Research Paper

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Chemical Composition of Essential Oils by GC-MS and Antibacterial Activity of their Compounds against Human Pathogenic Bacteria

Microbiology

KEYWORDS : Antibacterial activity, Essential oils, GC-MS, GC, pathogenic bacteria

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ABSTRACT

The essential oils of four plants such as Cymbopogon martini, Mentha piperita, Pelargonium graveolens and Rosemarinus officinalis were selected for chemical analysis and antibacterial activity on selected opportunistic bacterial pathogens. The chemical composition of the oils was analyzed by GC-MS technique and many compounds were identified. The purity of these chemical compounds was tested by GC. Four chemical compounds geraniol, trans-geraniol, citral and farnesol were chosen for in vitro antibacterial activity, which was evaluated by agar well diffusion method against five selected clinical isolates of bacterial species. All the four chemical compounds were found to be more effective against Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Bacillus cereus. The present study suggested that these chemical compounds can be used in treating human diseases caused by the test organisms.

Introduction

In the present time, drug resistance in microbes is a very serious problem. Hence, plant origin herbal medicines are considered as safe alternatives of synthetic drugs. There are varied methods of medicines like Ayurveda, Homeopathy and Unani, which utilize plant materials for drug production. Currently, Ayurveda considered as a vital system of medicine and governed the worldwide recognition and having non-toxic substances. However, newly discovered non-antibiotic substances such as certain essential oils (Sonboli *et al*., 2006) and their constituent chemicals (Chavan *et al*., 2006) have shown good fighting potential against drug resistant pathogens (Cowan, 1999; Ahmad and Beg, 2001). Essential oils are aromatic oily liquids, which are obtained from various plant parts such as flowers, buds, seeds, leaves, twigs, bark, woods, fruits and roots by steam distillation. Scientifically these oils have been proved highly potent antimicrobial agents in comparison to antibiotics. These plant essential oils are rich source of scents and used in food preservation and aromatherapy. These possess multiple antimicrobial i.e., antibacterial (Ozcan *et al*., 2006), antifungal (Cafarchia *et al*., 2002), anticancer, antiviral and antioxidant properties (Salehi *et al*., 2005; Vardar-Unlu *et al*., 2003), against viruses, bacteria and fungi (Kalemba and Kunicka, 2003). Some essential oils such as aniseed, calms, camphor, cedar-wood, cinnamon, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, rosemary, basil, vetiver and w inter green are traditionally used by people in different parts of the world. Cinnamon (Prabuseenivasn *et al*., 2006), clove, rosemary and lavender oils have shown both antibacterial and antifungal properties (Quale *et al*., 1996; Chang *et al*., 2001; Wilkinson and Cavanagh, 2005). Besides this, Cinnamon oil possesses anti-diabetic and anti-inflammatory activity (Mitra *et al*., 2000), while lemon, rosemary and peppermint exhibit anticancer activities (Imai *et al*., 2001).

Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants. They may provide potential alternatives to the control agents currently used because the compositions of essential oils are rich of bioactive chemicals and commonly used as fragrance and flavouring agents for food and beverage (Isman, 2000). They were previously reported to have biological activity antibacterial (Dorman *et al*., 2000)

Materials and methods

Essential oils preparation

Air-dried to a constant weight, plant material (2 x 3 batches of about 500 g for each sample) was subjected to hydrodistillation with approximately 2 L of distilled water for 2.5 h using the original Clevenger-type apparatus (Radulovic *et al*., 2009) .The obtained oils were separated by extraction with freshly distilled diethyl ether (Merck, Germany), dried over anhydrous magnesium sulfate (Aldrich, USA), and immediately analyzed.

Chemicals and microorganisms

All chemicals with the highest purity available and culture media were purchased from Himedia Mumbai, Maharashtra (India). *Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* (Clinical isolates) was used as test organisms. The bacterial cultures were obtained from Microlabs Institute of Research and Technology Arcot, Tamil Nadu (India).

Gas Chromatography-mass spectrometry

GC-MS was done at South Indian Textile Research Association Coimbatore, Tamil Nadu (India) and analysis was carried out using a Hewlett-Packard 5890/5971A system fitted with a HP1 column (50 m x 0.20 mm fused silica capillary column; film thickness, 0.5 µm). GC oven initial temperature was 60°C and was programmed to 220°C at a rate of 2°C/min and 220°C during 120 min under the following operation conditions: vector gas, He; injector and detector temperatures, 250°C; injected volume: 0.2 µl, with a ratio split of 1/100. Retention indices were determined with Hexane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35 - 400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (Wiley, MassFinder 2.1 Library, NIST98) and home-made libraries and MS literature data (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998; Joulain *et al*., 2001)

Gas chromatography

GC (FID) analyses were carried out under the same experimental conditions using the same column and same gas chromatograph type as described for the GC-MS. The percentage composition was computed from the GC peak areas without the use of correction factors. The results of four essential oils showed 99.99 % purity.

Determination of antibacterial activity

In this study standard agar well diffusion method was followed (Perez *et al*., 1990; Perez *et al*., 1999; Erdemoglu *et al*., 2003; Bagamboula *et al*., 2004). Antibacterial activity was performed for four essential oil chemical compounds Geraniol, Trans-geraniol, Farnesol and citral using bacterial cultures, *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* as test organisms which are of clinical isolates. The well diffusion technique was performed for the

essential oil chemical compounds with standard antibiotic disc at the concentration ciprofloxacin (15 µg/mL) and nutrient broth cultures was swabbed on the surface on Muller Hinton agar. The results were recorded by measuring the zone of inhibition around the well and antibiotic disc. The experiments were done in triplicate.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1

Results

Chemical composition of essential oils of GC-MS

Chemical compositions of essential oils are shown in Tables 1to 4. As seen, major components of *Cymbopogon martinii oil, Mentha piperita oil, Pelargonium graveolens* and *Rosemarinus officinalis* were: Geranyl acetate (0.37%), farnesol (0.37%), trans-Geraniol (0.37%) and farnesol (0.19 %), 2Z,6E- Farnesol (0.19 %), d-Nerolidol (0.19 %) and farnesol(0.36%),trans-Geraniol(0.36%), cis-Farnesol (0.36%) and Geraniol (0.04%), trans-Geraniol (0.04%), Cyclopentane, bromo- (CAS) (0.04%), respectively.

Table 1
Chemical composition of *Cymbopogon martinii* oil

Compound name	Retention time (min)	Amount %
Geraniol	16.11	0.37
Lavanduyl acetate	16.11	0.37
Trans sesquilavandulol	16.11	0.37
Citral	16.11	0.37
Cis-farnesol	16.11	0.37
Geranyl acetate	16.11	0.37
3,7-Dimethyl-2,6-octadien-1-yl propionate	16.11	0.37
Geranyl propionate	16.11	0.37
farnesol	16.11	0.37
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-(CAS)	16.11	0.37
Geranyl acetate	16.11	0.37
Cis-farnesol	16.11	0.37
á-Citronellol	29.33	0.06
Neryal acetate	22.44	0.59

Table 2
Chemical composition of *Mentha piperita* oil

Compound name	Retention time (min)	Amount %
Nerolidol	16.08	0.19
d-Nerolidol	16.08	0.19
(-) - Nerolidol	16.08	0.19
Nerolidol isomer	16.08	0.19
Farnesol	16.08	0.19
2Z,6E- Farnesol	16.08	0.19
Cis-sesquilavandulol	16.08	0.19
Nerolidol Z and E	16.08	0.19
(Z)-2-(Propen-2'-yl)oct-2-en-1-ol	16.08	0.19

Table 3
Chemical composition of *Pelargonium graveolens* oil

Compound Name	Retention time(min)	Amount %
Citral	8.87	0.36
Lavanduyl acetate	8.87	0.36
Farnesol	8.87	0.36
Cyclohexene, 4-ethenyl-1,4-dimethyl- (CAS)	8.87	0.36
Geranyl acetate	8.87	0.36
Trans-sesuilavandulol	8.87	0.36
Geranyl propionate	8.87	0.36
Cis-farnesol	8.87	0.36
Trans- geraniol	8.87	0.36
Neryl acetate	11.38	3.45

Table 4
Chemical composition of *Rosemarinus officinalis* oil

Compound name	Retention time (min)	Amount %
3-methyl-3-nitro-1-butene	28.33	0.04
Trans-geraniol	28.33	0.04
Geranyl acetate	28.33	0.04
Geraniol	28.33	0.04
Farensol	28.33	0.04
Cyclopentane, bromo- (CAS)	28.33	0.04
2Z,6E-Farnesol	28.33	0.04
Trans sesquilavandulol	28.33	0.04
Cis-farnesol	28.33	0.04
dl- lemonene	28.33	0.04
Neryal acetate	28.33	0.20

Antibacterial activity in vitro

Each essential oils chemical compounds showed notable antibacterial activities against *Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*. In (Table 5) Farnesol was very highly active against *Escherichia coli* (9.30±0.44) and least against *Bacillus cereus* (3.00±0.10). (Table 6) Geraniol was highly active against *Salmonella typhi* (6.00±0.00) and least against *Escherichia coli* (3.00±0.00). (Table 7) trans-geraniol was highly active against *Staphylococcus aureus* (5.00±0.00) and least against *Salmonella typhi* (2.00±0.00).(Table 8) geranyl acetate showed high activity against *Salmonella typhi* (11.00±0.28) and low activity against *Bacillus cereus* (7.00±0.17). (Table 9) Citral showed very high activity against *Bacillus cereus* (30.00±0.57) and low activity against *Salmonella typhi* (9.00±0.28).All bacteria were found to be sensitive to all test essential oils chemical compounds and mostly comparable to the standard reference antibacterial drug ciprofloxacin was highly effective.

Table 5
Antibacterial activity of essential oils chemical compound farnesol

Microorganisms	Farnesol	Farnesol 1:10	Farnesol 1:100	Ciprofloxacin
Salmonella typhi	14.00 ± 0.28^{a}	5.06 ± 0.06^{a}	10.16 ± 0.15^{a}	17.33 ± 0.33^{a}
Pseudomonas aeruginosa	11.00 ± 0.28^{b}	4.10 ± 0.10^{b}	8.30 ± 0.26^{b}	12.50 ± 0.28^{b}
Staphylococcus aureus	11.83 ± 0.16^{cb}	4.16 ± 0.08^{cb}	8.10 ± 0.08^{cb}	21.66 ± 0.33^{c}
Bacillus cereus	12.96 ± 0.26^{d}	3.00 ± 0.10^{d}	9.10 ± 0.00^{d}	18.83 ± 0.44^{d}
Escherichia coli	15.66 ± 0.33^{e}	9.30 ± 0.44^{e}	12.83 ± 0.44^{e}	20.00 ± 0.57^{e}

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values represented as Mean ± SD of chemical compound farnesol. chemical compound farnesol has significant effect at 0.05 level

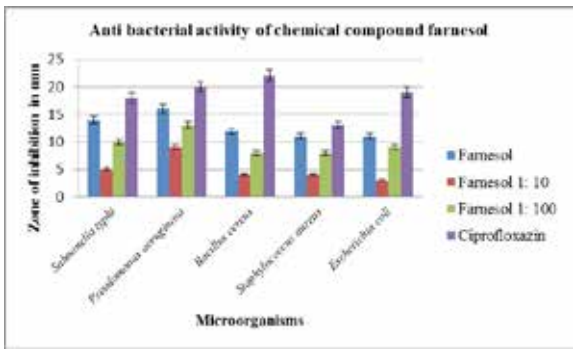

Fig. 1.Inhibition of growth of selected bacteria by essential oils chemical compound farnesol

Table 6
Antibacterial activity of essential oils chemical compound Geraniol

Microorganisms	Geraniol	Geraniol 1:10	Geraniol 1:100	Ciprofloxacin
Salmonella typhi	10.26±0.14^{a}	6.00±0.00^{a}	8.06±0.06^{a}	16.66±0.33^{a}
Pseudomonas aeruginosa	12.00±0.28^{b}	3.00±0.00^{b}	6.06±0.06^{b}	22.22±0.88^{b}
Staphylococcus aureus	12.16±0.16^{cb}	3.00±0.00^{cb}	9.16±0.16^{c}	18.50±0.28^{c}
Bacillus cereus	8.76±0.14^{d}	4.00±0.00^{d}	7.06±0.06^{d}	14.83±0.44^{d}
Escherichia coli	11.00±0.28^{e}	3.00±0.00^{e}	6.93±0.06^{eb}	11.83±0.44^{e}

Values represented as Mean ± SD of chemical compound geraniol has significant effect at 0.05 level

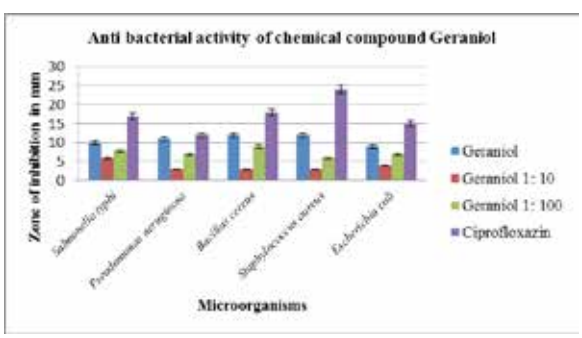

Fig. 2.Inhibition of growth of selected bacteria by essential oils chemical compound Geraniol

Table 7
Antibacterial activity of essential oils chemical compound Trans-geraniol

Microorganisms	Trans-geraniol	Trans-Geraniol 1:10	Trans-geraniol 1: 100	Ciprofloxacin
Salmonella typhi	12.00±0.28^{a}	2.00±0.00^{a}	5.76±0.14^{a}	17.66±0.33^{a}
Pseudomonas aeruginosa	8.93±0.06^{b}	5.06±0.06^{b}	6.93±0.06^{b}	12.50±0.28^{b}
Staphylococcus aureus	11.80±0.41^{c}	5.00±0.00^{cb}	8.76±0.14^{c}	17.83±0.44^{ca}
Bacillus cereus	8.83±0.16^{db}	3.00±0.00^{d}	6.03±0.08^{db}	15.66±0.33^{d}
Escherichia coli	10.00±0.28^{e}	3.03±0.03^{ed}	7.03±0.08^{e}	15.50±0.28^{ed}

Values represented as Mean ± SD of chemical compound trans-geraniol has significant effect at 0.05 level

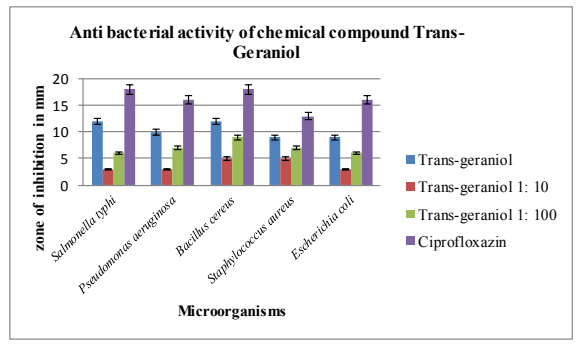

Fig. 3.Inhibition of growth of selected bacteria by essential oils chemical compound trans-geraniol

Table 8
Antibacterial activity of essential oils chemical compound geranyl acetate

Microorganisms	Geranyl acetate	Geranyl acetate 1: 10	Geranyl acetate 1: 100	Ciprofloxacin
Salmonella typhi	11.00±0.28^{a}	5.03±0.08^{a}	6.93±0.06^{a}	16.83±0.44^{a}
Pseudomonas aeruginosa	9.00±0.11^{b}	3.00±0.00^{b}	8.26±0.14^{b}	15.83±0.44^{b}
Staphylococcus aureus	8.06±0.17^{c}	3.00±0.00^{cb}	4.00±0.00^{c}	22.00±0.57^{c}
Bacillus cereus	7.00±0.17^{d}	4.00±0.11^{d}	8.16±0.08^{db}	15.00±0.57^{db}
Escherichia coli	8.90±0.20^{ec}	2.00±0.00^{e}	5.00±0.11^{e}	16.00±0.28^{ea}

Values represented as Mean ± SD of chemical compound geranyl acetate has significant effect at 0.05 level

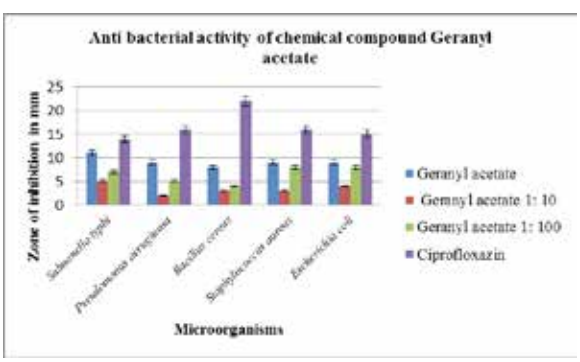

Fig. 4.Inhibition of growth of selected bacteria by essential oils chemical compound geranyl acetate

Table 9
Antibacterial activity of essential oils chemical compound citral

Microorganisms	Citral	Citral 1: 10	Citral 1: 100	Ciprofloxacin
Salmonella typhi	9.00±0.28^{a}	3.00±0.00^{a}	5.96±0.03^{a}	14.83±0.44^{a}
Pseudomonas aeruginosa	9.93±0.23^{ba}	4.00±0.05^{b}	7.00±0.11^{b}	11.83±0.44^{b}
Staphylococcus aureus	10.50±0.28^{c}	4.03±0.08^{cb}	7.06±0.12^{cb}	14.00±0.28^{ca}
Bacillus cereus	30.00±0.57^{d}	7.06±0.20^{d}	12.00±0.28^{d}	22.83±0.44^{d}
Escherichia coli	10.73±0.37^{ec}	7.00±0.11^{ed}	9.00±0.11^{e}	15.83±0.44^{e}

Values represented as Mean ± SD of chemical compound citral. The chemical compound citral has significant effect at 0.05 levels.

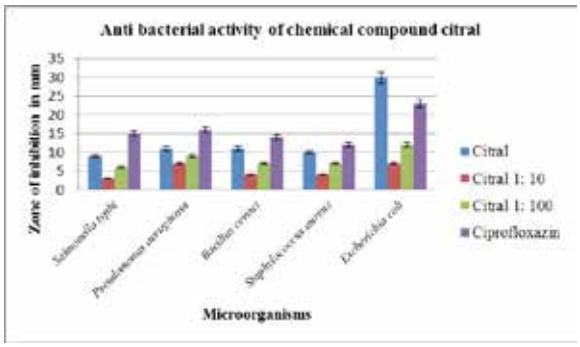

Fig. 5.Inhibition of growth of selected bacteria by essential oils chemical compound citral

Discussion

Plant essential oils and extracts have been used for many thousands of years (Jones, 1996) in food preservation, pharmaceuticals alternative medicine and natural therapies (Reynolds, 1997; Lis-balchin *et al*., 1997). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds (Mitscher *et al*., 1987) especially against bacterial pathogens. *In vitro* studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak (Zakia, 1988).

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Knobloch *et al*., 1986; Sikkema *et al*., 1994). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Denyer and Hugo, 1991). Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria (Zakia, 1988). In the present study, cinnamon, lime, geranium rosemary, orange, lemon and clove oils were found to be equally effective against both gram-positive and gram negative organisms.

Various essential oils obtained from the plants showed antimicrobial activity against a range of microorganisms including Gram's positive bacteria, Gram's negative bacteria and fungi. However, the differences may be explained by susceptibility, testing conditions, physico-chemical characteristics of the oil and strain differences (Badar *et al*., 2008). The earlier studies on essential oil of *Pelargonium* sp. (Rajeswara Rao et al., 1996) reported that the terpenoid composition of the essential oil was strongly influenced by the seasonal climatic changes. During the summer citronellol (40.80%) and linalool (13.27%) were the main component; wile during the winter geraniol (26.62%) was the main compound. when the chemical profile of the studied essential oil is compared to previously studied essential oil of *Pelargonium* sp from Portugal (Gomes *et al*., 2007), it appears that citronellol (26.9%) and citronellyl formate (13.2%) were the main components, while in comparison with our sample the second component is totally absent. The essential oil composition of *Pelargonium* sp. cultivar 'Kelkar', grown in the agroclimatic conditions of the western Himalayas, showed that citronellol (36.79%) and geraniol (22.12%) were present in high amounts (Babu *et al*., 2005). However, the composition of the essential oil of Indian pelargonium showed that geraniol (21.3–38.4%) and linalool (14.7–19.6%) were the major constituents (Rajeswara Rao *et al*., 2002). It is previously mentioned that the essential oil of *P. graveolens* exhibit a significant antibacterial activity against five strains of *C. albicans* (Rosato *et al*., 2008), and against *B. subtilis* (Rosato *et al*., 2007).

Conclusion

In our study the four essential oils chemical compounds geraniol, trans-geraniol, geranyl acetate, citral, and farnesol have shown a good antibacterial activity at low concentrations. These chemical compounds were identified by GC-MS and the purity of these essential oils chemical compounds was tested by GC. The antibacterial activity of these chemical compounds may be attributed to their chemical structure similar to various active principles of the essential oils. Further studies are needed to characterize the bioactive principles to develop new antibacterial drugs.

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