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Research Paper

Otitis Externa –Bacterial Isolates and Their Sensitivity to Essential Oils of Selected Indigenous Plants

Zoology

KEYWORDS : Antibacterial, Essential oils, Otitis externa, Ear infection, indigenous plants, Agar well diffusion

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ABSTRACT

Many pathogens and opportunistic pathogenic agents are increasingly encountered in ear infections. Bacteria such as Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, and Proteus mirabilis isolated from otitis externa infections cases were tested for their sensitivity to essential oils from Cymbopogon citratus, Cymbopogon martinii, Cinnamomum zeylanicum, Rosmarinus officinalis, Mentha piperita, Pelargonium graveolens, and Vitex negundo.In agar well diffusion method the selected essential oils were effective against both gram positive as well as gram negative organisms. For e.g, C. citratus was highly active against S.pneumoniaeand least against S. aureus. C. martinii was highly active against S.typhiand least against S. aureus. C.zeylanicumwas highly active against S.pneumoniaeand least against P.mirabilis. R..officinaliswas highly active against P.mirabilisand least against P.aeruginosa. The minimal inhibitory concentration (MIC) of essential oil of C.zeylanicumagainst all test organisms was best (0.25 mg/ml) and it was quite comparable with the reference drug Ciprofloxacin (0.25 mg/ml). The results for all other essential oils were moderate, when compared with the reference drug. The results for minimal bactericidal concentration (MBC) were similar to minimal inhibitory concentration results. As no organism was found to be resistant to the tested essential oil, the results indicated that those essential oils could be used (aromatherapy) in different forms for the prevention, control and treatment of bacterial infections caused by those organisms particularly, for otitis externa patients.

Introduction

The important role of bacteria in otitis media (OM) pathogenesis has long been acknowledged, however the aetiology of recurrence and persistence of this condition is not well understood. Many characteristics suggest that chronic otitis media with effusion (COME) and recurrent acute otitis media (rAOM) are biofilm related (Park et al., 2004). Biofilms are defined as clusters of bacteria embedded in a polymeric matrix with increased resistance to antibiotics and host defense mechanisms when compared to their “planktonic” or “free floating” counterparts (Burmolle et al., 2010). While biofilm has been demonstrated in OM animal models, (Ehrlich et al., 2002) there is limited data available on biofilm formation in the middle ears of children with OM (Coates, 2004).

Otitis externa is also known as external otitis and swimmer’s ear(Rapini Ronald et al., 2007) is an inflammation of the outer ear and ear canal. Along with otitis media, external otitis is one of the two human conditions commonly called “earache”. It also occurs in many other species. Inflammation of the skin of the ear canal is the essence of this disorder. The inflammation can be secondary to dermatitis (eczema) only, with no microbial infection, or it can be caused by active bacterial or fungal infection. In either case, but more often with infection, the ear canal skin swells and may become painful or tender to touch.

In contrast to the chronic otitis externa, acute otitis externa (AOE) is predominantly a bacterial infection, (Rosenfeld et al., 2014), occurs rather suddenly, rapidly worsens, and becomes very painful. The ear canal has an abundant nerve supply, so the pain is often severe enough to interfere with sleep. Wax in the ear can combine with the swelling of the canal skin and any associated pus to block the canal and dampen hearing to varying degrees, creating a temporary conductive hearing loss. In more severe or untreated cases, the infection can spread to the soft tissues of the face that surround the adjacent parotid gland and the jaw joint, making chewing painful.

The skin of the bony ear canal is unique, in that it is not movable but is closely attached to the bone, and it is almost a paper thin. For these reasons it is easily abraded or torn by even minimal physical force. Inflammation of the ear canal skin typically begins with a physical insult, most often from injury caused by attempts at self-cleaning or scratching with cotton swabs, pen caps, finger nails, hair pins, keys, or other small implements. Another causative factor for acute infection is prolonged water exposure in the forms of swimming or exposure to extreme humidity, which can compromise the protective barrier function of the canal skin, allowing bacteria to flourish; hence the name “swimmer’s ear”. Constriction of the ear canal from bone growth (Surfer’s ear) can trap debris leading to infection. Saturation divers have reported Otitis externa during occupational exposure. (Ahlén et al., 1998) Even without exposure to water, the use of objects such as cotton swabs or other small objects to clear the ear canal is enough to cause breaks in the skin, and allow the condition to develop. Once the skin of the ear canal is inflamed, external otitis can be drastically enhanced by either scratching the ear canal with an object, or by allowing water to remain in the ear canal for any prolonged length of time.

The two factors that are required for external otitis to develop are (1) the presence of germs that can infect the skin and (2) impairments in the integrity of the skin of the ear canal that allow infection to occur. If the skin is healthy and uninjured, only exposure to a high concentration of pathogens, such as submersion in a pond contaminated by sewage, is likely to set off an episode. However, if there are chronic skin conditions that affect the ear canal skin, such as atopic dermatitis, seborrheic dermatitis, psoriasis or abnormalities of keratin production, or if there has been a break in the skin from trauma, even the normal bac-

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teria found in the ear canal may cause infection and full-blown symptoms of external otitis (Kang and Stevens, 2003).

The goal of treatment is to cure the infection and to return the ear canal skin to a healthy condition. When external otitis is very mild, in its initial stages, simply refraining from swimming or washing hair for a few days, and keeping all implements out of the ear, usually results in resolution. External otitis is often a self-limiting condition. However, if the infection is moderate to severe, or if the climate is humid enough that the skin of the ear remains moist, spontaneous improvement may not occur.

Effective solutions for the ear canal include acidifying and drying agents, used either singly or in combination (Vikingo,.2007). When the ear canal skin is inflamed from the acute otitis externa, the use of dilute acetic acid may be painful. Burow's solution is a very effective remedy against both bacterial and fungal external otitis. This is a buffered mixture of aluminium sulfate and acetic acid, and is available without prescription in the United States.Topical solutions or suspensions in the form of ear drops are the mainstays of treatment for external otitis. Some contain antibiotics, either antibacterial or antifungal, and others are simply designed to mildly acidify the ear canal environment to discourage bacterial growth. Some prescription drops also contain anti-inflammatory steroids, which help to resolve swelling and itching. Oral antibiotics should not be used to treat uncomplicated acute otitis externa. Oral antibiotics are not a sufficient response to bacteria which cause this condition and have significant side effects including increased risk of opportunistic infection.

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. Essentialoils 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. Various essential oils and their components possess pharmacological effects, demonstrating anti-inflammatory, antioxidant and anti-cancerogenic properties (Golab et al., 2005; Naser et al., 2005; Ito et al., 2008).

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).

The present study was carried out to identify the effectiveness of seven essential oils against bacterial pathogens isolated from otitis externa cases, because of the lesser works done in the area.

MATERIALS AND METHODS

Essential oils

Seven essential oils such as Lemongrass oil (Cymbopogon citratus-Graminae), Palmarosa oil (Cymbopogon martini-Graminae), Cinnamon bark oil (Cinnamomum zeylanicum-Lauraceae), Rosemary oil (Rosmarinus officinalis-Labiatae), Geranium oil (Pelargonium graveolens-Geraniaceae), Peppermint oil (Mentha piperita-Labiatae),and Chaste tree leaf oil (Vitex negundo-Lamiaceae) were obtained from Aromax Trading Co, India (commercial producers of plant essential oils and aromatic substances) were used in this study. Quality of the oils was ascertained to be more than 98% pure. The oil was stored in the dark at 4°C until used within a maximum period of one week.

Qualitative chemical analysis essential oils

The essential oils were subjected to qualitative chemical analysis for secondary metabolites, such as alcoholic compounds, aldehydes, terpenoides, alkaloids, phenolic compounds and flavonoids in accordance with Trease and Evans,(1989) and Harborne,(1998) with little modification and Sofowora (1984).

Collection of Specimens

Samples of ear discharge were collected from the patients using sterile swab.

Isolation and Identification of Bacteria

Standard bacteriological techniques were followed to isolate and identify the bacteria present in the collected samples.

ANTIBACTERIAL ASSAY

Agar well diffusion method

In this study standard agar well diffusion method was followed (Erdemoglu et al., 2003). 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. Antimicrobial 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. The tests were carried out in duplicates.

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&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 105 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. Chloramphenicol 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

Qualitative chemical analysis essential oils

Alcohols, terpenoids, phenolic compounds, flavonoids, alkaloids, aldehydes were identified in essential oils all four such as lemongrass oil, Palmarosa oil, cinnamon bark oil and rosemary. Geranium oil showed positive results for alcohol, terpenoids and alkaloids. In peppermint oil, alcohols, terpenoids, flavonoids and alkaloids were observed. Chaste tree leaf oil was found to possess phenolics,terpenoids, flavonoids and alkaloids.

Isolates

Based on morphological and cultural characters, isolateswere identified asBacteria such as Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogens, Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, and Proteus mirabilis.

Agar well diffusion method

In agar well diffusion method the selected essential oils were effective against both gram positive as well as gram negative organisms. C. citratus was highly active against Streptococcus pyogens and least against S. aureus. C. martinii was highly active against Micrococcus luteusand least against S. aureus. C.zeylanicumwas highly active against S.pneumoniaeand least against P. mirabilis. R.officinaliswas highly active against P.mirabilisand least against P. aeruginosa. M. piperita was highly active against E.coliand least against Micrococcus luteus.P.graveolenswas highly active against P.mirabilisand least against S. pneumoniae. All organismswere found to be resistant to the Vitex negundooils.

Results for minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of essential oils

The results of MIC of C.citratus, C. martinii,R. officnalis, M.piperita, and were moderate when compared with the reference drug Ciprofloxacin (0.25 mg/ml). The results of MIC of C. zeylanicumagainst all test organisms, was similar and it was 0.25 mg/ml which was quite comparable with the reference drug. The results of MIC of P.graveolensagainst all test organisms were similar and it was 2.00 mg/ml and it was very high dose when compared to the reference drug.V. negundowas not used in this analysis since it has not produced any observable results in preliminary agar well diffusion test.

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.3 and figure in no.3.

Table 1-Antibacterial activity of essential oils ria from against bacte Otitis externa cases

Essential oils	Bacterial isolates /Zone of Inhibition in mm						
	Staphylococcus aureus	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Micrococcus luteus*	*Proteus mirabilis*
Cymbopogon citratus	9.98±0.53	11.30±0.90	12.85±0.56	11.58±0.61	7.98±0.45	12.24±0.81	13.16±1.00
Cymbopogon martini	11.28±0.87	12.96±0.51	14.16±0.84	11.95±0.39	11.54±0.54	12.92±0.36	11.98±0.45
Cinnamomum zeylanicum	20.15±1.31	22.39±0.91	19.03±0.61	20.98±1.46	17.23±0.65[c]	14.10±0.64	12.08±0.60
Rosmarinus officinalis	10.12±0.36	10.11±0.56	13.03±0.64	11.12±0.37	8.21±0.44	11.08±0.59	14.06±0.47
Mentha piperita	8.86±0.31	9.27±1.34	8.08±0.61	9.16±0.91	8.68±0.84	8.30±0.96	7.39±0.96
Pelargonium graveolens	7.83±0.76	3.43±0.94	6.11±0.67	8.26±1.0	6.84±0.77	7.53±0.53	6.94±0.90
Vitex negundo	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ciprofloxacin©	20.27±1.15	20.30±0.91	18.65±0.72	16.43±0.52	12.51±0.51	18.08±0.59	17.05±0.65

© - 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 oils against bacteria from Otitis externa cases

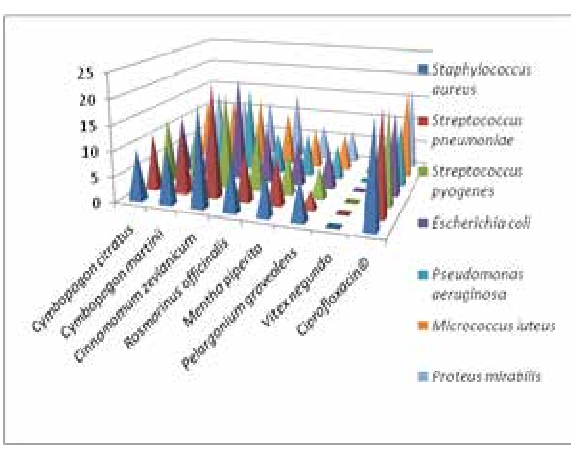


Table 2. MIC of essential oils against clinical bacterial isolates from Otitis externa cases

Organisms	Essential oil / MIC in mg/mL							
	Cymbopogon citratus	*Cymbopogon martinii*	*Cinnamomum zeylanicum*	*Rosmarinus officinalis*	*Mentha piperita*	*Pelargonium graveolens*	*Vitex negundo*	*Ciprofloxacin©*
Staphylococcus aureus	1.00	0.50	0.25	0.75	2.00	2.00	0.00	0.25
Streptococcus pneumoniae	1.00	0.50	0.25	0.75	2.00	2.00	0.00	0.25
Streptococcus pyogenes	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25
Escherichia coli	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25
Pseudomonas aeruginosa	2.00	2.00	0.25	1.50	2.00	2.00	0.00	0.25
Micrococcus luteus	2.00	2.00	0.25	2.00	2.00	2.00	0.00	0.25
Proteus mirabilis	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25

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

Fig 2- MIC of essential oils against clinical bacterial isolates from Otitis externa cases

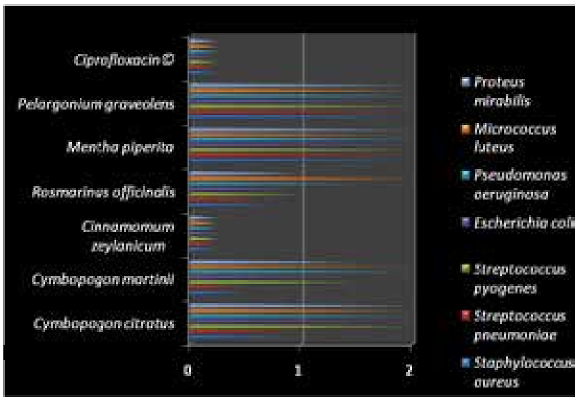
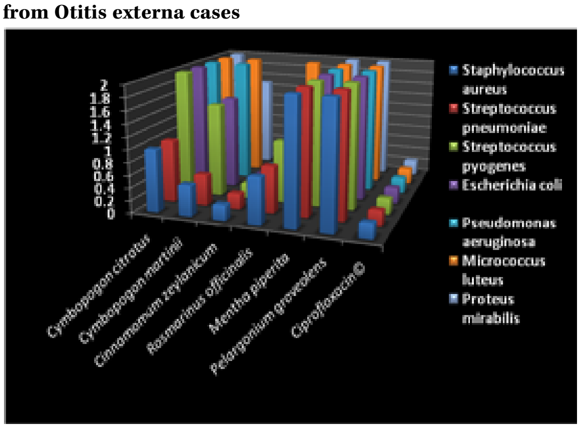


Table 3- MBC of essential oils against clinical bacterial isolates from Otitis externa cases

Organisms	Essential oil / MIC in mg/mL							
	Cymbopogon citratus	*Cymbopogon martinii*	*Cinnamomum zeylanicum*	*Rosmarinus officinalis*	*Mentha piperita*	*Pelargonium graveolens*	*Vitex negundo*	*Ciprofloxacin©*
Staphylococcus aureus	1.00	0.50	0.25	0.75	2.00	2.00	0.00	0.25
Streptococcus pneumoniae	1.00	0.50	0.25	0.75	2.00	2.00	0.00	0.25
Streptococcus pyogenes	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25
Escherichia coli	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25
Pseudomonas aeruginosa	2.00	2.00	0.25	1.50	2.00	2.00	0.00	0.25
Micrococcus luteus	2.00	2.00	0.25	2.00	2.00	2.00	0.00	0.25
Proteus mirabilis	2.00	1.50	0.25	1.00	2.00	2.00	0.00	0.25

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

Fig 3-MIC of essential oils against clinical bacterial isolates from Otitis externa cases



DISCUSSION

All the selected essential oils were found to be active against both gram positive and gram negative bacterial isolates from ocular infection cases. The isolate may be a pathogen or an opportunistic pathogen depending upon condition of the patient's immune system, which has not been focused in this study.Cinnamon oil was more active compared to other oils among the 7 oils tested. Lemongrasses, Palmarosa, rosemary were found to be more than the moderate range. The others such as geranium, peppermint and chaste tree leaf oil. As far as the chemical analysis, and antibacterial study, the following studies were comparable to the results with the present study. Lemongrass (C. citratus) was found to be as effective in a 2.5% cream as four other commercial creams against ringworm and clinical isolates of four dermatophytes in vitro (Wannissorn et al., 1996). Each of the commercial creams had clotrimazole, isoconazole nitrate, ketoconazole, benzoic acid, and salicylic acid as their main active ingredients. (Onawunmi,,&Ogunlana, (1986), found lemongrass effective against E.coliand 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 (Hmamouch et al., 1990). Gachkar et al.,(2007), reported the chemical composition, antibacterial, antioxidative and radical–scavenging properties of the essential oils of R.officinalisobtained by steam distillation (Santoyo et al., (2005), attributed the antimicrobial property of the essential oil of R.officinalisto 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.coliand K. pneumoniae) bacteria strains.

Multiple bacterial species were present in all specimens suggesting that OM is a polymicrobial infection. For example, S. pneumoniae and M. catarrhalis were found in the same specimen in two of five cases (40%). M. catarrhalisand H. influenzae were found together in two out of six specimens (33%) tested. This may be important clinically as evidence in animal models suggests that in a polymicrobial biofilm bacterial species are able to confer protection from host defenses and antimicrobials to each other and thus more resistant that single-specie biofilms (Armbruster et al., 2010). Furthermore, in the nasopharynx of mouse OM models, bacterial composition and viral infection are shown to have significant effects on OM incidence and severity (Krishnamurthy et al., 2009). Although S. pneumoniae and H. influenzae have been described to reside together in the middle ear of children with OM (Leibovitz et al., 2009), interestingly these pathogens were never observed together in the same specimen (0 of 5 specimens) in our study. While the number of samples tested for these species was small, our findings may reflect the competitive interactions observed between S. pneumoniae and H. influenzae (Lysenko et al., 2006). In a significant proportion of biopsy samples, multiple unidentified bacterial species were observed. These bacteria were often not cultured even when shown to be present using FISH or PCR. Other fastidious organisms such as Alloiococcusotitidis may also play a role in this disease as they have been found using PCR and culture in children with both OME and rAOM (Ashhurst-Smith et al., 2007). 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, & 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). 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). The findings of the chemical analysis of the present study were comparable to the results of the following. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds, which also in-pure form have been shown to exhibit antibacterial or antifungal activity (Conner, 1993). The antibacterial properties of these compounds are in part associated with their lipophilic character, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion (Conner, 1993). Chemical analysis of these oils have shown that the principal active compounds of these oils are principally carvacrol, thymol, citral, eugenol, 1–8 cineole, limonene, pinene, linalool and their precursors (Demetzos, &Perdetzoglou, 2001). However, there are often large differences in the reported antibacterial activity of oils from the same essence. The reasons for this variability can be due to the geographical sources, the harvesting seasons, the genotype, the climate, the drying and the distilled part of the plant which are significant factors influencing the chemical composition and relative proportions of the individual constituents in the essential oils of the plant (Juliano et al.,2000). Also, a number of essential oil constituents exhibit significant antimicrobial properties when tested separately (Lambert, et al., (2001). In conclusion, medicinal and aromatic plants are widely used today in modern phytotherapy (Demirci et al., 2004). The essential oils and their components are known to be active against a wide variety of microorganisms (Vukovic, 2007).

CONCLUSIONS

The essential oils 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 essential oil can be prepared in different forms such as topical agents, eye drops and other therapeutic agents of those essential oils may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of bacterial diseases in various patients, particularly, for otitis externa infection patients after further analysis on their side effects.

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REFERENCE

Ahlen C, Mandal LH, Iversen OJ (July 1998). "Identification of infectious Pseudomonas aeruginosa strains in an occupational saturation diving environment". Occup Environ Med 55 (7): 480–4. | Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol (1998 ;) 62:: 183–93. | Armbruster CE, Hong W, Pang B, Weimer KE, Juneau RA, Turner J, Swords WE: Indirect Pathogenicity of Haemophilusinfluenzaeand Moraxella catarrhalis in Polymicrobial Otitis Media Occurs via Interspecies Quorum Signaling. MBio 2010, 1:(3). | Ashhurst-Smith C, Hall ST, Walker P, Stuart J, Hansbro PM, Blackwell CC: Isolation of Alloiococcusotitidis from Indigenous and non-Indigenous Australian children with chronic otitis media with effusion. FEMS Immunol Med Microbiol 2007, 51(1):163-170. | Bagamboula, C. F., Uyttendaele, M., &Debevere, J. (2003).Antimicrobial effect of pices and herbs on Shigellasonnei and Shigellaflexneri. Journal of Food Protection, 66, 668–673. | Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, GadhiCA.Antibacterial activity of Quercus ilex bark's extracts. JEthnopharmacol(2007;) 112:: 426–9. | Bower,C.K., K.F. Schilke and M.A. Daeschel, Antimicrobial properties of raisins in beef jerky preservation, Journal of Food Science 68 (2003), pp. 1485–1489. | Burmolle M, Thomsen TR, Fazli M, Dige I, Christensen L, Homoe P, Tvede M, Nyvad B, Tolker-Nielsen T, Givskov M, et al: Biofilms in chronic infections - a matter of opportunity - monospecies biofilms in multispecies infections. FEMS Immunol Med Microbiol 2010, 59(3):324-336. | Campo, M.J. Amiot and C. Nguyen-The, Antimicrobial effect of rosemary extracts, Journal of Food Protection 63 (2000), pp. 1359–1368. | Coates H: Chronic suppurative otitis media without cholesteatoma. In Advanced Therapy of Otitis Media.Edited by: Alper C, Bluestone, C., Dohar, J., Madel, E. &Casselbrant, M. Hamilton, Ontario. B.C. Decker Incorporated; 2004:299-305. | Conner, D. E (1993). Naturally occuring compounds. In P. Davidson & A. L. Branen (Eds.), Antimicrobials in foods (pp. 441–468). New York, NY: Marcel Dekker. | Cooper EL. CAM, eCAM, bioprospecting: the 21st century pyramid. Evid Based Complement Alternat Med (2005;) 2:: 125–7. | Cowan, 1999 M.M. Cowan, Plant products as antimicrobial agents, Clinical Microbiology Reviews 12, pp. 564–582. | Demetzos, C., &Perdetzoglou, D. K. (2001).Composition and antimicrobial studies of the oils of OriganumcalcaratumJuss.andO. scabrumBoiss. etHeldr. From Greece. Journal of Essential Oil Research, 13, 460–462. | Demirci B, Paper DH, Demirci F, Baser KH, Franz G. Essential oil of BetulapendulaRoth. buds. eCAM (2004;) 1:: 301–3. | Ehrlich GD, Veeh R, Wang X, Costerton JW, Hayes JD, Hu FZ, Daigle BJ, Ehrlich MD, Post JC: Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. Jama 2002, 287(13):1710-1715. | Erdemog¸lu, N., Ku¨ peli, E., Yes_ilada, E., (2003).Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine.J. Ethnopharmacol. 89, 123–129. | Gachkar, L., Yadegari, D., Rezaei, M. B., Taghizadeh, M., Alipoor, A.S., Rasooli, I. 320 (2007).Chemical and biological characteristics of Cuminumcyminum and 321 Rosmarinus officinalis essential oils. Food Chemistry 102: 898-904. | Golab M, Burdzenia O, Majewski P, Skwarlo-Sonta K. Tea tree oil inhalations modify immunity in mice. J Appl Biomed (2005;) 3:: 101–8. | Harborne JB (1998). Phytochemical Methods - A Guide to Modern Techniques of Plant analysis. Chapman and Hall, London. pp. 182-190. | Helander, I. M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. (1998).Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural and Food Chemistry, 46, 3590–3595. | Hmamouch, M., Tantaoui-Elaraki, A., Es-Safi, N., &Agoumi, A. (1990).Illustration of antibacterial and antifungal properties of eucalyptus essential oils.PlantesMedicinales. | Ito N, Nagai T, Oikawa T, Yamada H, Hanawa T. Antidepressant-like effect of l-perillaldehyde in stress-induced depression-like model mice through regulation of the olfactory nervous system. In:eCAM (2008;). | Juliano, C., Mattana, A., &Usai, M.(2000). Composition and in vitro antimicrobial activity of the essential oils of Thymus herba-baronaLoisel growing wild in Sardinia. Journal of Essential Oil Research, 12, 516–522. | Kang K, Stevens SR. Pathophysiology of atopic dermatitis. ClinDermatol 2003;21:116–121 | Krishnamurthy A, McGrath J, Cripps AW, Kyd JM: The incidence of Streptococcus pneumoniae otitis media is affected by the polymicrobial environment particularly Moraxella catarrhalis in a mouse nasal colonisation model. Microbes Infect 2009, 11(5):545-553. | Lambert, R. J. W., Skandamis, P. N., Coote, P., &Nychas, G.-J. E. (2001).A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91, 453–462. | Lataoui, N., &Tantaoui-Elaraki, A. (1994).Individual and combined antibacterial activity of the main components of three thyme essentials oils.RivistaItaliana EPPOS, 13, 13–19. | Leibovitz E, Serebro M, Givon-Lavi N, Greenberg D, Broides A, Leiberman A, Dagan R: Epidemiologic and microbiologic characteristics of culture positive spontaneous otorrhea in children with acute otitis media. Pediatr Infect Dis J 2009, 28(5):381-384. | Lysenko ES, Ratner AJ, Nelson AL, Weiser JN: The role of innate immune responses in the outcome of interspecies competition for colonization of mucosal surfaces. PLoSPathog 2005, 1(1):e1. | Naser B, Bodinet C, Tegtmeier M, Lindequist U. Thujaoccidentalis (Arbor vitae): A review of its pharmaceutical, pharmacological and clinical properties. eCAM (2005;) 2:: 69–78. | Onawunmi, G.,&Ogunlana, E. (1986).Astudy of the antibacterial activity of the essential oil of lemongrass. Int J Crude Drug Res, 24(2), 64-68. | Park CW, Han JH, Jeong JH, Cho SH, Kang MJ, Tae K, Lee SH: Detection rates of bacteria in chronic otitis media with effusion in children. J Korean Med Sci 2004, 19(5):735-738. | Rapini, Ronald P.; Bolognia, Jean L.; Jorizzo, Joseph L. (2007). Dermatology: 2-Volume Set. St. Louis: Mosby. ISBN 1-4160-2999-0. | Rosenfeld, R. M.; Schwartz, S. R.; Cannon, C. R.; Roland, P. S.; Simon, G. R.; Kumar, K. A.; Huang, W. W.; Haskell, H. W.; Robertson, P. J. (3 February 2014). "Clinical Practice Guideline: Acute Otitis Externa Executive Summary". Otolaryngology -- Head and Neck Surgery 150 (2): 161–168. | Salomao K, Pereira PR, Campos LC, Borba CM, Cabello PH, Marcucci MC, de Castro SL. Brazilian Propolis: correlation between chemical composition and antimicrobial activity. eCAM (2008;) 5:: 317–24. | Santoyo, S., Cavero, S., Jaime, L., Ibanez, E., Senorans, F. J. &Reglero, G. (2005). 377 Chemical composition and antimicrobial activity of Rosmarinus officinalis L. 378 essential oil obtained via supercritical fluid extraction. Journal of Food 379 Protection, 68(4): 790-795. | Sofowora A (1984). Medicinal plants and traditional medicine in Africa published in association with spectrum Books Ltd. Ibadan by John Wiley and Sons, NY pp 142-143. | Trease GE, Evans WC (1989) Textbook of Pharmacognosy. 12thEdn.Balliere, Tinadl London. | Van der Berghe, D.A., Vlietinck, A.J., (1991). Screening methods for antibacterial agents from higher plants. In: Dey, P.M., Harborne, J.B., Hostettman, K. (Eds.), Methods in Plant Biochemistry. Assay for Bioactivity, vol. 6. Academic Press, London, pp. 47–69. | Vikingo .2007. "Swimmers Ear – Additional Advice About A Pesky and Sometimes Painful Problem". Diver's Alert Network: Alert Diver Magazine. Retrieved 2008-07-22 | Vukovic N, Milosevic T, Sukdolak S, Solujic S. Antimicrobial activities of essential oil and methanol extract of Teucriummontanum. eCAM. (2007;) 4:: 17–20. | Wannissorn, B., Jarikasen, S., &Soontorntanasart, T. (1996).Antifungal activity of lemongrass oils and lemongrass oil cream.Physiotherapy Research, 10, 551-554. |