



## Essential Oils and their Antimicrobial Activity: A Review

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### Abstract

Essential oils (EOs) have been used in ethno-medicine as an effective and safe antimicrobial agents for a long time. They have been evaluated for the bactericidal, fungicidal, antiparasitical, insecticidal, virucidal, medicinal and cosmetics applications. EOs are derived from various species of edible and medicinal plants including herbs and spices. They are liquid and volatile complex mixture of compounds obtained from different parts of plants such as tea tree, oregano tree, clove, thyme, citrus and mint. Terpenes and terpenoids and their derivatives are the major constituents of EOs while aromatic and aliphatic components are the minor constituents. EOs possess strong antibacterial and antifungal properties for both susceptible and resistant strains, where they have the ability to damage the structural integrity of cell membrane, induce leakage of cell constituents, influencing the cell metabolism and eventually causing cell death.

### Keywords:

### INTRODUCTION

Essential oils (Eos) have long been used in ethno-medicine as effective and safe antimicrobial agents. The research on EOs and closely related components has been recently intensified, due to their biological, antioxidant and antimicrobial properties (Bukvicki *et al.*, 2014; Ayazet *al.*, 2015). Essential Oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetics applications, especially nowadays in pharmaceutical, sanitary, agricultural and food industries (Anonymous, 2008).

Essential oils represent a cheap and effective antiseptic topical treatment option even for antibiotic-resistant strains as MRSA and antimycotic-resistant *Candida* species where large prevailing effective zones of inhibition were observed for Thyme white, Lemon, Lemongrass and Cinnamon oil (Patrick *et al.*, 2009).

Plant-derived essential oils of various species of edible and medicinal plants, herbs and spices have been used as natural agents for food preservation in the food industry due to the presence of antimicrobial compounds (Dahiya and purkayastha, 2012) and they have been evaluated as natural sources for controlling spoilage bacteria during food storage (Zhang *et al.*, 2017).

Therefore, essential oils could be used in food preservation technologies where they can act as bio-preservatives, reducing or eliminating pathogenic bacteria and increasing the overall

quality of animal and vegetable food products. Although clinical studies are scarce, the uses of EOs for topical administration and as penetration enhancers for antiseptics are promising. Little information exists for oral administration (Solórzano-Santos and Miranda-Novales, 2012).

### Essential oils (EOs) and their Sources

Essential oils are liquid, volatile and rarely colored containing complex mixture of compounds (Bakkali *et al.*, 2008), and they are obtained from different plant parts such as leaves, seeds, flowers, bark, fruits, and roots (Burt, 2004). EOs are synthesized naturally in different plant parts during the process of secondary metabolism (Swamy *et al.*, 2016).

Tea tree oil is an increasingly popular ingredient in a variety of household and cosmetic products, including shampoos, massage oils, skin and nail creams, and laundry detergents. Known for its potential antiseptic properties, it has been shown to be active against a variety of bacteria, fungi, viruses, and mites. It is well known for its medicinal and potential cosmetic uses and is reportedly used for its antiseptic, antifungal and anti-viral properties (David and Sharon, 2012).

Oregano essential oil is obtained from different genera such as *Origanum*, *Thymus*, *Coridothymus*, *Thymbra*, *Satureja* and *Lippia* and is rich in carvacrol, a monoterpenic phenol isomeric with thymol. Oregano is mainly used in food, spice and pharmaceutical industries. Carvacrol is responsible for the biological activities of oregano.

Many diverse activities of carvacrol such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, anti-inflammatory, angiogenic, anti-parasitic, antiplatelet, ache inhibitory, insecticidal, anti-hepatotoxic and hepatoprotective activities and uses such as feed additive, in honeybee breeding and in gastrointestinal ailments have been shown (Baser, 2008).

#### Essential oil composition

Essential oils are made from a very complex mixture of volatile molecules that are produced by the secondary metabolism of aromatic and medicinal plants and can be obtained by different methods, including the use of low or high pressure distillation of different parts of plants or the employment of liquid carbon dioxide or microwaves (Chouhan *et al.*, 2017).

EOs can be applied in liquid or vapor phase, however, there is a growing evidence that EOs in vapour phase are effective antimicrobial systems and they could have advantages over the use of EOs in liquid phase, especially in a hospital environment (Mandras *et al.*, 2016). The different antifungal activity in liquid and vapour phase could be due to the characteristics of EOs such as high hydrophobicity and volatility. In the liquid phase, the activity depends upon the diffusibility and solubility of the EOs in the medium while in the vapour assay it depends upon the volatility (Tyagi and Malik 2010).

Generally, the biological properties of the essential oils are determined by their major components including two groups of distinct bio-synthetic origin which are terpenes and terpenoids comprising the main groups whereas aromatic and aliphatic constituents comprise the other group, all characterized by low molecular weight (Anonymous, 2008).

The major constituents (terpene and terpenoid origin) of EOs can constitute up to 85%, whereas other components (aromatic and aliphatic origin) are present in trace amounts, examples are;  $\alpha$ -phellandrene (36%) and limonene (31%) in *Anethum graveolens* leaf oil, d-limonene (over 80%) in citrus peel oils,  $\alpha$ -phellandrene (36%) and limonene (31%) in *Anethum graveolens* leaf oil, carvacrol (30%) and thymol (27%) in *Origanum compactum* oil,  $\alpha$ / $\beta$ -thujone (57%) and camphor (24%) in *Artemisia herba-alba* oil, carvone (58%) and d-limonene (37%) in *Anethum graveolens* seed oil, and menthol (59%) and menthone (19%) in *Menthapiperita* oil (Shaaban *et al.*, 2012).

Terpenoids can be sub-divided into alcohols, phenols, esters, aldehydes, ethers, ketones, and epoxides, examples are; thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol. A relatively small

proportion of essential oils is composed of phenylpropenes, and the phenylpropenes that have been most thoroughly studied are safrole, eugenol, isoeugenol, vanillin, and cinnamaldehyde (Guleria *et al.*, 2008). Thymol and carvacrol are among the most active natural antioxidants and antimicrobials found in EOs (Tyagi and Malik 2010; Fontenello *et al.*, 2011).

Carvacrol has specific effects on *S. aureus* and *S. epidermidis*, while Perilla oil suppresses expression of  $\alpha$ -toxin, *Staphylococcus* enterotoxin A and B and toxic shock syndrome toxin, and geraniol shows good activity in modulating drug resistance in several gram-negative species (Solórzano-Santos and Miranda-Novales, 2012).

According to Mandras *et al.* (2016), thyme was also generally more effective than clove in the volatile experiment, tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Yadav and Munin, 2011).

#### Antimicrobial Activity of EOs

Plant essential oils have been known to possess various capacity to inhibit microorganisms (Chou *et al.*, 2013), and have been screened as potential sources of novel antimicrobial compounds (Solórzano-Santos and Miranda-Novales, 2012). However, it is difficult to compare the data with the literature because the antimicrobial activity of EOs and their components are influenced by several factors including chemical compositions and experimental conditions (Mandras *et al.*, 2016).

Antimicrobial activity of EOs have been shown against Gram-positive and Gram-negative bacteria, although Gram-negative organisms are slightly less susceptible than Gram-positive bacteria (Burt, 2004; Huang *et al.*, 2014; Azhdarzadeh and Hojjati, 2016). This can be attributed to the fact that Gram-negative bacteria have an outer membrane which is rigid, rich in lipopolysaccharide (LPS) and more complex, thereby limiting the diffusion of hydrophobic compounds through it, while this extra complex membrane is absent in Gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane (Hyldgaard *et al.*, 2012; Zinoviadou *et al.*, 2009).

Moreover, Gram-positive bacteria may ease the infiltration of hydrophobic compounds of EOs due to the lipophilic ends of lipoteichoic acid present in cell membrane (Chouhan *et al.*, 2015).

Researchers have focused on the antimicrobial activity of EOs against bacteria with a strong activity (Bilia *et al.*, 2014), such as the antibacterial activity of oregano EO against *E. coli* in eggplant salad reported by Skandamis and Nychas (2000), the superior antibacterial activity of *Thymus daenensis* EO against *E. coli* (Moghimi *et al.*, 2016) and the bacterial activity against meat-borne *E. coli* of black pepper EO (Zhang *et al.*, 2017).

As proven *in vitro*, Patrick *et al.* (2009) showed that many plants' essential oils such as tea tree oil and eucalyptus oil (and several others) have demonstrated promising efficacy against several bacteria and have been used clinically against multi-resistant strains. Another *in vitro* studies by Burt (2004) have demonstrated antibacterial activities of EOs against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10µl/ml.

Moreover, eugenol has also been shown to cause deterioration of the cell wall, lysis of cells, and prevention of enzyme action in *Enterobacter aerogenes*. Also, cinnamaldehyde of cinnamon trees and other species of the genus *Cinnamomum* is found as growth inhibitor of *Escherichia coli* and *Salmonella typhimurium* but does not disintegrate the outer membrane or deplete the intracellular ATP pool (Jess *et al.*, 2015). The EOs of *Forsythia koreana* is rich in oxygenated diterpenes, oxygenated monoterpenes and alcohols, it also have significant antibacterial activity against foodborne and pathogenic bacteria such as *S. enteritidis*, *E. coli*, *S. aureus*, *L. monocytogenes* (Yang *et al.*, 2015).

A study reported eugenol as the antifungal bioactive molecule from *Cinnamomum tamala*, with a minimum inhibitory amount of 9.5 and 8.2 µg against *Alternaria alternata* and *Curvularia lunata*, respectively (Heeret *et al.*, 2017). In a study by Guleria *et al.* (2008), α-cedrol was reported as the bioactive constituent of the essential oil from fresh leaves of *Thuja orientalis* with a minimum inhibitory amount (MIA) of 30.5 µg against *A. alternata*. Oregano essential oils showed high levels of antifungal activity against the fluconazole-susceptible *Candida glabrata* group, whereas the cinnamon essential oil showed the best antifungal activity against the fluconazole-resistant *C. glabrata* isolates

(Soares *et al.*, 2015). High antifungal activity of examined EOs, also against antibiotic-resistant isolates is according to recent evidence of some authors (Soares *et al.*, 2015; Rajkowska *et al.*, 2017; Flores *et al.*, 2016).

#### Mechanism of Action of Some EOs

The composition of EOs varies significantly because of plant different species, chemotypes, geographical origin, season and extraction procedure (Mandraset *et al.*, 2016). Some data suggests that components presented in small amounts in EOs, such as carvacrol, also play an important role in antimicrobial activity due to the possible synergistic action with other components (Bogavaset *et al.*, 2015; Chaftaret *et al.*, 2016; Moon and Rhee, 2016).

Factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions (Chouhan *et al.*, 2015). And because of the heterogeneous composition of EOs, it seems unlikely that there is only one mechanism of action or that only one component is responsible for the antimicrobial action (Zhang *et al.*, 2017).

There are different targets for mechanism of action of EOs on microbial cell, these include; Cell wall and membrane disturbance, ATP production, Protein synthesis, DNA damage, Intracytoplasmic changes, pH disturbance and Quorum sensing (Faleiro, 2011). Antimicrobial activity of EOs is therefore attributed to a cascade of reactions involving the entire bacterial cell (Macwan, 2016). It has been shown that the bioactive components present in EOs might attach to the surface of the cell, and thereafter penetrate to the phospholipid bilayer of the cell membrane. The structural integrity of cell membrane is disturbed by their accumulation, which can detrimentally influence the cell metabolism causing cell death (Bajpai *et al.*, 2013; Lvet *et al.*, 2011).

Moreover, Cui, *et al.* (2015) and Lakehal (2016) have reported that action of EOs on the integrity of cell membrane changes the membrane permeability which leads to loss of vital intracellular contents like proteins, reducing sugars, ATP and DNA, while inhibiting the energy (ATP) generation and related enzymes leading to the destruction of cell and leakage of electrolytes.

EOs components are characterized by their hydrophobicity, which enables them to partition into the lipids of the bacterial cell membrane, disturbing the cell structure, rendering them more permeable and leading to lysis and leakage of intracellular compounds (Bajpai *et al.*, 2013).

EOs can act as peroxidants in eukaryotic cells effecting inner cell membranes and organelles such as mitochondria, they also exhibit cytotoxic effects on living cells depending on type and concentrations, but they are usually non genotoxic (Anonymous, 2008).

Zhang *et al.* (2017) determined the mechanism behind the antibacterial activity of cinnamon EO against *E. coli* and *S. aureus* and reported that the bacterial cell membrane was destroyed after addition of cinnamon EO at the MIC level, whereas addition of cinnamon EO at the MBC levels resulted in the killing of the bacterial cell. EO of *Dipterocarpus gracilis* inhibited the growth of *Bacillus cereus* and *Proteus mirabilis* by acting on the cytoplasmic membrane as one of its targets. These activities could be exploited for food preservation in the food industry (Kolli *et al.*, 2016).

Also, mechanism of action of black pepper against *E. coli* indicates by the breakage of permeability of cell membrane which led to leakage of electrolytes, ATPs, protein and DNA materials which cost disorder, decomposition and eventually death (Zhang *et al.*, 2017). EOs of *F. koreana* caused physical destruction through loss of cytoplasm integrity (Chiang *et al.*, 2010).

Li *et al.* (2014) reported that the kinetic curves (antibacterial) of *Litsea cubeba* oil at 0.0625% (v/v) was able to prolong the lag phase growth of *Escherichia coli* cells to approximate 12 h while the cells were completely killed at 0.125% (v/v) within 2 h, as shown by transmission electron microscopy. Destruction of the *E. coli* outer and inner membrane might be due to the penetration of the *Litsea cubeba* oil with the observation of many holes and gaps on the damaged cells, which led to killing them eventually. The time-kill assay of *Foeniculum*

*vulgare* (Fennel) oil against *Shigella dysenteriae* revealed destruction of the membrane integrity (Diao *et al.*, 2014).

Moreover, Ahmad *et al.* (2011) showed that antifungal activity of *Coriaria nepalensis* essential oil (CNEO) against *Candida* isolates is due to the inhibition in the biosynthesis of ergosterol and disruption in the integrity of membrane. Similarly, another study described the utility in designing new formulations for candidosis treatment because of the antifungal activity of coriander essential oil on *Candida spp.*, in which it was reported that the fungicidal effect of coriander essential oil is a result of damage in the membrane of cytoplasm and subsequent leakage of intracellular components such as DNA (Silva, *et al.*, 2011).

Likewise, disruption of the fungal cell endomembrane system including the plasma membrane and mitochondria, i.e., the inhibition of ergosterol synthesis, malate dehydrogenase, mitochondrial ATPase, and succinate dehydrogenase activities was related to the antifungal activity of natural essential oil (EO) derived from turmeric (*Curcuma longa* L.) against *Aspergillus flavus* (Hua, *et al.*, 2017).

#### Conclusion and Recommendations

Essential oils (EOs) have strong ability as antimicrobial agents as they are shown in various researches to have antibacterial and antifungal activity because of their composition and nature of their functional group and synergistic effect. They have the ability to disrupt cell membrane, damage DNA, intrupts cell metabolism and protein synthesis. This paper review *invitro* studies on the effect of EOs on different strains of bacteria and fungi. Further studies are needed to determine and evaluate the *in vivo* activity of these EOs and their mechanism of action at molecular level.

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