As free radicals play an important role in the pathogenesis of gastroduodenal mucosal inflammation, peptic ulcer disease and probably even gastric cancer, various micronutrients are considered to protect the gastric mucosa by scavenging the free radicals.

It was found that the crude extract of *Salvia officinalis* reduced the minimum inhibitory concentration of aminoglycosides in vancomycin resistant *enterococci*, then the effective compound was isolated. Carnosol, the active compound showed weak antimicrobial activity and greatly reduced the MICs of various aminoglycosides [30]. Abietane diterpenoids extracted from *Salvia sclarea* were shown to be bacteriostatic and bactericid for the cultures of *S. aureus* and *S. epidermidis* strains, regardless to their antibiotic susceptibility profile [31].

Plants may have direct antimicrobial effects and may act indirectly by stimulating the defence mechanisms, stimulating the immune response. *Echinacea* is a well-known immunostimulant and anti-inflammatory drug. The immunostimulant effect is complex. It has been reported that *E. purpurea* has an interferon (IFN)-like effect, activating macrophages, T-lymphocytes and inducing the production of cytokines. Low concentration of *Echinacea* extracts induce higher levels of cytokines (TNF-α, IL-1, IL-6, IL-10) in macrophages, which is consistent with an immune activated antiviral effect [32,33].

Reversal of bacterial resistance by plant derived compounds

Antibiotic resistance provides a great therapeutical and economic burden in the treatment of infectious diseases and it may threaten the success of antimicrobial chemotherapy. It is estimated that antibiotic resistance double hospital stay and morbidity. Single antibiotic resistance itself is a great problem, however the appearance of multiple antibiotic resistant (MDR) strains cause a more pronounced obstacle for patients and healthcare professionals [34,35].

Resistant strains appeared soon after the introduction of an antibiotic and initially showed up in hospitals, where most antibiotics were used, causing clinical difficulties for nosocomial treatment on a global scale. Antibiotic resistance has been unavoidable from an evolutionary perspective, since the antibiotic pressure provides the potential for resistant bacteria to acquire an important advantage. Discretionary use of antibiotics lead to the spread of resistant strains, which causes the most expressed problem at nosocomial circumstances, mainly in immunocompromized patients. Important contributing factors to community acquired infection with resistant bacteria may be the poor hand-hygiene among healthcare workers and device-associated infections.

Antibiotics can be disseminated into the environment from agricultural sources as well, of which importance has recently been recognized and studied extensively. Antibiotics are widely used as growth promoters in veterinary practice in sub-clinical concentrations and this poses a potential risk for the selection of resistant bacterial strains, which may be transmitted to humans. Since the growing rate of bacterial resistance is of great medical and public concern, there is an urgent need to develop appropriate methods and protocols to overcome or at least reduce the incidence and spread of resistant strains [36,37,38].

The mechanisms of resistance show a versatile picture, however certain bacterial strains have intrinsic resistance against antimicrobial agents, acquired resistance against antimicrobial chemotherapy possess a larger risk of therapeutical failure. Antibiotic resistance in bacteria can be divided into the following major groups based on the mechanism involved: Resistance may develop due to the presence of an enzyme that inactivates (beta-lactamases) or modifies the antibiotic (aminoglycoside, due to the presence of an alternative enzyme for that inhibited by the antibiotic), modification of the antibiotic-target, reduced permeability and active efflux may also lead to resistance [39].

The genetic determinants encoding antimicrobial resistance can be located on the bacterial chromosome or on plasmids, which may replicate independently from the chromosome. Normally susceptible populations of bacteria may become resistant to antimicrobial agents through mutation and selection, or by acquiring from other bacteria the genetic information, that encodes resistance. The acquisition of such genetic elements may occur through transfer of genetic mechanisms i.e. conjugation, transduction and transformation [40]. These mechanisms are in the background of infectious resistance of bacteria. Development of novel antimicrobial agents or substances that target the resistance of bacteria on genetic or protein level would be a possible attempt to overcome the problem. Several in vitro studies prove that herbal medicines or plant-derived compounds may serve sources for this purpose.

Many attempts has been made to investigate the potential role of plant extracts and some active compounds for their efficacy to combat the problems of drug resistance in bacteria by reversal of resistance, efflux inhibition, inhibition of biofilm formations, interference in bacterial quorum sensing etc. Some of these fin dings are discussed below.

One attempt is the selective inhibition of efflux mechanisms related to antibiotic resistance in order to potentiate the chemotherapeutic agent. Martins *et al* found that the extract of *Carpobrotus edulis* is effective in this respect. The genus *Carpobrotus* is a perennial succulent horizontally low-

growing sub-shrub. It is traditionally utilized for its medicinal properties: the leaf juice is used for a wide range of bacterial and fungal infections and in the treatment of sinusitis, diarrhoea, infantile eczema, tuberculosis and other internal chest conditions. The crude extract of the leaves was proved to have antibacterial effects against several human pathogenic bacteria. The methanol extract of that plant is able to enhance the killing activity of human peripheral blood monocyte-derived macrophages against intracellular *S. aureus*. It is hypothesised that it may be attributed to the ability of macrophages to concentrate the extract *in vitro*, which might be based on the inhibition of efflux functions [41,42,43,44].

A single pump can provide bacteria with resistance to a wide array of chemically and structurally diverse compounds. Microbial efflux was first reported for the efflux of tetracycline by *E. coli*. Five major families of efflux transporters can be distinguished: the major facilitator superfamily, the resistance-nodulation-division superfamily, the small MDR family, the ATP binding cassette family and the multiple antibiotic and toxin extrusion family. However there does not exist a combination of antibiotic and efflux inhibitor in the treatment of infections, plant derived compounds seem to be potential drug candidates in this respect. The antihypertensive plant alkaloid reserpine was first isolated from the roots of *Rauwolfia vomitoria* and its efflux inhibitory effect was demonstrated on various bacterial strains. However, this compound seemed ideal as an MDR modifier according to *in vitro* studies, the further investigation of this substance is highly limited by its neurotoxic effects in the concentration of efflux inhibition [45]. There have also been a number of methoxylated flavones and isoflavones described as putative inhibitors of MDR pumps. These effects are mainly exhibited by potentiation and not by direct inhibition [46].

In several microbial infections, biofilm formation plays a major role in the ineffectivity of antibiotic therapy, therefore the inhibition of biofilm formation would be a possible approach to find resistance modifiers. Biofilm formation is generally regulated in a population-density dependent manner via quorum sensing. An important consequence of biofilm growth is the markedly enhanced resistance to antimicrobial agents where biofilm-associated microorganisms are estimated to be 50 to 500 times more resistant than their planktonic counterparts. (Figure 1.)

Quorum-sensing activities have been described for the sesquiterpenoid farnesol. In this role, farnesol produced extracellularly prevented the transition from yeast to hyphal growth in *Candida albicans* and greatly compromised biofilm formation by this fungus. Some studies indicated a possible interaction of farnesol with cell membranes of certain bacterial species including *Streptococcus mutans*. This natural compound has the potential to inhibit the development of

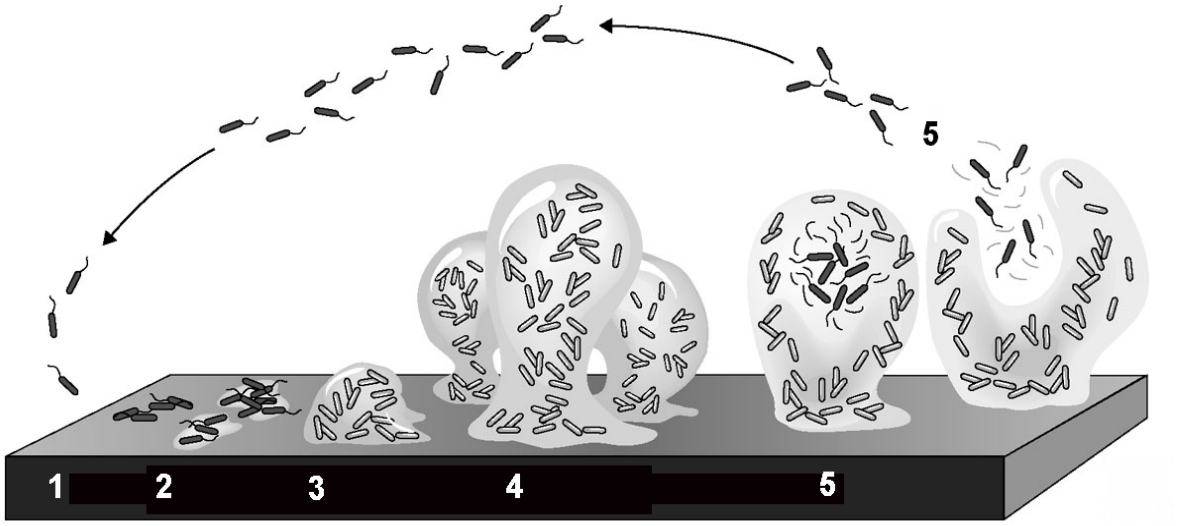

Figure 1. Stages of biofilm formation. In certain microbial infections, biofilm formation plays an important role in the loss of activity of antimicrobial therapy, therefore the inhibition of quorum sensing mechanisms related to biofilm formation would serve a possible approach in the design of antibiotics with new targets. (http://biology.binghamton.edu/davies/images/biofilm.jpg).

dental caries via the inhibition of biofilm formation. The use of the compound would be a novel approach in oral hygiene, since this compound does not necessarily have a major effect on the viability of the oral flora population, but rather it may disrupt the accumulation and the polysaccharide content of dental plaque related to this common oral disease [47]. By monitoring changes in the amount of K^+ ions in the presence of terpene alcohols, the rate of leakage of the ion from the bacterial cells was found to increase with increasing concentrations of the compounds added. Farnesol is naturally found in the essential oil of citrus fruits and was shown to devoid of toxic effects and non-mutagenic *in vitro* and *in vivo*.

Inhibition of formation and accumulation of biofilm communities by affecting the synthesis of polysaccharides is an attractive route for preventing biofilm-related infections. Relatively low concentration of farnesol was sufficient to inhibit biofilm formation, as was shown by viability assays and fluorescence microscopy for both of MRSA and MSSA strains. The hydrophobic nature of farnesol favors its accumulation in the mambrane, possibly causing membrane leakage. It was also successful at enhancing the antibacterial efficacy of antibiotics to which *S. aureus* strains were somewhat susceptible. This ability of farnesol to sensitize *S. aureus* to such a heterogeneous group of antibiotics underlines the non-specific nature of this enhancing activity [48]. Farnesol may be a component of adjuvant therapy of skin infections [49,50,51].

The Australian red macroalga *Delisea pulchra* produces a range of halogenated furanone compounds that display antimicrobial properties. It is

hypothesized that furanones of *D. pulchra* constitute a specific means of eukaryotic interference with bacterial signalling process via competing with the quorum-sensing signals. Some derivatives of these compounds were shown to repress quorum sensing in *P. aeruginosa* and reduce virulence factor expression [52,53].

Resistance of *enterococci* is a great concern in public health, several strains exhibit multiple antibiotic resistance including that to both ampicillin and vancomycin. Flavonoids i.e. galangin and 3,7-dihydroxyflavone were shown to restore the vancomycin sensitivity of *Enterococcus faecalis* and *E. faecium*, by lowering the MICs to the level of vancomycin sensitive strains. It is suggested that the alternatively synthesized disaccharide peptide (ala-lac) production might be initially inhibited by the presence of flavonoid. It seems likely that in the presence of both sub-MIC levels of flavonoids and vancomycin that the production of disaccharide peptides ala-ala and ala-lac were inhibited and cell wall biosynthesis was interrupted as a result of cessation of peptidoglycan synthesis [54].

Antibacterial effects of essential oils

The antiseptic properties of aromatic plants and their extracts have been recognised since antiquity and are still used in the medicine, food and cosmetic industry. There appears to be a revival in the use of traditional approach to protecting livestock and food from disease and spoilage in industrial countries. Bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents. These therapeutic effects can be generally attributed to the essential oils of these plants rather than their extracts [55]. Essential oils are generally isolated from non-woody plants by distillation methods, mainly by steam or hydrodistillation. Essential oils are usually made up of terpenoids, specifically monoterpenes and sesquiterpenes, but diterpenes may also be present and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, coumarines and homologues of phenylpropanoids. Most terpenes are derived from the condensation of branched five-carbon isoprene units and are categorized according to the number of these units present in the carbon skeleton [56]. The mechanism of action of terpenes is not fully understood, it is assumed that membrane disruption by the lipophilic components is involved in the antibacterial action. *In vitro* studies proved that increasing the hydrophilicity of kaurene diterpenoids by the addition of a

methyl group drastically reduced their antibacterial activities [57]. Terpenoids may serve as an example of lipid soluble agents which affect the activities of membrane-catalysed enzymes, for example their action on respiratory pathways. Certain components of essential oils can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation. Specific terpenoids with functional groups, e.g. phenolic alcohols or aldehydes, also interfere with membrane-integrated or associated enzyme-proteins, stopping their production or activity [58]. The mode of action also depends on the microorganism and is mainly related to its cell wall structure. Gram-negative bacteria have intrinsic resistance against toxic components, since they have a permeability barrier against toxic agents. Hydrophobic macromolecules, such as essential oil constituents, are unable to penetrate the barrier. On the other hand, essential oils usually express low aqueous solubility, which prevents them from reaching a toxic level in cellular membranes [59]. In case of tea tree oil, the antimicrobial effect is based on the denaturation of membrane proteins, resulting in the outer membrane disruption, subsequent K^+ leakage, respiration inhibition and cell lysis [60,61]. Essential oils are also able to inhibit the synthesis of DNA, RNA, proteins and polysaccharides in fungal and bacterial cells [62].

Since *in vitro* tests also proved the efficacy of certain essential oils as resistance modulators, there is every likelihood that these plant secondary metabolites might be used in the clinical practice as complementary agents in the treatment of resistance bacterial infections. Nevertheless their antimicrobial action is based on non-specific mechanisms (i.e. membrane disruption), thus the clinical use might be limited to superficial infections.

Antibacterial assays for essential oils

For the establishment of antimicrobial properties of essential oils, conventional methods of testing are usually applied. The agar diffusion and broth dilution methods are the most widely used. The assessments of antimicrobial activities by these methods are limited due to the volatility, water insolubility and complexity of essential oils. The agar diffusion method is not considered a perfect method for essential oils, as their volatile components are likely to evaporate with the dispersing solvent during the incubation time, while their poorly soluble components do not diffuse well in the agar broth, but it still remains the most common technique. The inhibitory effect of essential oils in the test tubes and microtitre plates is measured turbidimetrically or with the count plate method. In experiments testing the essential oils activity towards microorganisms, the result depends mainly on

the method used, however a number of other factors should also be considered. Culture conditions are predominantly influencing the study; therefore these should be precisely being stated in reports. [58].

Antimicrobial properties of selected essential oil producing herbs

Peppermint (*Mentha piperita*) is a perennial flowering member of the mint family. The medicinal use of peppermint and other mint plants date back to the ancient Greece, where peppermint leaf was used internally as a digestive aid and for the management of gallbladder disease and it was also used in the treatment of upper respiratory diseases and for cough. Extracts of peppermint are used as flavouring in many alimentary and cosmetic products and in over the counter medicines. Peppermint oil has become a popular treatment for a variety of conditions. *In vitro* research shows peppermint oil to be effective in relaxing gastrointestinal smooth muscle. This finding has led to the popularity of enteric-coated peppermint formulations [63]. Since essential oils were found to exert antimicrobial effect on various bacterial strains *in vitro*, the beneficial effects may be partly attributed to this activity [64,65]. Studies examining the effect of peppermint oil on bowel motility have shown that mechanisms may include calcium channel blocking on a local level, causing smooth muscle relaxation [66,67]. Peppermint oil may reduce spasm during endoscopies and barium enema [68,69,70]. The antibacterial effect of peppermint oil can be used in those conditions which are in relation with the bacterial overgrowth of the small intestine. A number of functional somatic disorders are included like irritable bowel syndrome, fibromyalgia and chronic fatigue syndrome. In these conditions, peppermint oil preparations seem efficient to alleviate the symptoms [71,72].

The antimicrobial effects of peppermint oil and its components have been proved in several studies. The MIC values of the oil is relatively high (may reach 5.0 mg/mL depending on the source), therefore the medicinal use of the oil is limited [73]. Due to its toxicity, the external use of the oil would be preferable, but successful internal application was reported in enteric-coated form. Peppermint oil and its main constituent menthol was described as possible agent in reversal of multidrug resistance in bacteria via their plasmid curing effects. Bacterial plasmids may carry resistance genes for different antibiotics and structurally unrelated chemicals. These plasmids can be transferred by conjugation between different bacterial strains or genera, which are a major cause of the spread of multiple resistance to antibiotics of bacteria. The inhibition of this process by the elimination of resistance-gene

carrying plasmids seems a promising perspective to overcome the problem. Molnar *et al* investigated a great number of chemical agents, predominantly tricyclic antipsychotic drugs and carried out a systematic assay in order to find structure-activity relationship in the antiplasmid activities of the investigated substances and it has been proposed that the HOMO orbital energy, the conjugated π-electron system of the tricyclic skeleton and the symmetric π-electron distribution to the L-molecular region and the superdelocalizability of the π-electron system on 10, 12, 13 atoms have special importance in the antiplasmid activity [74,75,76]. Promethazine was one of the most effective plasmid eliminators and was tested in *vivo* studies as well. Promethazine in combination with gentamycin reduced the number of recurrences in urinary tract infections as compared with the control (gentamycin only) group in children with frequently recurring pyelonephritis and had beneficial effects in urinary tract infections in adult patients [77,78,79,80]. Peppermint oil and menthol, the major component was tested for plasmid curing activity in *in vitro* experiments on the metabolic plasmid of *E. coli* F'lac K12 LE140. The plasmid curing activities were relatively high (Table 1.) [65].

Table 1. Antiplasmid activities of peppermint oil and menthol. The plasmid curing activities are designated in percentage.

Sample	Concentration (mg/ml)	Bacterial growth after 24 h x10^8 CFU/5 ml	Plasmid elimination (%)
MTY Control	0	0	-
Promethazine	0.04	36.0	0.0
	0.05	24.5	0.1
	0.06	3.3	15.0
	0.07	2.3	82.0
	0.08	2.0	55.9
	0.09	0.4	2.5
	0.10	-	-
Peppermint oil	0.18	21.6	0
	0.27	5.3	0.4
	0.36	2.5	2.9
	0.45	1.3	9.0
	0.54	0.4	37.5
	0.63	-	-
Menthol	0.250	3.5	7.1
	0.275	5.3	82.0
	0.300	1.5	74.0
	0.325	3.1	96.0
	0.350	1.3	64.0
	0.375	3.9	8.7
	0.400	-	-

Figure 2. Plasmid elimination of the F'lac metabolic plasmid of *E. coli* K12 LE 140. Bacteria with functioning plasmid form dark colonies on the surface of eosine methylene blue agar, while those cells, of which F'lac plasmid has been eliminated develop transparent colonies due to the loss lactose fermentation.

Garlic (*Allium sativum* L.) has had an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects are ascribed to specific oil- and water-soluble organosulfur compounds, which are responsible for the typical odour and flavour of garlic. During crushing or cutting the clove, the odourless amino acid alliin is cleaved by the enzyme allinase to yield allicin and other thiosulfinates that are the source of the characteristic odour of garlic. The crude extracts of garlic exhibit a broad antimicrobial spectrum against gram-positive and gram-negative bacteria.[81,82]

Gastric cancer is a major neoplastic diseases on a global scale. *Helicobacter pylori* is implicated in the aetiology of the development of the disease. It has been revealed that the incidence of stomach cancer is lower in individuals with a high intake of garlic vegetable. *In vitro* studies proved the anti-*Helicobacter* properties of garlic, therefore we may conclude that the lower incidence can be attributed to the antibacterial effect of the higher garlic intake, however there have not been made a sufficient number of randomized clinical trials [82].

Thiosulfonates play an important role in the antibacterial activity of garlic. It was shown that the antimicrobial activity of garlic is completely abolished when the thiosulfinates are removed from the extract. The major

volatile component responsible for the antimicrobial effect is allicin. The antibacterial effect of allicin is of a broad spectrum, even resistant bacterial strains e.g. MRSA, MDR enterotoxigenic strains (ETEC, *Enterococci*, *Shigellae*) were found to be sensitive to allicin. Allicin also had an *in vivo* antibacterial activity against *S. flexneri* when tested in the rabbit model of experimental shigellosis [83]. The mechanism by which allicin exert its action is presumably based on the rapid reaction with thiol groups of thiol-containing enzymes. It was also found in *in vitro* assays that allicin exhibits its activity via immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target [84].

Australian tea tree (*Melaleuca alternifolia*) lives on low-lying, swampy coastal ground in New South Wales in Australia and unlike several other *Melaleuca* species it does not occur naturally outside Australia. The essential oil of that plant is employed largely for its antimicrobial properties. Tea tree oil (TTO) is incorporated as active ingredient in many topical formulations used to treat cutaneous infections. The earliest reported use of *M. alternifolia* plant that presumably exhibited antibacterial activity was the traditional use by Australian aboriginals in New South Wales for coughs and colds. Several *in vitro* studies proved the antimicobial properties of TTO against gram-positive and gram-negative bacterial strains [85,86,87,88]. The activity of TTO against antibiotic-resistant bacteria has attracted considerable interest, with methicillin-resistant *Staphylococcus aureus* receiving the most attention so far. Subsequent reports on the susceptibility of MRSA to TTO have not shown great differences compared to antibiotic-sensitive organisms [89]. Despite major advances in wound management, infection still remains an important factor in wound healing. In burns, majority of burns are due to complications with sepsis resulting from wound infection. A considerable proportion of wound become colonised by resistant strains of *S. aureus*. An *in vitro* study highlights the potential use of TTO impregnaterd dressings for treating wounds infected with MRSA [90]. The mechanism of TTO against microorganisms is partly elucidated. Via disruption of membrane integrity, it is able to permeabilize model liposomal membranes, and in bacterial cells inhibits respiration. Treatment of *S. aureus* with TTO resulted in leakage of K^+ ions, sensitized bacteria to sodium chloride and produced morphological changes apparent electron microscopy, but not whole-cell lysis [91]. In contrast with the absence of whole-cell lysis in *S. aureus*, lysis occurs in *E. coli* after the treatment with TTO and this effect is further exacerbated by co-treatment with EDTA. All of these effects confirm that TTO compromises the structural and functional integrity of bacterial membranes. It has also been reported that the susceptibility of bacteria may differ in different growth

phases, which suggests that in the effects of TTO targets other than the cell membrane may be involved [92].

In parallel with the characterization of in vitro antimicrobial activity of TTO, the clinical efficacy of the oil has also been the subject of investigation. In a single-blind randomized trial, the 5% lotion of tea tree oil (TTO) and benzoyl peroxide (control) were investigated in the treatment of acne. However, the benzoyl peroxide group showed a more rapid remission, the skin discomfort reported during the trial was less frequent in the TTO group [93]. A combination of a 4% tea tree oil nasal ointment and 5% TTO body wash was compared with a standard 2% mupirocin nasal ointment and triclosan body wash for the eradication of ethicillin resistant *S. aureus* carriage. TTO combination appeared to perform better than the standard combination [94]. In a randomized controlled trial of tea tree topical preparations versus standard preparations (mupirocin nasal ointment, chlorhexidine gluconate soap and silver sulfadiazine cream) were studied. Mupirocin was significantly more effective at clearing nasal carriage of MRSA than tea tree cream, but Tea tree treatment was more effective than chlorhexidine and silver sulfadiazine at clearing superficial skin sites and lesions. Since tea tree preparation was well tolerated by the treated patients, it may considered in regimens for eradication of MRSA carriage [95]. A patient with MRSA osteomyelitis was reported to have alternative anti-inflammatory therapy of a TTO preparation. During treatment, percutaneous TTO containing calcium sulphate pellets were given to the bone. Over a three-month period, the symptoms resolved with a healing response on X-ray [96].

Thyme is stated to possess carminative, antispasmodic, antitussive, expectorant, secretomotor, bactericidal, anthelmintic and astringent properties. Due to its antimicrobial properties, thyme oil is considered a potent food preservative. It has antilisterial activity in relatively low concentration. The effects on the bacterial cells were examined by electron microscopy and the following findings were taken: the bacterial cells shrank, cell wall exhibited budding scars and degenerative changes showing splitting of the wall layers. Lack of cytoplasm and cell membrane disruption was evident at an early stage. These results on the mechanism of action of thyme oil on the inactivation of *L. monocytogenes* will help in the development or modification of the processing conditions, or the implementation of a new preservation factor to complement those already employed in food preservation and safety [97,98].

Contamination of food comodities with aflatoxin resulting from fungal attack can occur before, after and during the harvest and storage operations. One of the characteristics of aflatoxin deactivation is that it should destroy the mycelia and spores of the toxic fungi, which may proliferate under favorable conditions. The essential oils of thymus species seem good candidates

for that purpose. In an *in vitro* study, the essential oil-related inhbition in mycelial growth was observed to be associated with significantly decreased levels of aflatoxin production. 8- and 4-fold dilutions of the essential oils of T. eriocalyx and T. x-porlock are effective in complete retardation of fungal growth respectively and a somewhat lower concentrations are suficient to significantly inhibit aflatoxin production. Contribution of thymol to the positive results seems to be significant [99].

Origanum vulgare is a well known spice mainly in the Mediterraneum. Recently, origanum essential oil has been studied as a potential natural preservative in food manufacturing. Origanum essential oil exerts antimicrobial effect via cell membrane damage, with envelope disruption, formation of blebs and lack of cytoplasmic material. These alterations correlate with the ability of hydrocarbons to interact with hydrophobic structures, like bacterial membranes. These findings are based on investigations of *E. coli* and *S. aureus* by transmission electron microscopy, which allows to study the possible bacterial ultrastructural alterations [100].

Antimicrobial activities of individual oil components

Essential oils are mainly composed of terpenoids: monoterpenes and sesquiterpenes. Terpenes have been reported to be very active against bacteria. Many essential oil components are chiral compounds. The enantiomers very often show different biological activity. (+)-pinene and (-)-pinene serve a good example, since these enantiomers act differently: (+)-pinen was found to exert more pronounced antimicrobial effect on different bacterial and fungal strains [58].

Carvacrol is one of the most common essential oil components which exert antibacterial effects, it is the major component of oregano and thyme. Carvacrol was investigated in *E. coli* O157:H7 ATCC 43895 for its effects on the protein synthesis. The presence of 1mM carvacrol during overnight incubation caused the bacterial strain to produce significant amounts of heat shock protein 60 (HSP60) and inhibited the flagellin synthesis highly significantly causing cells to be aflagellate and therefore nonmotile [101]. Addition of carvacrol of the culture of *B. cereus* results in an increase of membrane fluidity by changing their fatty acid and head-group composition. Carvacrol also caused depletion of the intracellular ATP pool and an increased permeability of the cell membrane for K^+ upon exposure to carvacrol. K^+ plays a role in the activation of cytoplasmic enzymes, the maintenance of turgor pressure, and possibly the regulation of the cytoplasmic pH. Different studies showed that an efflux of K^+ is a first indication of membrane damage in bacteria. [102,103].

The antifungal activities of carvacrol and eugenol were investigated in immunosuppressed rats for the treatment of oral candidiasis induced by *Candida albicans*. The anticandidal activity was established by microbiological and hystopathological methods. Nystatin was used as positive control. Microbiologically carvacrol and eugenol significantly reduced the number of colony forming units sampled from the oral cavity of rats treated. Histologically, the untreated control animals showed numerous hyphae on the epithelium of the dorsal surface of the tongue. In contrast, no hyphal colonisation was seen in carvacrol-treated animals, while in rats treated with eugenol, only a few focalized zones of the dorsal surface of the tongue was occupied by hyphae. Carvacrol and eugenol could be proposed as therapeutic agents in the treatment of oral candidiasis. [104]

Elastase, a serine proteinase released by human neutrophils, can degrade a wide variety of biomacromolecules including elastin and is considered a biomarker of inflammatory diseases. Thymol, which is a potent antibacterial components of thyme essential oil can approach the ion channel proteins through the lipid phase of the membrane due to its hydrophobic nature and alters the local environment of calcium channels inhibiting capacitative calcium entry. This leads to a corresponding reduction in elastase. The antimicrobial, antioxidant and anti-elastase properties of thymol may have helpful effects in controlling the inflammatory processes present in many infections. [105]

In a non-randomized clinical trial the extract of *Thea assamica* was tested on patients with impetigo contagiosa. The extract was as effective as the antibiotic control [106]. The essential oil of Chenopodium botrys has antibacterial activity on selected gram-positve and Gram-negative bacterial strains, furthermore the extract was proved to have antifungal activity against the ATCC strains of *Aspergillus niger* and *Candida albica*ns [107].

Summary

There is resurgence in the use of herbal medicines worldwide. An estimated one third of adults in the Western world use alternative therapies, including herbs. These herbs may be used either in their primary forms or combined in mixtures. In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines. However, problems may arise due to intrinsic toxicity, adulteration, substitution, contamination, misidentification, drug-herb interactions and lack of standardization [108]. This unfavourable fact urges the study of medicinal plants and plant derived compounds used in medicine and food industry.

A great deal of essential oils has beneficial properties, such as antioxidant, anti-inflammatory and antimicrobial properties. The main constituents of esential oils- mono- and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones – are responsible for the fragrant and biological properties of aromatic and medicinal plants. Despite the development of antibiotics, bacterial and fungal infections are still a major issue in medicine, and the presence of multidrug resistant strains poses a great challenge. Recently, there has been a growing interest in natural products due to their availability and better biodegradebility. In this regard, essential oils may offer a great potential and these plant secondary metabolites may be used as alternative anti-infective and food preservatives [58]. The use of essential oils in the treatment of infectious diseases is limited due to their toxicity but in the treatment of superficial infections or respiratory diseases via aromatherapy may be possible applications. In food industry, the shelf-life of foods is often extended by the addition of antibacterial chemicals, but there is a need for preservatives, which do not alter the organoleptic properties of foods. Some of the essential oils seem good candidate for that purpose to prevent food spoilage as well.

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