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Review Article :

Essential oils: their antimicrobial activity and potential application against pathogens by gaseous contact – a review

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ABSTRACT

Essential oils (EOs) have been long recognized for their antibacterial, antifungal and antiviral properties. They are widely used in medicine for these purposes. The increased interest in alternative natural substances is driving the research community to find new uses and applications of these substances. EOs and their components show promising activities against many pathogens and spoilage microorganisms when tested *in vitro*. The use of combinations of EOs and their isolated components are thus new approaches to increase the efficacy of EOs in microorganisms control, taking advantage of their synergistic and additive effects. The purpose of this review is to survey of the methods used for the determination EOs activity by gaseous contact and mechanisms involved in the antimicrobial activities are also reported. EOs and their volatile constituents are used widely to prevent and treat human diseases. The possible role and mode of action of these natural products are discussed, as well as their bioactivity as antimicrobial agents.

Their application as natural products enhanced drug delivery and the therapeutic properties of essential oils in aroma therapy will also be outlined. Their antimicrobial properties and low toxicity make them ideal as additives in food, cleaning products, medicine aromatherapy.

INTRODUCTION

EOs has a wide spectrum of different impressive qualities (Pisseri *et al.*, 2008). Due to their multifunctional, it found a huge application area in medicine and aromatherapy. EOs show significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria, fungi, yeasts and viruses. Therefore, plants were used for the treatment of infectious illnesses since ancient times even though (Rios and Recio, 2005).

Medicinal plants are still in use nowadays, but now the investigation of the active agents is possible by modern means. EOs is becoming more popular because many synthetic drugs are connected with severe side effects. Volatile oils also represent an interesting alternative due to emerging resistance of microorganisms against synthetic agents. The *in vitro* antimicrobial activity of EOs has been studied against a number of microorganisms, usually using direct-contact antimicrobial assays, such as different types of diffusion or dilution methods, as reviewed by some literatures (Holley and Patel, 2005; Janisiewicz and Korsten, 2002; Tripathi and Dubey, 2004; Burt, 2004). Due to high hydrophobicity and volatility of the EOs, the direct-contact assays face many problems. In opposite, there were several attempts to utilize the volatile nature of EOs, which lead to high degree of inhibition by volatile components of EOs in vapor phase (Paster, *et al*., 1995; Hartmans, *et al*., 1995; Delaquis, *et al*., 1999; Inouye, *et al*., 2001a; Weissinger, *et al*., 2001; Suhr and Nielsen, 2003; Lopez, *et al*., 2005; Fisher, *et al*., 2009). Until now, no standard screening assay exists, and there are many methods used by different investigators, but any of them is suitably adapted for fast screening of large quantities of samples. This paper reviews the current knowledge concerning the vapor phase application methods to assess antimicrobial activity of EO, also their advantages were considered.

Essential oils

EOs are volatile substances with an oily consistency typically produced by plants. They can be liquid at room temperature and showing different colors ranging from pale yellow to emerald green and from blue to dark brownish red (Balz, 1999). They are synthesized by all plant organs and are stored in secretary cells, cavities, canals, epidermis cells or glandular trichomes (Bakkali *et al*., 2008). Several techniques can be used to extract EOs from different parts of the aromatic plants, including water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions. To evaluate EO quality several procedures are known, namely sensory evaluations, physicochemical tests and chromatrospectral techniques (Baser and Demitri, 2007). The latter allow a detailed qualitative and quantitative characterization of the EO, being capillary gas chromatography and mass spectrometry the main techniques employed (Lahlou, 2004; Rubiolo *et al*., 2010). Analytical guidelines published by several institutions such as European Pharmacopoeia, ISO, WHO are available and must be followed to assure the good quality of the commercialized EO and of the plants from which they are obtained. EOs, are complex mixtures of volatile constituent's biosynthesized by plants, which mainly include two biosynthetically related groups (Pichersky *et al*., 2006). These main groups include (terpenes, terpenoids) and (aromatic, aliphatic) constituents. Most of the antimicrobial activity in EOs is found in the oxygenated terpenoids (*e.g*., alcohols and phenolic terpenes), while some hydrocarbons also exhibit antimicrobial effects (Delaquis *et al*., 2002; Burt, 2004; Koroch *et al*., 2007).

Historical use

The term “essential oil” was used for the first time in the 16th century by Paracelsus von Hohenheim, who referred to the effective component of a drug as “Quinta essential” (Guenther, 1955). The first bactericidal experiment of EOs have been carried out by De la Croix in 1881 (Boyle, 1955). However, since those times the use of EOs in medicine gradually decreased (Guenther, 1948). Distillation as a method for extraction EOs was first used in the East (Egypt, India and Persia) (Guenther, 1948) more than 2000 years ago and was improved in the 9th century by the Arabs (Bauer *et al.*, 2001). By the 13th century EOs were being made by pharmacies and their pharmacological effects were described in pharmacopoeias (Bauer *et al.*, 2001) but their use does not appear to have been widespread

in Europe until the 16th century, from which time they were traded in the City of London (Crosthwaite, 1998). The use of tea tree oil for medical purposes has been documented in Australia at the end of the 18th century (Carson and Riley, 1993). The first experimental measurement of the bactericidal properties *via* vapors of EO has been carried out by De la Croix in 1881 (Boyle, 1955). Then proposed as early as 1960 (Maruzzella and Sicurella, 1960) and fully described by Lopez *et al*., 2005, is just a simple modification of disc diffusion assay used for nonvolatile compounds. Paper disc is moved from agar surface to the opposite site, on the lid of the Petri dish (PD).

Current use

The well-known use of EOs in aromatherapy constitutes is little more than 2% of the total market (Van de Braak and Leijten, 1999). The antibacterial properties of essential oils and their components are exploited in such diverse commercial products as dental root canal sealers, (Manabe *et al.*, 1987), antiseptics (Cox *et al.*, 2000) and feed supplements (Ilsley *et al.*, 2002).

***In vitro* methods to assess antimicrobial activity of essential oils**

The principles and practice of these test methods are explained in the literature (Barry, 1976; Davidson and Parish, 1989; Hodges and Hanlon, 1991). The NCCLS method for antibacterial susceptibility testing, which is principally aimed at the testing of antibiotics has been modified for testing EOs (Hammer *et al.*, 1999; NCCLS, 2000). Researchers adapt experimental methods to better represent possible future applications in their particular field. A number of methods used for antimicrobial activity studies have been surveyed in Table 1.

Table 1: Test methods used to assess the antimicrobial activity of EOs and their constituents

Test method	Purpose	EO or its constituents	Microorganism	References
Paper disc diffusion	activity	Citral, geraniol, carvacrol	*Salmonella typhimurium*	Kim *et al*., 1995
		Cinnamomum zeylanicum, Allium sativum, A. cepa, Thymus vulgaris, T. capitatus, Ocimum basilicum, Eugenia caryophyllata	*Bacillus cereus, Staphylococcus aureus, S. enteritidis, Aspergillus flavus, A. oryzae A. parasiliensis*	Dobre *et al*., 2011
		Satureja hortensis	11 bacterial and 3 fungal strains	Mihajilov-Krstev *et al*., 2009
Agar well diffusion	activity	*Cinnamomum zeylanicum*	*Aspergillus* spp.	Carmo *et al*., 2008
Agar dilution	strength	*Cymbopogon martini, Eucalyptus globulus, C. zeylanicum*	*A. fumigatus, A. niger*	Bansod and Rai, 2008
		Origanum vulgare	*A. flavus, A. fumigatus, A. parasiliensis, A. terreus, A. ochraceus*	Mitchell *et al*., 2010
Broth micro dilution	strength	*Cuminum cyminum, Satureja hortensis*	11 bacterial and 3 fungal strains	Carmo *et al*., 2008
Disc volatilization method	activity	*Cinnamomum zeylanicum, Allium sativum, A. cepa, Thymus vulgaris, T. capitatus, Ocimum basilicum, Eugenia caryophyllata*	*Bacillus cereus, Staphylococcus aureus, S. enteritidis, Aspergillus flavus, A. oryzae A. parasiliensis*	Dobre *et al*., 2011
		Cinnamon, Thyme, peppermint, tea tree, lavender, eucalyptus	*Haemophilus influenza, Streptococcus pneumonia, S. pyogenes, S. aureus*	Inouye *et al*., 2001a and b
		Clove	*Candida albicans, Dermophyton fluccasum, Microspoum, Trichophyton mentagrophytes, T. rubrum*	Chee and Lee, 2007
		Citron, lavender, tea tree, lemongrass, thyme, cinnamon	*A. fumigatus*	Inouye *et al*., 2000
Air washer coupled with air sampler	Activity with air-borne microbs	Citral, trans-cinnamaldehyde, perillaldehyde, citronellal, eugenol and carvacrol	All germ count present in air	Sato *et al*., 2006
End point titration	Antiviral activity	*Euphorbia cotinifolia, E. tirucalli*	*Herps simplex virus type- 2* (HSV-2)	Betancur-Galvis *et al*., 2002
Direct bioautography	Activity	the tea-tree oils, terpinen-4-01, a-terpineol and a-pinene	*Staphylococcus aureus, S. epidermidis and Propionibacterium acnes*	Raman *et al*., 1995

Antibmicrobial activity assessments of EOs based on gaseous contact

There are many difficulties on the determination of the antimicrobial activity of Eos *via* liquid contact. This is mainly due to its volatile properties as well as their insolubility in water. In particular their hydrophobic nature and high viscosity, which causes an irregular distribution throughout the culture medium as well as an unequal dilution. The essential oils activity in vapor phase has been less explored. The demand for new means to replacing the use of chemicals and the knowledge regarding the potential inhibition activity by volatile components of Eos (Caccioni *et al*., 1997) had forced the search for new control agents and new methods to evaluate the volatile components, especially for elimination of resistant bacterial species such as methicillin resistant *Staphylococcus aureus* (MRSA) and *Legionella pneumophila* (Doran *et al*., 2009, Mondello *et al*., 2009). Assurance of pharmaceutical processing environments can be attained by the use of essential oils in the vapor phase (Chapin and Musgnug, 2004; Lanciotti *et al*., 2004). Few studies are available on vapor phase antimicrobial activity of essential oils and these are concerned with cinnamon (*Cinnamon zeylanicum*), clove (*Syzygium aromaticum*), basil (*Ocimum basillicum*), rosemary (*Rosmarinus officinalis*), dill (*Anethum graveolens*), and ginger (*Zingiber officinalis*) (Lopez *et al*., 2005; Goni *et al*., 2009). Inouye *et al*., 2001a and b, investigated the antibacterial activity of 14 essential oils in gaseous phase against respiratory tract pathogens. Tyagi and Malik, 2010, studied the anticandidal activity (in liquid and vapor phase) of the lemon grass, menthe and eucalyptus Eos. The *in vitro* antimicrobial activity of several commercial EO against clinical strains isolated from onychomycosis was also studied by Tullio *et al*., 2007. For most strains, lower minimum inhibitory concentrations (MIC's) were obtained using the vapor phase method compared with direct contact.

The principles and practice of vapor phase test methods are explained in the literature (Inouye *et al*., 2006 and Tullio *et al*., 2007) but it appears that no standardized test has been developed for evaluating the antimicrobial activity against microorganisms. A number of researchers have surveyed the methods used for antimicrobial activity studies *via* vapor phase with EOs as shown in Table 2.

Inverted Petri dish technique

The so-called microatmosphere method (Lee *et al*., 2008). It was first reported by Maruzzella *et al*., 1959 and 1960; Kienholz 1959, and then has been used by subsequent researchers (Gocho, 1991). Disc moistened with essential oil is attached to the lid of a Petri dish, which is then inverted and incubated. The results are presented as the diameter of the microorganism growth inhibition zone (Didry *et al*., 1993; Bishop and Thornton, 1997; Domokos *et al*., 1997).

Disc volatilization method

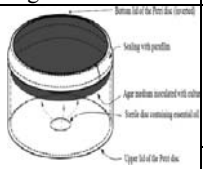
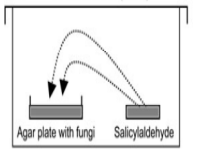
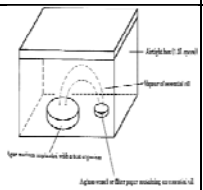
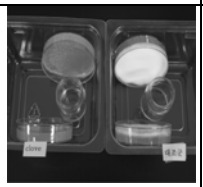
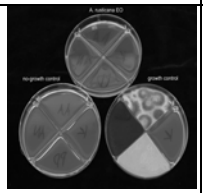
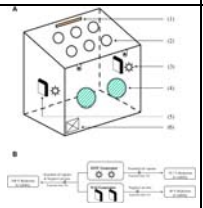
Solution of essential oil was added to 6 mm diameter sterile blank filter discs and placed in the center of the cover of the Petri dish in which was previously covered with a thin layer of medium to avoid the adsorption of essential oils to the cover. The dishes were then sealed using sterile laboratory parafilm to avoid evaporation of the essential oils then followed by incubation (Lopez *et al*., 2005). The effectiveness of the essential oils was calculated by measuring the diameter (in mm) of the zone of microorganism growth inhibition above the disc.

Vapor-agar contact method

Antifungal activity was determined by the vapor-agar contact method previously described by Sekiyama *et al*., 1996 with a slight modification by Nakahara *et al*., 2003. Fungal spores were inoculated in the center of PDA plates (40 mm diameter) which were aseptically placed in a chamber (capacity, 300 mL) without lids. Tested volatile compounds were introduced into the chambers followed by proper sealing and incubation. The inhibitory activity was evaluated by measuring the diameter of

colonies formed by the tested microorganisms.

Table 2: Overview of studies testing the antimicrobial activity of essential oils or their components by gaseous contact method.

Test method	Purpose	Essential oil	Microorganism	Figure	Reference
Inverted Petri dish	activity	*Leptospermum petersonii*	*A.ochraceus, A. flavus, A. niger*		**Kim E. and Il-Kwon P. (2012)**
Disc volatization	activity	*C. zeylanicum, T. capitatus, Eugenia caryophyllata*	*B. cereus, S. aureus, S. enteridis, E. coli*		**Dobre *et al.*, 2011**
Vapor agar contact	activity	*Cymbopogon nardus*	*Asergillus spp., Penicillium spp., Eurotium spp.*		**Nakahora *et al.*, 2003**
		salicylaldehyde	*A.parasiticus*		**Kim *et al.*, 2011**
Airtight box	strength	*clove*	*C. albicans, Epidermophyton floccosum, Microsporum audouinii, T. mentagrophytes, T. rubrum*		**Chee and Lee, 2007**
Phytatray chamber	strength	*lemongrass*	*H. influenza, S. pyogenes, S. pneumonia, S. aureus, E. coli*		**Inouye *et al.*, 2001a**
Divided Petri dish	activity	*A.sativum,* *O. compactum, O.vulgaris*	*S,aureus, S.enteritidis, P.aeruginosa,* *A.niger*		**Kloucek *et al.*, 2012**
Kill time	strength	*lemongrass*	*C.albicans*		**Tyagi and Malik, 2010; Tyagi *et al.*, 2012**

Airtight box

Under the prior conditions, the air space is too small to measure the vapor concentrations of EO. So that Inouye *et al*., 2001b employed an airtight box of 1L air capacity for the measurement Eos vapor activity. Subsequently, the antimicrobial activity of essential oils in the gaseous state was evaluated in a closed system using an airtight box capacity until 1.3L (Inouye, 2003).

The gaseous activity was expressed by a minimum inhibitory dose (MID) per unit space of air that allowed no microbial growth after incubation. Now the airtight box manufactured in Jalle Co., Tokyo, Japan.

Phytatray chamber assay

Disposable Phytatray chamber with sterilized lid was used as a chamber containing EO and microorganism. The method compare between more than one microorganism at the same EO concentration. Inhibitory activities of essential oil were investigated as radial growth or spore germination (Chee and Lee, 2007).

Divided Petri dish method

The tests were performed in 90 mm Petri dishes (PD) divided into four sections according to Kloucek *et al*., 2012. Into each section five ml of warm agar were poured, as well as into the lid. After solidification, three different microorganisms were inoculated into three sections; the fourth one was left uninoculated as a contamination control. Eos solution was pipetted on round sterile filter paper, and left to dry for one minute. Finally, the filter paper was laid into the PD on walls, to be the distance between paper and agar surface was approximately 2 mm. The PD was closed with its lid containing solidified agar then incubated. Blank filter papers with and without ethyl acetate served as negative control.

Kill time method

Avila *et al*., 1999 have conducted the kill time method for the first time. Tyagi and Malik 2010 and Tyagi *et al*., 2012, studied efficient essential oil vapours in a compact chamber made up of acrylic material (size 50 cm × 50 cm; W× L). The height of the chamber was 50 cm on the back side and 25 cm at the front side. The front side of the chamber had gloves through which the things inside the chamber could be handled without opening the chamber. Prior to exposure the chamber was cleaned with ethanol and UV sterilized. Two essential oil evaporating machine were fixed in this chamber as described earlier (Tyagi *et al*., 2008). Appropriate serial dilution of the culture was plated on PDA plates. After a particular time period the plates were detached, closed and incubated (Tyagi and Malik, 2010; Tyagi *et al*., 2012).

EOs antimicrobial activity vapor terms

Many terms have cited by most researchers and not surveyed yet. For example the minimum inhibitory concentration (MIC) is cited by most researchers as a measure of the antimicrobial performance of EOs. The definition of the MIC differs between publications and this is another obstacle to compare between studies. In some cases the minimum bactericidal concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC. In addition, the term minimum cidal concentration' has been used but is not defined (Hammer *et al*., 1999). The terms minimum lethal dilution (or concentration) (Janssen, 1989; Janssen, *et al*., 1987) and minimum inhibitory dilution (Janssen, 1989) appear to have fallen out of use. A list of the most frequently used terms in antimicrobial activity testing of EOs by gaseous contact are surveyed in Table 3.

Mode of essential oil vapors action

Eos has been proven to perform well *in vitro* as antimicrobials but their mode of action is still largely unknown. EOs vapors adhere around microbial cells, enables to traverse the bacterial cell wall causing increased permeability and leakage of ions and other essential molecules to bacteria **(**Burt, 2004). Phenolic compounds involve disruption of the bacterial cell membrane, proton motive force, electron flow and active transport as well as coagulation of cell contents (Burt, 2004). Figure 1a, summarized the sites of action for EO components (data source, Burt, 2004).

Table 3: Terms used in antibacterial activity testing

Term	Definition	Reference
Gaseous activity	Expressed by minimum inhibitory dose (MID) per unit of air space that allowed no microbial growth after incubation	**Inouye *et al*., 2003**
Vapor phase	The gaseous state of EO, that allows free attachment to the microorganism (indirect action)	**Inouye *et al*., 2003**
	In comparison, the direct action result from liquid of EO, which allowed to contact directly to the microorganism in broth or solid media	**Pinto *et al*., 2009**
Minimum inhibitory concentration (MIC)	Defined as the MID per unit air space required to suppress the growth of microorganism in a closed system	**Souse *et al*., 2012**
	Expressed as weight per unit volume (mg/L air), that did not allow bacterial growth	**Inouye *et al*., 2001a**
	Defined as lowest concentration (mg/L in air) of volatile compounds which inhibited colony formation of test fungi by 50%.	**Nakahara *et al.*, 2003**
	Determined as the lowest concentration at which no growth of fungal cells were observed	**Chee and Lee, 2007**
	Determined as the lowest concentration of EO preventing visible growth of *C. albicans*	**Tyagi and Malik, 2010**
	Expressed as the lowest volume of EO per volume unite of atmosphere, which absolutely inhibit visible growth of the microorganism	**Marija *et al*., 2009; Kloucek *et al*., 2012**
Fungicidal concentration	The lowest concentration at which the fungal pathogen failed to grow and were not regrow after transfer to EO0free plate	**Tyage and Malik, 2010**
Fungistatic concentration	The lowest concentration at which the fungal pathogen failed to grow but was regrow after transfer it onto EO-free plate.	**Tyage and Malik, 2010**
Bactericidal concentration	Lowest concentration at which bacterial pathogen failed to grow in broth, and are not cultured when broth is plated onto agar	**Smith-Palmer *et al*., 1998**
Bacteriostatic concentration	Lowest concentration at which bacterial pathogen failed to grow in broth, but are cultured when broth is plated onto agar	**Smith-Palmer *et al*., 1998**

Also mechanism of action by carvacrol in cytoplasmic membrane (data source Ultee *et al*., 2002 and Calsamiglia *et al*., 2007) showed in figure 1b. Generally, we can survey the mode of action of EO towards microorganisms as follow:

1- Cell morphology

a- Forming elongated filamentous forms on *E. coli* after treatment with essential oil; normal cells: 3–5 μm in length; elongated cells: 10–25 μm in length (Pattnaik *et al*., 1995).

b- Alteration of cell shape: wild type cells of *M. ssential* exhibit a flask-shaped morphology, whereas tea tree oil-treated strains form ovoid or round cells after treatment with tea tree oil (Harkenthal *et al*., 2000).

c- Changes in cell morphology and damages to cell wall Rammanee and Hongpattarakere (2011).

2- Disruption of outer membrane in Gram-negative bacteria

a- Damages to the outer membrane was recognized according to, Helander *et al*., 1998; Fisher and Phillips, 2009.

3- Cytoplasmic membrane

a- Inhibition of cell respiration sites of *E. coli; S. aureus; Candida albicans* after treatment with tea tree oil. (Carson *et al*., 2006; Cox *et al*., 1998 and 2000).

b- Inhibition of oxygen uptake, respiratory electron flow and oxidative phosphorylation of *R. sphaeroides* after treatment with thymol, carvacrol and other monoterpene alcohols (Knobloch *et al*., 1986).

c- K^+ leakage in *E. coli* and *S. aureus* caused by tea tree oil, farnesol and nerolidol (Cox *et al*., 1998 and 2000; Shepira and Mimran, 2007).

d- Depletion of intracellular ATP concentration in *E. coli* and *L. monocytogenes* after treatment with oregano and cinnamon oils (Helander *et al*., 1998; Oussalah *et al*., 2006).

e- Changes in membrane permeability induced on *C. albicans, C. glabrata* and *Saccharomyces cerevisiae* by treatment with tea tree oil (Hammer *et al*., 2004).

f- Changes in membrane fluidity in *Candida albicans; C. glabrata* and *S. cerevisiae* caused by tea tree oil (Hammer *et al*., 2004).

g- Reduction of ergosterol content in the cell membrane in *Aspergillus fumigates* by treatment with *Thymus pulegioides* oil. Also, Changes in yeast cell's ergosterol biosynthesis (Ahmad *et al*., 2011)

h- ATP leakage from the cells (Oussalah *et al*., 2006).

i- Changes in membrane properties: Effects on membrane melting temperature, fluidity and phase separation (Pérez-Fons *et al*., 2006; Cristani *et al*., 2007)

4- Cell wall

a- Formation of extracellular blebs in *E. coli* after treatment with tea tree oil and lemongrass (Ogunlana *et al*., 1987; Gustafson *et al*., 1998).

b- Cell lysis in *S. ssential; E. coli* and *B. subtilis* caused by oregano oil, thyme oil; oregano oil and clove oil (Horne *et al*., 2001; Rhayour *et al*., 2003).

c- Eos hydrophobicity enables them to traverse the bacterial cell wall causing increased permeability and leakage of ions and other essential molecules to bacteria (Burt, 2004).

5- Cell division

a- Total inhibition of cell division caused by tea tree oil (Reichling *et al*., 2002).

6- Anti-resistance plasmid activity

a- Elimination of resistant-plasmids in *E. coli* after treatment with peppermint, rosemary, eucalyptus and menthol oils (Schelz *et al*., 2006).

7- Cell cytoplasm

a- Formation of condensed, filamentous, electron-dense material in the cytoplasm in *S. aureus* after treatment with tea tree oil (Reichling *et al*., 2002).

8- Intracellular

a- pH disturbance in the intracellular of *E. coli* and *S. typhi* when the bacterial cells were treated with the MIC value of mustard essential oil (Turgis *et al*., 2009). In another study, oregano oil caused an increase in potassium and phosphate leakage in *S. aureus* and *P. aeruginosa* as well as a marked decrease in the internal pH for both bacteria (Lambert *et al*., 2001).

b- In the study conducted by Becerril *et al*., 2007, *E. coli* cells treated with oregano EO exhibited intracytoplasmic changes, whereas coagulated material appeared in specific areas located to the cell wall and apical ends.

Quorum sensing (QS): the EO of rose, geranium, lavender, rosemary and clove seem to be very effective on as QS inhibitors (Szabó *et al*., 2010).

10- Inhibition of particular enzymes

a- Inhibition of the cell wall synthesizing enzymes β-(1,3)-glucan synthase and chitin synthase (Bang *et al*., 2000)

11- Complex reaction mechanism

a- Reaction with thiol groups in a variety of targets (Luciano and Holley, 2009) and competitive binding of thiol groups (Juven *et al*., 1994).

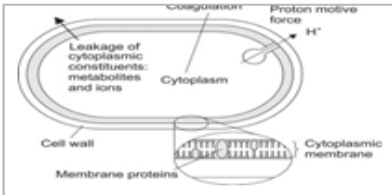


Fig. 1a: Locations and mechanisms in the bacterial cell sites of action for EO components: degradation of the cell wall (Helander *et al*., 1998); damage to cytoplasmic membrane (Ultee *et al*., 2002); damage to membrane proteins (Ultee *et al*., 1999); leakage of cell contents (Lambert *et al*., 2001); coagulation of cytoplasm (Gustafson *et al*., 1998) and depletion of the proton motive force (Ultee and Smid., 2001). Data source: Burt, 2004.

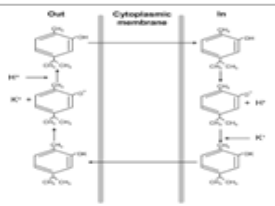


Fig. 1b: Hypothesized mechanism of action of carvacrol in the cytoplasmic membrane (Ultee *et al*., 2002). The undissociated carvacrol diffuses through the cytoplasmic membrane toward the cytoplasm and dissociates, thereby releasing its proton to the cytoplasm. Later, undissociated carvacrol returns by carrying a potassium ion (or another ion) from the cytoplasm to the extracellular medium. Carvacrol liberates this ion in the extracellular medium and recovers additional hydrogen to close the cycle. Data source: Calsamiglia, 2007.

Factors affecting the efficacy of EOs activity by gaseous contact

Several factors are distinguished as key factors in essential oil activity evaluation by gaseous contact method as follow:

a. Volatility: the difficulties on the determination of the antimicrobial activity of EOs are well recognized and it's mainly due to its volatile properties. Also, vapor pressure of each component of EO starts to spread according to their volatility (Rios *et al*., 1988). Although, carvacrol possesses low volatility, its vapors has been reported to be absorbed into the agar layer in large amounts (Inouye *et al*., 2001a).
b. Evaporation speed and stability: EOs activity strongly depends on speed of evaporation of its active constituents and its stability (Friedman *et al*., 2002).
c. Exposure time: Antimicrobial activity of EO vapors was dependent on exposure time. Inouye *et al*., 2003, demonstrated that the air vapor concentration of wild thyme was maximal at 1h and then decreased gradually (after 24h).
d. Chemical structure: The chemical structure of the individual EO components affects on their antimicrobial activity (Dorman and Deans, 2000). The importance of the presence of the hydroxyl group in phenolic compounds such as carvacrol and thymol has been confirmed (Ultee *et al*., 2002). In this way, carvacrol, a phenolic compound containing an alcohol group in its chemical structure seems to be a good barrier compared to aldehyde compounds (e.g., cinnamaldehyde, citral) because the hydroxyl group has less affinity for water than for the carbonyl groups.
e. Temperature: EOs especially the active components was a potent inhibitor (*via* vapor phase) against microbes at ambient temperatures (Nakahara *et al*., 2003). The activity of most EOs increases as the temperature increases. Greater temperature causes degradation to EO vapors and weakens its activity. Generally, increased temperature can accelerate the migration or evaporation of the active agents in EOs, while refrigeration slows down the migration rate (Quintavalla and Vicini, 2002).
f. Growth phase and location of microorganism: The larger number of microbs requires more time to destroy all of them. 30 minutes are required to kill 10 *Bacillus atrophaeus* spores but 3 hours to kill 100,000 spores. The location of microorganisms also must be considered when factors affecting the efficacy of Eos are assessed. Crevices, joints and channels are more difficult to disinfect than are flat-surface equipment.
g. Innate resistance of microorganisms: Microorganisms vary greatly in their resistance to chemical, EO liquids, vapors and processes, according to resistance mechanisms. For example, spores are resistant to EO because its coat and cortex acts as a barrier, also mycobacteria have a waxy cell wall that prevent EO entry. To destroy the most resistant types of microorganisms, we needs to increase the exposure times and concentration to achieve complete destruction.
h. Concentration: The more concentration of any disinfectant lead to greater its efficacy and shorter time necessary to active microbial kill. Generally, that fact not recognized with EO vapors, however all EOs are not similarly in affected time, it depend on the potency of the EO.

Application of essential oil vapors as antimicrobial agents

a- Aromatherapy vapor inhalation

Inhalation by EOs vapors used as aromatherapy treatment *via* gaseous technique. Since many EO are used to alleviate respiratory diseases by steam inhalation which was a very popular application method. Five drops from EOs were added to steaming water and inhale the aroma using a towel tent placed around head. It seems that EOs vapors not only works through inhalation, but also through absorption into the tissues of the chest (Amrish and Kumar, 2009; Cal and Sopala, 2008). Applications of EOs vapors inhalation provide benefit for both purulent and non-purulent respiratory problems, such as bronchitis, asthma and chronic obstructive pulmonary diseases. Inhalation of peppermint essential oil vapors has been suggested as an adjunct in combined multidrug therapy in patients with disseminated and infiltrative pulmonary tuberculosis. The action of the oil is mainly due to the antimicrobial activity of its volatile constituents (Shkurupii *et al.*, 2002). Cinnamon and clove oils also showed an inhibition to different Gram (+)ve and Gram (−)ve pathogenic bacteria from the vapor phase (Lopez *et al.*, 2005). In striking case according to Sherry and Warnke (2004), 28-years-old female [diagnosed with tuberculosis (TB) by sputum culture and chest x-ray], who had refused conventional treatment, employed *Eucalyptus globulus* EO inhalation (3 ml EO to 500 ml boiling water) three times daily for three weeks. After 10 days the malaise reduced, appetite improved, cough subsided and weight was gained. Objectively, the temperature normalized and sputum cultures were negative, although erythrocyte sedimentation rate remained high at 110 (normal range 0-20) and there was no change in the chest x-ray.

b- EOs as air disinfectants

The EOs of *Pelargonium graveolens* and *Cymbopogon flexuosus* were used in a mixture which contained geranial (22.3%) and b-citronellol (18.4%) (as the major constituent in each oil, respectively). The antimicrobial agency of this mixture used as vapor and evaluated in different tests using a special vapor machine. Therefore the number of air-borne bacteria was reduced to 11% in an office room within 15h. This EOs blend could be applied as air disinfectant. Moreover, it demonstrated inhibitory activity against *A. baumanii*, *C. difficile*, MRSA and vancomycin-resistant *Enterococcus faecium* (VRE) strains in *in vitro* tests (Doran *et al*., 2009). *Salvia officinalis* contained an EO which contained bthujone (17.8%), 1,8-cineole (16.3%) and camphor (14.2%). Due to the observed high vapor agency, it might find application as disinfectant against airborne microorganisms (Bouaziz *et al*., 2009). That result indicated that EOs can reduce the number of air-borne bacteria. This indicates their possible application as air disinfectants.

c- Healthcare Environments

EOs vapors can be used as hospital wards and communal areas. Also, in community healthcare environments such as: care homes, nursing homes, surgeries rooms and ambulances.

6- Advantages of vapour contact technique

a- It can treat large area.
b- Do not require direct contact with liquid oils, so that, it suitable for use as disinfectant of rooms and cleaning products.
c- The oil might be also used as inhalation therapy against most pathogens such as respiratory tract.
d- Vapour phase method allows best results due to EO high volatility.
e- EOs are highly effective at ambient temperature.
f- Most EOs constituents is more stable in gaseous contact, which lead to more potent.
g- Lower concentrations can be used (lower MIC) compared with liquid contact.
h- Lipophilic volatiles nature are thought to be absorbed by fungal mycelia efficiently *via* gas phase.
i- Highly active against fungi because lipophilic nature of

mycelia coupled with large surface area relative to the volume of fungus.

7- Safety

- Safety confirmed by National Toxicology Program (NTP) in lifetime animal studies (1983, Technical Report No. 223, NTP)
- Salmonella assay also showed eugenol to be antimutagenic (1995, Azizan & Blevins, East Tennessee State University).
- Animals studies at the University of Wisconsin Medical School achieved similar results in an animal model.
- The Joint FAO/WHO Expert Committee on Food Additives estimated an acceptable human daily intake of eugenol of up to 2.5mg / kg body weight
- The German Commission monograph prescribes mouthwashes consisting of 1 to 5% clove essential oil as an oral antiseptic and topical anesthetic, stating that it has "antibacterial, antifungal, antiviral" action

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ARABIC SUMMARY

الزيوت الطياره: كمضادات ميكروبية والتطبيقات المحتملة لها ضد مسببات الأمراض نتيجة لأبخرتها الغازية – بحث مرجعى

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عرفت الزيوت الطياره كمضادات للبكتيريا والفطريات والفيروسات منذ فترة طويلة ولهذا تستخدم على نطاق واسع في الطب لهذه الأغراض. الاهتمام المتزايد بالمواد الطبيعية البديلة يقود المجتمع البحثى لإيجاد استخدامات وتطبيقات جديدة للزيوت الطياره. الزيوت الطياره ومكوناتها الأساسيه تظهر أنشطة واعدة ضد العديد من مسببات الأمراض (الكائنات الدقيقة)عند دراستها مخبريا. استخدام مجموعات من الزيوت الطياره أو مكوناتها الأساسيه المعزولة منها تؤدى الى نهج جديدة لزيادة فعالية تلك الزيوت في مجال مكافحة الكائنات الحية الدقيقة وذلك للاستفادة من آثارها و مؤازره بعضها البعض . و الغرض من هذا الاستعراض هو التعرف على الطرق المستخدمة لتحديد نشاط تلك الزيوت الطياره عن طريق أبخرتها وآليات عملها كمضادات ميكروبيه. تناولت المقاله ايضا مناقشة لدور تلك الزيوت وطريقة عملها كمنتجات طبيعية ، فضلا عن نشاطها الحيوي بالمقارنه بالمضادات الحيويه .

أيضا تطبيقها على النحو المبين (كأبخره غازيه) يعزز استخدامها كمنتجات طبيعية وكذلك خصائصها العلاجية. خصائص هذه الزيوت كمضادات للميكروبات بالأضافه الى عدم سميتها تجعلها مثالية كمواد علاجيه أو كإضافات في المواد الغذائية أو منتجات التنظيف والتطهير.