

## Antibacterial activity of six essential oil against some pathogenic bacteria

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### Abstract

In the present study effort have been made to find the anti-bacteria effect of six medicinal plant essential oil namely, *Ocimum basilicum* (Basil oil), *mentha arvensis* (Cormint oil), *Zingiber officinale* (ginger oil), *citrus limon* (lemon oil), *thymus mastichina* (Marjoram oil) and *mentha spicata* (Spearmint oil) were assessed for anti bacterial activity against therefore pathogenic bacteria such as *B.cereus*, *E.aerogens*, *E.coli*, *K.oxytoca*, *K.pneumoniae*, *L.vulgaris*, *P.aeruginosa*, *P.mirabilis*, *S.paratyphi*, *S.facsalis*, *S.typhi*, *S.aureus* and *V.cholera*. The activity was measured in terms of zone of growth inhibition in mm. These included animal and plant pathogens, food spoising and spoilage bacteria. The essential oils exhibited considerable inhibitory effects against all organism. The essential oil show the strong growth inhibiting activity was found in the lemon oil and moderate growth inhibition was found in the essential oil of Basil oil, ginger oil Marjoram oil, and spearmint oil. However, the lowest range of growth inhibition was found in the cormint oil. © 2010 IJRSR. All rights reserved.

Key words: Antibacterial activity, essential oil, growth inhibition.

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### 1. Introduction

Chemicals from nature have been a part of human civilization ever since our early ancestors began exploiting natural compounds to improve and enrich their own lives Agosta, (1996). In spite of modern improvements in chemotherapeutic techniques, infectious disease are still an increasingly important public health issue world resource institute (2000). Now-a-days the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of he currently available antimicrobial agents. Action must be taken to reduce this problem such as controlling the use of antibiotics, carrying out research to investigate drugs from natural sources and also drugs that can either inhibit the growth of pathogen or kill them and have to or least toxicity to the host cell are considered conditions for developing new antimicrobial drugs. 252 traditional medicines have been selected by WHO, of which 11.1% come from plants and 8.7% from animals Marques (1997).bThe antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s (Martindale 1910; Hoffman & Evans 1911). Plant volatile oils are generally isolated from nonwoody plant material by distillation methods, usually steam or hydrodistillation, and are vairbale mixtures of principally terpenoids, specifically monoterpenes (C<sub>10</sub>) and sesquiterpenes (C<sub>15</sub>) although diterpenes (C<sub>20</sub>) may also be present, and a variety of low molecular weight aliphatic hydrocarbons (linear, ramified, saturated and unsaturated),

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acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen – and sulphur – containing bcompounds, coumarins and homologues of phenylpropanoids. Terpenes are amongst the chemicals responsible for the medicinal, culinary fragrant uses of aromatic and medicinal plants. 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 (Dorman 1999). The antimicrobial properties of plant volatile oils and their constituents from a wide variety of palnts have bene assessed (Lis-Balchin & Deans 1997) and reviewed (Janssen et al. 1987; Jain & Kar 1971; Inouye et al. 1983; garg & Dengre 1986; Rios et al. 1987; Sherif et al. 1987; Deans & Svoboda 1988, 1989; Cruz et al. 1989; Recio et al. 1989; Crespo et al. 1990; Carson et al. 1995; Larrondo et al. 1995; Pattnaik et al. 1995; Carson et al. 1996; Nenoff et al. 1996; Rios et al. 1988). It is clear from these studies that these plant secondary metabolites have potential in medical procedures and applications in the cosmetic, food (Ueda et al. 1982; Shelef 1983; Jay & Riverse 1984; Gallardo et al. 1987; Baratta et al, 1998a, b; Youdim et al. 1999) and pharmaceutical industries (Janssen et al. 1988; Pelissier et al. 1994; Shapiro et al. 1994; Cai & Wu 1996). Investigations into the antimicrobial activities, mode of actions and potential uses of plant volatile oils have regained momentum. There appears to be a revival in the use of traditional approaches to protecting livestock and foods from disease, pests and spoilage in industrial courtiers. This is especially true in regard to plant volatile oils and their antimicrobial evaluation, as can be see from the comprehensive range of organisms against which volatile oils have been tested. These have included food spoiling organisms (Zaika et al. 1983, 1984b; Connor and Beuchat 1984a; Janssen et al. 1988; Ouattara et al. 1997)

and food poisoning organisms (Beuchat 1976; Tharib et al. 1983; Deans & Ritchie 1987; Lis-Balchin and Deans 1997), spoilage and mycotoxigenic filamentous fungi (Knobloch et al 1989), Pathogenic and dimorphic yeasts (Boonchild & Flegel 1982; Ghannoum 1988) and animal and plant viruses (Ieven et al. 1982; Ghannoum 1988) and animal and plant viruses (Ieven et al., 1982; Romerio et al. 1989). The aims of the present investigation were to assess the antimicrobial activities of the six essential oil against thirteen pathogenic bacteria.

## 2. Materials and methods

### 2.1. Collection of plants

Six essential oil yielding plants species from three families (Table 1) were collected between March and April 2009. The plant samples were then dried for several weeks at room temperature. The voucher specimen has been deposited in the laboratory of Zoology, Annamalai University, Annamalai Nagar Tamil Nadu.

### 2.5. Assessment of inhibition of bacterial growth

The measurement of growth inhibition was carried out in agreement with the method of Deans & Ritchie (1987) using Iso-Sensitest agar. Cells from cultures grown on Iso-Sensitest agar. Cells from cultures grown on Iso-Sensitest slopes were inoculated using a sterile loop into fresh Iso-Sensitest broth and incubated overnight at 25°C. (10 ml volume, 10<sup>5</sup> ml<sup>-1</sup> final concentration). 1ml amounts of each culture were pipetted into separate sterile Petri dishes to which 20 ml amounts of molten Iso-Sensitest agar (45°C) were added. Once set, wells of 6mm diameter were made in the centre of each agar plate using a Pharmacia gel punch (Uppsala, Sweden), into which 15µl test substance was added. The plates were then left undisturbed to allow diffusion of the sample into the agar, and incubated inverted in the dark at 25°C for 48h. Following this, zones of growth inhibition were measured using Vernier calipers.

## 3. Results

In the present study effort have been made to find

**Table 1: Investigated plant material**

Botanical name	Family	Part used	Oil name	Yield (%)
<i>Ocimum basilicum</i>	Lamiaceae	Herb	Basil	3.3
<i>Mentha arvensis</i>	Lamiaceae	Herb	Cornmint	1.7
<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	Ginger	4.3
<i>Citrus limon</i>	Rutaceae	Peel	Lemon	5.6
<i>Thymus mastichina</i>	Lamiaceae	Flower/leaf	Marjoran	3.4
<i>Mentha spicata</i>	Lamiaceae	Flower herb	Spearmint	3.8

### 2.3. Essential oil distillation

The essential oils were obtained by hydrodistillation in a Clevenger type apparatus for eight hours. The oil thus obtained were dried over

### 2.4. Bacterial strains

Thirteen bacterial strains were used to assess the antimicrobial properties of the test samples. Were carried out thirteen bacterial strains – *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lactobacillus vulgaris*, *Pseudomonas aeruginosa*, *Protens mirabilis*, *Salmonella paratyphi*, *Streptococcus faecalis*, *Salmonella typhi*, *Staphylococcus aureus*, and *Vibrio cholera*. Thirteen bacterial strains were maintained on 130-sensitest agar slopes (cm 471) (Oxoid, UK) at room temperature. All bacterial strains were obtained from the Department of medical microbiology, Raja Muthiah Medical College, Annamalai University, Tamil Nadu, India. All strains were subcultured every 2 weeks.

the anti-bacteria effect of six medicinal plant essential oil namely, *Ocimum basilicum* (Basil oil), *mentha arvensis* (Cormint oil), *Zingiber officinale* (ginger oil), *citrus limon* (lemon oil), *thymus mastichina* (Marjoram oil) and *mentha spicata* (Spearmint oil) were assessed for anti bacterial activity against therefore pathogenic bacteria such as *B.cereus*, *E.aerogenes*, *E.coli*, *K.oxytoca*, *K.pneumoniae*, *L.vulgaris*, *P.aeruginosa*, *P.mirabilis*, *S.paratyphi*, *S.facalis*, *S.typhi*, *S.aureus* and *V.cholera*. The activity was measured in terms of zone of growth inhibition in mm. These included animal and plant pathogens, food poisoning and spoilage bacteria. The essential oils exhibited considerable inhibitory effects against all organism. The essential oil show the strong growth inhibiting activity was found in the lemon oil table 2 (> 90.0 mm was inhibiting the eight pathogenic bacteria viz., *E.coli*, *K.oxytoca*, *K. Pneumonia*, *P.mirabilis*, *S.Paratyphi*, *S.typhi*, *S.aureus* and *V.cholera*) and moderate growth inhibition was found in the essential oil of Basil (>90.0 mm was inhibiting the five pathogenic bacteria viz., *K.oxytoca*, *K.pneumoniae*, *S.paratyphi*, *S.aureus* and *V.cholera*), ginger oil (> 90.0 mm was inhibiting the four pathogenic bacteria. viz., *K.oxytoca*,

*P.aeruginosa*, *S.facsalis*, and *V.cholera*), majoram oil (> 90.0 mm was inhibiting the three pathogenic bacteria viz., *L.vulgaris*, *S.paratyphi*, and *S.typhi*, ). Spearmint oil (> 90.0 mm was inhibiting the two pathogenic bacteria viz., *K.oxytoca* and *S.typhi*) However the lowest range of growth inhibition was found in the corumint oil (10.0 ± mm in *K.pneumoniae*, and 11.1±0.1 mm in *E.aerogens*)

### 3.1. Antibacterial activity of plant volatile oils

The antibacterial activities of the plant essential oils are in general agreement with previously reported studies on the volatiles oils of *P.nigrum* (Deans & Ritchie 1987; Ouattara et al. 1997), *S. aromaticum* (Deans et al. 1995; Cai and Wu 1996; Hao et al. 1998; Smith – Plamer et al. 1998), *P.graveolens* (Pattnaik et al. 1996), *M. fragrans*, *O.ulgare* (Kivanc & Akgul 1986) and *T. vulgaris* (Kinanc & Akgul 1986; Smith-Plamer et al. 1998). All the bacterial strains demonstrated some degree of sensitivity to the plant volatile oils tested, although the growth of a number of bacteria were uninhibited by specific volatile oils.

## 4. Discussion

The activity of the oils would be expected to relate to the respective composition of the plant volatile

bactericidal or bacteriostatic agents, depending upon the concentration used (Pelczer et al., 1988). These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data (Nadal et al., 1973; Suresh et al., 1992; Lattaoui & Tantaoui-Elaraki 1994; Belaiche et al 1995; Jeongmok et al 1995; Chari et al 1996; Mahmoud 1994; Meena & Sethi 1994; Shapiro et al., 1994; Sivropoulou et al., 1996; Hili et al., 1997; Lis – Balchin & Deans 1997). Furthermore, the stereochemistry has an influence on bioactivity. It was observed that  $\alpha$ -isomers are inactive relative to  $\beta$ -isomers, e.g.  $\alpha$ -pinenel cis-isomers are inactive contrary to trans-isomers, e.g. geraniol and nerol; compounds with methyl-isopropyl cyclohexane rings are the most active; or unsaturating of the cyclohexane ring further increases the antibacterial activity, e.g. terpinolene, terpineol and ter-pineolene (Hinou et al., 1989). Investigations into the effects of terpenoids upon isolated bacterial membranes suggest that their activity is a function of the lipophilic properties of the constituent terpenes (Knobloch et al. 1986), the potency of their functional groups and their aqueous solubility (Knobloch et al., 1988). Their site of action appeared to be at the phospholipid bilayer, caused by biochemical mechanisms catalysed by the phospholipid bilayers of the cell.

**Table 2 .Zone of growth inhibition (mm) showing antibacterial activity for a six selected medicinal plant essential oil; well diameter 6.0 mm.**

Bacterial strain	Basil	Marjoram	Ginger	Comment	Spearmint	Lemon
<i>B. Cereus</i>	53.8±1.2	11.40.6	46.06.7	No activity	25.52.9	29.50.1
<i>E.aerogens</i>	35.9±4.4	18.60.5	33.90.4	11.10.1	21.60.9	15.50.6
<i>E-coil</i>	31.80.5	No activity	16.30.8	No activity	No activity	>90.0
<i>K. oxytoca</i>	>90.0	29.60.8	>90.0	18.70.6	>90.0	>90.0
<i>K.pneumoniae</i>	>90.0	22.20.3	28.51.0	10.01.9	15.80.7	>90.0
<i>L.vulgari</i>	21.60.9	>90.0	37.11.1	No activity	18.70.6	44.64.9
<i>P.aeruginosas</i>	39.170.8	53.81.2	>90.0	30.95.4	No activity	41.80.8
<i>P. mirabilis</i>	25.52.9	32.40.1	18.70.6	No activity	No activity	>90.0
<i>S.Paratyphi</i>	>90.0	>90.0	15.8.0.7	18.51.2	24.60.4	>90.0
<i>S.Faccalis</i>	29.80.1	23.10.6	>90.0	33.60.2	31.20.8	52.51.5
<i>S.typhi</i>	37.13.2	>90.0	23.80.3	No activity	>90.0	>90.0
<i>S.aureus</i>	>90.0	29.53.4	44.64.9	16.30.8	18.90.4	>90.0
<i>V.cholera</i>	>90.0	14.90.1	>90.0	No activity	19.01.5	>90.0

Values for zone of growth inhibition are presented as mean±SEM.

Oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic inter-actions between components. A correlation of the antimicrobial activity of the compounds tested and their relative percentage composition in the plant volatile oils used in this study, with their chemical structure, functional groups and configuration, suggests a number of observations. Members of this class are known to be either

These processes include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions (Knobloch et al., 1988). Their activity in whole cells appears more complex (Knobloch et al., 1988). Although a similar water solubility tendency is observed, specific statements on the action of single terpenoids *in vivo* have to be assessed singularly, taking into account not only the structure of the terpenoid, but also the chemical composition of the cell

wall (Knobloch et al. 1988). The plant extracts clearly demonstrate antibacterial properties, although the mechanistic processes are poorly understood. These activities suggest potential use as chemo-therapeutic agents, food preserving agents and disinfectants.

Chemotherapeutic agents, used orally or 25 ically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious disease. The tested plant products appear to be effective against a wide spectrum of microorganisms, both pathogenic and nonpathogenic. Administered orally, these compounds may be able to control a wide range of microbes but there is also the possibility that they may cause an imbalance in the gut microflora, allowing opportunistic pathogenic coliforms to become established in the gasrrintestinal tract with resultant deleterious effects. Further studies on therapeutic applications of volatile oils should be undertaken to investigate these issues, especially when considering the substantial number of analytical studies carried out on these natural products.

The volatile oils and their component volatility and lack of solubility make these plant extracts less appealing for general disinfectant applications. However, a role as disinfectants of rooms has been reportedly investigated in a classical study (Kellner & Kober 1954). Their volatility would be a distinct advantage in lowering microbial contamination in air and on difficult to reach surfaces. Although the minimum inhibitory concentrations for a selection of oils tested in a closed chamber were lower in the vapour phase (Inouye et al., 1983), evidence suggests that such applications may have merit (Taldykin 1979; Makarchuk et al., 1981). As food preservatives, volatile oils may have their greatest potential use. Spices, which are used as integral ingredients in cuisine or added as flavouring agents to foods, are present I in insufficient quantities for their antimicrobial properties to be significant. However, spices are often contaminated with bacterial and fungal spores due to their volatile oil content, often with antimicrobial activity, being enclosed within oil glands and not being released onto the surface of the spice mater. Volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, if added to foodstuffs, would cause no loss of organoleptic properties, would retard microbial contamination and therefore reduce the onset of sopoilage. In addition, small quantities would be required for this effect. Furthermore, evidence suggests that these oils possess strong antioxidant activities (Dorman 1999; Youdim et al. 1999), which are favourable properties to combat free radical – mediated organoleptic deterioration.

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