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Research Article

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Antibacterial activity of selected compounds of essential oils from indigenous plants

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ABSTRACT

Opportunistic infections are common and in the cases of cancer patients, they are very harmful. Bacteria such as Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa,Shigella dysenteriae, and Proteus mirabilis isolated from cancer and other immunosuppressed patients were tested for their sensitivity to compounds of essential oils such as Citral, Limonene, Menthone and Thymol. In agar well diffusion method the selected essential oils were effective against both gram positive as well as gram negative organisms. For e.g, Citral was highly active against S.pneumoniae and least against S. aureus. Limonene was highly active against S.typhi and least against S. aureus. Menthone was highly active against P.mirabilis and least against P.aeruginosa. The results for Thymol were moderate, when compared with the reference drug ciprofloxacin. The results for minimal bactericidal concentration (MBC) were similar to minimal inhibitory concentration (MIC) results. As no organism was found to be resistant to the tested essential oil compounds, the results indicated that they could be used in different forms for the prevention, control and treatment of opportunistic bacterial infections caused by those organisms, particularly in cancer patients.

Key words: Antibacterial activity, opportunistic bacteria, essential oils, Citral, Limonene, Menthone, Thymol,.

INTRODUCTION

Most opportunistic infections occur during poorly controlled malignancy in the setting of cytotoxic and immunosuppressive therapy (Shannon Smiley,et al.,2005). Infections are a major cause of morbidity and mortality in cancer patients undergoing antineoplastic treatment. (Hughes et al. 2002),An increased rate of opportunistic infections was observed with chronic imatinib treatment in animal studies(Sinai et al.,2007). Plants can resist parasitic attacks using several defense mechanisms. One of such is the synthesis of antimicrobial compounds which elicit defense substances called phytoalexins. Plant defense substances belong to a wide range of different chemical classes including flavonoids, terpenoids, alkaloids, steroidal saponins, tannins, phenolic acids, lactones, quinones essential oil, and polyphenols (Cowan, 1999). Herbal and alternative medicines are popular in the general population worldwide. A great number of modern drugs are still derived from herbs (Cooper, 2005).In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Essential oils, odors and volatile products of plant secondary metabolism, have a wide application in folk medicine as well as in fragrance industries. Essential

oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam-distillation. The main constituents of essential oils, for example, monoterpenes and sesquiterpenes and phenylpropanoids including carbohydrates, alcohols, ethers, aldehydes and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants (Reichling, 1999). Various essential oils and their components possess pharmacological effects, demonstrating antiinflammatory, antioxidant and anticancerogenic properties (Golab et al., 2005; Naser et al., 2005; Ito et al., 2008). Burt, 2004) have demonstrated that oxygenated monoterpenes had a higher antimicrobial activity than did hydrocarbons. Among oxygenated monoterpenes detected in mandarin EO, carvone and limonene oxide were active against a wide spectrum of pathogenic fungi and bacteria tested (Aggarwal et al., 2002).

In addition to inducing resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immunosuppression and allergic reactions (Ahmad et al., 1998). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (Berahou et al., 2007; Salomao et al., 2008). Many plant materials have been investigated for their antimicrobial activity. The addition of raisins to the formulation of beef jerky had a marked inhibitory effect on pathogenic bacteria (Bower et al., 2003). Rosemary extract has demonstrated antimicrobial activity against a number of food borne pathogenic bacteria (Campo et al., 2000).

EXPERIMENTAL SECTION

Essential oil compounds

Four essential oil components such as Citral, Limonene, Menthone, Thymol were obtained from commercial producers of plant essential oils(****) and aromatic substances were used in this study. Quality of the oils were ascertained to be more than 98% pure by Gas Chromatography. The oil is stored at 4°C under dark condition until further use.

Test bacteria

Microorganisms (Clinical bacterial isolates) such as *S.aureus*, *S.pneumoniae*, *S.typhi*, *E.coli*, *P. aeruginosa*, *S.dysenteriae*, and *P.mirabilis* were obtained from the Microlabs Institute of research and technology, India. The cultures were maintained in their appropriate agar slants (slant names) at 4°C throughout the study and used as stock used as test bacteria in the appropriate medium.

Antibacterial Assay

Agar well diffusion method

In this study standard agar well diffusion method was followed (Perez, et al.,(1999); Bagamboula,, 2004; Erdemoglu et al.,2003; Perez, et al., 1990). Each bacterial isolate was suspended in Brain Heart Infusion (BHI) (Himedia, India) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were "flood inoculated" onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 100µl of the samples solutions were delivered into the wells. The plates were incubated for 18 h at 37°C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ethanol was used as solvent control. Ciprofloxacin was used as reference antibacterial agent.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The estimation of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out by the broth dilution method (Van der Berghe, and Vlietinck,1991). Dilutions of essential oil from 2.0 to 0.075 mg/ml were used. Test bacteria culture was used at the concentration of 10^5 CFU/ml. MIC values were taken as the lowest essential oil concentration that prevents visible bacterial growth after 24 h of incubation at 37°C, and MBC as the lowest concentration that completely inhibited bacterial growth. Ciprofloxacin was used as reference and appropriate controls with no essential oil were used. Each experiment was done in triplicate.

Statistical analysis

Data were analyzed using Least Significant Difference(LSD) test following – (***)way analysis of variance (ANOVA) using SPSS 10.0 computer software package. Difference on statistical analysis of data were considered significant at $p<0.05$.

RESULTS

Agar well diffusion method

In agar well diffusion method the selected essential oils were found to be effective against both gram positive as well as gram negative organisms. Citral was highly active against *S. typhi* and least against *S. aureus*. Limonene was highly active against *S.typhi* and least against *S. aureus*. Menthone was highly active against *P.mirabilis* and least against *P. aeruginosa*. Thymol was highly active against *P.mirabilis* and least against *S. aureus*. None of the organisms were found to be resistant to the test oils. The results are interpreted in table-1 and figure-1.

Results for minimum inhibitory concentration (MIC) and Minimumbactericidal concentration (MBC) of essential oil compounds

The results are shown in table no.3 and figure in no.2. The results of MIC of Citral, Limonene, Menthone, were found to be effective when compared with the reference drug Chloramphenicol (0.25 mg/ml). The results of MIC of Thymol against all test organisms was similar (2.00 mg/ml) and it was very high dose when compared to the reference drug. The results for Minimum Bactericidal Concentration (MBC) were similar to Minimum Inhibitory Concentration (MIC) results, but in MBC confirmation was made by the presence and absence of culture. The results are shown in table no.2.

Table 1. Antibacterial activity of essential oil compounds against clinical bacterial isolates

Essential oil compounds	Organisms (Zone of Inhibition (mm)[a])						
	S. aureus	***S. pneumoniae***	***S. typhi***	***E. coli***	***P. aeruginosa***	***S. dysenteriae***	***Pr. mirabilis***
Citral	9.98±0.53 [a]	11.30±0.90 [a]	12.85±0.56 [a]	11.58±0.61[ah]	7.98±0.45 [a]	12.24±0.81[a]	13.16±1.00 [a]
Limonene	11.28±0.87 [a]	12.96±0.51 [b]	14.16±0.84 [b]	11.95±0.39 [a]	11.54±0.54 [b]	12.92±0.36 [a]	11.98±0.45 [a]
Menthone	10.12±0.36 [a]	10.11±0.56 [ae]	13.03±0.64 [ab]	11.12±0.37 [a]	8.21±0.44 [a]	11.08±0.59 [c]	14.06±0.47 [ae]
Thymol	7.83±0.76 [d]	13.43±0.94[db]	10.11±0.67[ed]	8.26±1.0[bf]	11.84±0.77[eb]	13.53±0.53[eba]	16.94±0.90 [c]
Ciprofloxacin ©	20.27±1.15[fb]	20.30±0.91[f]	18.65±0.72[gcd]	16.43±0.52[e]	12.51±0.51[gb]	18.08±0.59[g]	17.05±0.65[dc]

© - control antibiotic disc in 100 µg concentration

Different superscripts in the same column are significantly different at P<0.05 level (Least Significance Difference) mean followed by ± S.D.

Fig 1. Antibacterial activity of essential oil compounds against clinical bacterial isolates

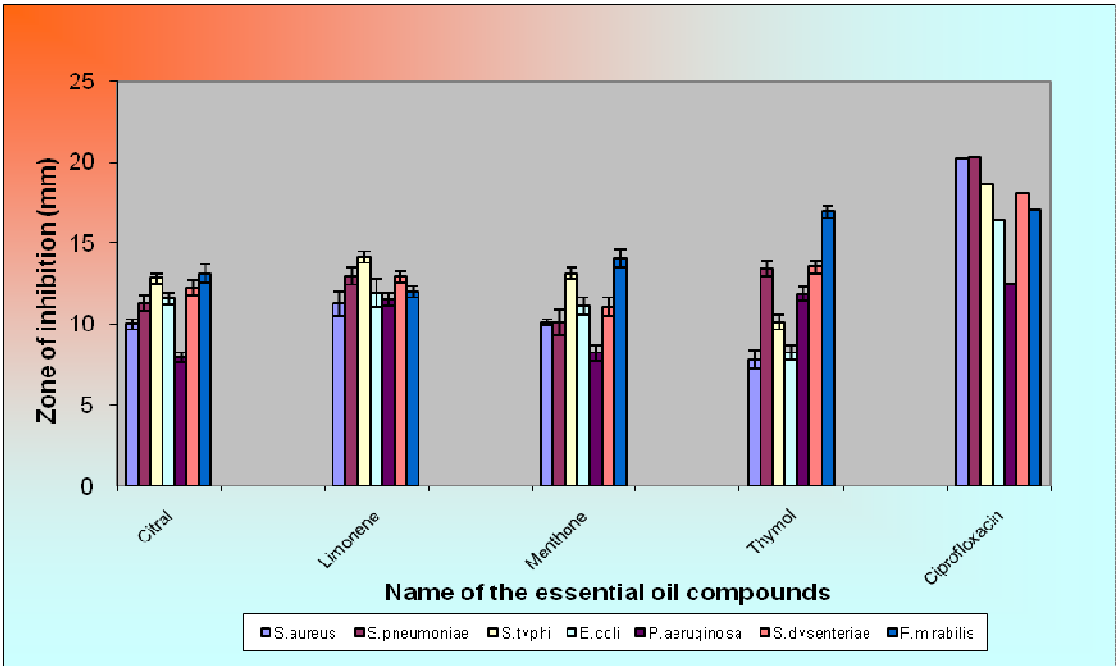

Table 2. MIC and MBC of essential oil compounds against clinical bacterial isolates

Organisms	Citral	Limonene	Menthone	Thymol	Ciprofloxacin ©
S. aureus	1.00	0.50	0.75	2.00	0.25
S. pneumoniae	1.00	0.50	0.75	2.00	0.25
S. typhi	2.00	1.50	1.00	2.00	0.25
Escherichia coli	2.00	1.50	1.00	2.00	0.25
P.aeruginosa	2.00	2.00	1.50	2.00	0.25
S. dysenteriae	2.00	2.00	2.00	2.00	0.25
P. mirabilis	2.00	1.50	1.00	2.00	0.25

© - control antibiotic disc in 100 µg concentration MIC- Minimum Inhibitory Concentration MBC- Minimum Bactericidal Concentration

DISCUSSION

All the selected essential oils compounds were found to be active against both gram positive and gram negative bacterial isolates. Citral, Limonene, Menthone were found to be more than the moderate range. The others such as geranium were found to be moderate in activity. As far as the chemical analysis, and antibacterial study, the following studies were comparable to the results with the present study. Onawunmi, and Ogunlana, 1986, found lemongrass effective against *E.coli* and *Bacillus subtilis* in both broth dilution and agar diffusion tests. Lemongrass essential oil had an activity comparable to the standard antibiotic disks in the study, thus indicating that lemongrass is a viable option against certain pathogens (Dellacassa, 1989). Gachkar, et al., 2007 reported the chemical composition, antibacterial, antioxidative and radical–scavenging properties of the essential oils of *R.officinalis* obtained by steam distillation. Santoyo, et al., 2005, attributed the antimicrobial property of the essential oil of *R. officinalis* to the presence of -pinene, 1,8-cineole, camphor, verbinone and borneol with borneol being the most potent followed by camphor and verbinone. The quantities of these compounds were very high in our oils. The volatile oils of *R. officinalis* were screened against two Gram-positive (*S.aureus*, and *B.subtilis*) and two Gram-negative (*E.coli* and *K. pneumoniae*) bacteria strains. However, there is evidence that essential oils are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components; minor components appear, therefore, to play a significant role (Lataoui and Tantaoui-Elaraki, 1994).

Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Bagamboula, et al., 2003; Conner,1993; Conner and Beuchat, 1984; Dorman,and Deans, 2000;. Zaika,1988). More particularly, essential oils and their components are known to be active against a wide variety of microorganisms, including Gram negative bacteria (Helander, et al., 1998; Sivropoulou, et al., 1996). Onawunmi, and Ogunlana, 1986, found lemongrass effective against *E.coli* and *B. subtilis* in both broth dilution and agar diffusion tests. Lemongrass essential oil had an activity comparable to the standard antibiotic disks in the study, thus indicating that lemongrass is a viable option against certain pathogens (Dellacassa 1989). The findings of the chemical analysis of the present study were comparable to the results of the following. Citral is the major component of lemongrass oil which was extracted from its leaves, present at levels of, approximately, 65–85%. Citral (3, 7-dimethyl-2,6-octadienal) is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (trans-citral, citral A) and neural (cis-citral, citral B). In addition to citral, the lemongrass oil consists of small quantities of geranial, geranylacetate and monoterpene olefins, such as myrcene (Ferreira and Fonteles, 1989). Citral and its epoxide can act as bactericidal and fungicidal agents (Cristiane et al., 2008). The comparison between citral and lemongrass oil showed similar effect but citral epoxide showed more inhibitory effect, this can be explained on the basis that lemongrass oil contains of citral as the major component, small quantities of geranial, geranylacetate and monoterpene olefins, such as myrcene (Ferreira and Fonteles, 1989), which indicated significant association between their effects and the presence of citral in lemongrass oil (Onawunmi, 1989).

Thymol is an essential oil which is also used as a mouth rinse and was tested for its effect in inhibiting the development of plaque and gingivitis. Citral has been proved to be one of the most effective antifungal components of lemon and orange EOs (Caccioni, Ruberto et al., 1995).Citral (3,7- dimethyl-2,6-octadienal) is the major constituent of *Cymbopogon citratus* and has been used in perfumery, cosmetic and pharmaceutical industries for controlling pathogens (Guynot et al., 2003).In particular, the antimicrobial properties of plant essential oils (EOs) and their constituents have been widely demonstrated (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). The volatile constituents are a mixture of monoterpene (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, including aldehydes (such as citral), ketones, acids, alcohols (such as linalool) and esters (Flamini, Tebano, & Cioni, 2007). Limonene is the major chemical component of citrus EOs, ranging from 32 to

98% (Svoboda & Greenaway, 2003). The complexity of the composition of citrus essential oils induced (Caccioni et al.,1998) to propose a holistic approach to explain the antimicrobial capabilities of essential oils, whose performances could be the result of a certain quantitative balance of various components.The antimicrobial activity of this oil was confirmed in vitro and in fruit salads (Belletti et al., 2008), and was related to the high concentration of citral, whose antimicrobial potential is known, as reported by other authors (Rivera-Carriles et al., 2005). However, the overall bioactivity of an essential oil is the result of the bioactivity of the single constituents, whose effects can be additive, synergistic or antagonistic (Santiesteban-López et al., 2007).

CONCLUSION

The essential oil compounds as antimicrobial agents present two main characters: the first is their natural origin which means more safety to the people and the environment, the second is that they have been considered at low risk for resistance development by pathogenic microorganisms. The results of this study revealed that, the test compounds of essential oil can be prepared in different forms such as topical agents, suppositories, pessaries, mouth washes, nebulizer and other therapeutic agents of those essential oil compounds may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of bacterial diseases in various patients, particularly, immunosuppressed patients such as cancer patients.

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