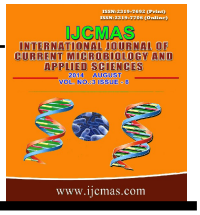
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 415-429**
http://www.ijcmas.com



Original Research Article

Otitis externa – Fungal isolates and their sensitivity to essential oils of selected herbals

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Keywords

Antifungal activity, opportunistic, pathogenic fungi, ear infections, essential oils, aromatherapy.

A B S T R A C T

Many pathogens and opportunistic pathogenic agents are increasingly encountered in ear infections. In this study fungi isolated from otitis externa cases were tested for their sensitivity to essential oils from *Cymbopogon citratus*, *Cymbopogon martinii*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis*, *Mentha piperita*, *Pelarogonium graveolens*, and *Vitex negundo*. In agar well diffusion method the selected essential oils were effective against all forms such as yeast like fungi and molds and mostly comparable to the standard reference antifungal Amphotericin – B. The minimal inhibitory concentration (MIC) of *C. citratus* and *C.martinii*were effective but to the least level, when compared with the reference drug. The MIC of *C.zeylanicum*was best and very much comparable to the reference drug. The MIC of *R.officinalis*was variable from more effective to least effective against different test organisms. The MIC of *M. piperita was* effective but to the least level (*1000* µg/ml), when compared with the reference drug. The MIC of *P.graveolens*, was effective but to the least level, when compared with the reference drug. The MIC of *Vitex negundo* was effective but to the least level, when compared with the reference drug. The results for minimum fungicidal concentration (MFC) were similar to MIC results, but in MBC confirmation was made by the absence of visible growth in culture media. The antifungal activity is attributed to the components of essential oils, which mostly interferes with the cell membrane structure and function. According to the results of this study, the essential oil or their ethanol extract of those essential oils may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of fungal diseases in various patients, particularly, for otitis externa patients.

Introduction

Fungal ear canal infections, also known as otomycosis, range from inconsequential to extremely severe. Fungus can be saprophytic, in which there are no symptoms and the fungus simply co-exists in the ear canal in a harmless parasitic relationship with the host, in which case the only physical finding is presence of the fungus. If

for any reason the fungus begins active reproduction, the ear canal can fill with dense fungal debris, causing pressure and ever-increasing pain that is unrelenting until the fungus is removed from the canal and anti-fungal medication is used. Most antibacterial ear drops also contain a steroid to hasten resolution of canal edema and pain. Unfortunately such drops make fungal infection worse. Prolonged use of them promotes growth of fungus in the ear canal. Antibacterial ear drops should be used a maximum of one week, but 5 days is usually enough. Otomycosis responds more than 95% of the time to a three day course of the same over-the-counter anti-fungal solutions used for athlete's foot.*Candida albicans* and *Aspergillus*species are the most common fungal pathogens responsible for the condition.

Although there is evidence that steroids are effective at reducing the length of treatment time required, fungal otitis externa (also called otomycosis) may be caused or aggravated by overly prolonged use of steroid-containing drops.

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 bacteria 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.

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 anti-inflammatory, antioxidant and anti-cancerogenic properties (Ito et al., 2008). In addition to inducing resistance, antibiotics are sometimes associated with opposing effects such as hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). Therefore, there is a need to

develop alternative antimicrobial drugs for the treatment of infectious diseases (Salomao et al., 2008). It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher et al., 1987). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Lis-Balchin and Deans, 1997). All the oils tested exhibited different degrees of antifungal activity against *A. fumigatus* and *A. niger*. The maximum antimycotic activity was shown by *C. martinii* followed by *C. citratus*, *Eucalyptus globulus*and *C.zeylenicum*. Aggarwal et al., (2000) reported antimycotic activity of *C. martinii* against *A. niger*. The oil of *C. citratus* was effective against fungal pathogens causing diseases in plants and human beings (Singh, 2000).

Quale et al., (1996) treated infections caused by *Candida* in AIDS patients with a drug based on Cinnamon. In our study we also found that essential oil extracted from *C. zeylanicum* demonstrated strong antifungal activity on both the species of *Aspergillus*. The antimycotic activity of cinnamon bark due to presence of cinnamaldehyde is well known (Viollon and Chaumont, 1994). Similarly, *in vitro* antimicrobial activity of *C.zelyanicum*(bark) against human pathogenic fungi and commensally bacteria was studied by Chaumont, (2003)andMatan et al.,, (2006). The oils of *M.spicata, Azadirachtaindica, Eugenia caryophyllata, Withaniasomnifera* and *Zingiberofficinale*exhibited moderate activity. The essential oil of mint was found to have strong antimycotic activity against *C. albicans (*Kishore et al., 1993).

The main advantage of essential oils is that they can be used in any foods and are considered generally recognized as safe (GRAS) (Kabara, 1991), as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food. Such antimicrobial activity is due to the presence of bioactive substances such as flavonoids, terpenes, coumarines and carotenes (Tepe et al., 2005).

The objective of this work was to study the effect of the essential oils of Lemongrass oil, Palmarosa oil, Cinnamon bark oil, Rosemary oil, Geranium oil, Peppermint oil, and Chaste tree leaf oil on the growth of fungus commonly associated with ocular infection cases.

The interplay of plants and human health has been documented for thousands of years(Newmanet al., 2003). Herbs have been integral to both traditional and non-traditional forms of medicine dating back at least 5000 years (Koehn and Carter, 2005). The enduring popularity of herbal medicines may be explained by the tendency of herbs to work slowly, usually with minimal toxic side effects.

The present study was carried out to identify the effectiveness of seven essential oils against fungal pathogens isolated from fungal 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 martinii*-

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) andHarborne,(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 Fungi

Standard mycological techniques were followed to isolate and identify the fungi present in the collected samples.

Antifungal activity

Agar well diffusion method

In this study standard agar well diffusion method was followed (Bagamboula et al., 2004; Erdemogilu et al., 2003). Each fungal isolate was suspended in Sabouraud's Dextrose (Himedia, India) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were "flood inoculated" onto the surface of Sabouraud's Dextrose 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 48 h at Room Temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ethanol was used as solvent control. Amphotericin –B was used as reference antibacterial agent. The tests were carried out in triplicate.

Minimum inhibitory concentration (MIC) and Minimal fungicidal concentration (MFC)

Antifungal activity was measured using a dilution in agar technique (Alves&Cury, 1992). The essential oil (100 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially two fold diluted in Yeast Nitrogen Base Phosphate (YNBP) broth (Merck, Germany) to obtain a concentration range of 15.6-1000 µl/ml.

YNBP broth containing only DMSO diluted in the same way, which did not influence fungal growth, were included as controls. All fungal strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an OD (530 nm) of 0.05 (equivalent to 1 X 10^6 CFU/ml). This suspension was used as the inoculum for the test in the agar plates.

Fungal suspensions (3µl) were inoculate using a automatic micropippete (Transasia), and plates (diameter: 25 cm) were incubated at 37^{o}C for 48 h. the minimal inhibitory concentration (MIC) was defined as the minimal concentration of the essential oil which completely inhibited the visible growth of the fungus and MFC as the lowest

concentration that completely inhibited fungal growth in plate. An antifungal agent Amphotericin –B included as reference antifungal agent, was tested using the same technique. All antifungal assays were tested in duplicate.

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 and Discussion

Isolates

Based on morphological and cultural characters, isolateswere identified as *Candida albicans, Candida tropicalis, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium Sp, Mucor Spp,* and *Rhizopus Spp.*

Results for agar well diffusion method

In agar well diffusion method the selected essential oils were effective against all forms such as yeast, yeast like fungi and molds. *C. citratus* was highly active against *Mucor spp(*14.25±0.97), *Penicillium Sp(*13.0±0.51) and least against *A.flavus spp*. (8.13±0.636) *C.martinii*was highly active against *C.albicans*(13.25±0.90), *C.tropicalis*, (13.16±0.53),*C.neoformans (* 13.89±0.43) and least against *A.flavus*(11.03±0.48). *C.zeylanicum*was highly active against *C.neoformans*(21.83±0.74) *A.niger*(21.15±0.86) *C.albicans*, (19.23±0.80) and least against *Mucor spp*(11.87±0.77). *R. officinalis* was highly active against *Mucor spp(14.8*±0.70), *C.albicans*(13.12±0.80) and least against. *A.flavus*(7.97±0.73).*M.piperita*was highly active against *Rhizopus spp*(21.06±0.67),*C.albicans*(19.40±0.73) and least against *A.fumigatus*(*8.11*±0.37). *P. graveolens* was highly active against *Rhizopus spp*(18.04±0.55) *Mucor spp*, (17.51±0.96) and least against *A.niger (*9.03±0.58). *V.negundo*was highly active against *Rhizopus spp*(13.09±0.43), *A.niger*(13.03±0.81), *C.albicans*(12.09±0.59) and least against *C.tropicalis*(8.99±0.47). All fungi were found to be sensitive to all test essential oils and mostly comparable to the standard reference antifungal drug Amphotericin –B.

Results for minimum inhibitory concentration (MIC) of essential oils

The minimal inhibitory concentration of *C. citratus* was 250 μg/ml *against C.albicansC.tropicalis*and *PenicilliumSp*and it was 500 μg/ml, for *A.niger, A.flavus, A.fumigatus Mucor spp*and *Rhizopus spp.* The results showed that they effective but to the least level, when compared with the reference drugAmphotericin –B. The minimal inhibitory concentration of *C. martinii* was similar to *C. citratus.* The minimal inhibitory concentration of *C.zeylanicum*was 15.6 μg/ml against *C.albicans*, *C.tropicalis*, and *Penicillium Spand* 31.25 μg/ml *against A.niger, A.flavus, A.fumigatus*, *Mucor spp*, and *Rhizopus spp*.. The results were best and very much comparable to the reference drug.

The minimal inhibitory concentration of *R.officinalis*was 125 μg/ml against *C.albicans*and *C.tropicalis,*and it was 250 μg/ml *Penicillium Sp*and 500 μg/ml for *A.nigerA.flavus*, *A.fumigatusMucor spp*and *Rhizopus spp.* The results showed that they were active against *C.albicans*and *C.tropicalis*effective; moderate to *Penicillium Sp*but to the least level against

other organisms, when compared with the reference drug. The minimal inhibitory concentration of *M. piperita* was 1000 µg/ml for all the test fungi. The minimal inhibitory concentration of *P.graveolens,*was 500 µg/ml for all the test fungi. The minimal inhibitory concentration of *Vitex negundo* was 500 µg/ml for all the test fungi. The results showed that they were effective but to the least level, when compared with the reference drug.

Results for Minimum fungicidal concentration (MFC) of essential oils

The results for minimum fungicidal concentration (MFC) were similar to minimum inhibitory concentration (MIC) results, but in MBC confirmation was made by the absence of visible growth in culture media.

Chronic otitis externa (COE) is a chronic relapsing disease for which current medical options prove ineffective(HajioffandMackeith, 2010; Wright et al., 2009; Caffieret al., 2007)1–3.Over the last decade; we have perfected a safe and simple therapeutic technique for the management of COE known as chemical ear peeling (CEP). The clinical results for a group of 28 patients have been recently published (Fusconi, 2010). The procedure allows the debridement of the infected external auditory canal (EAC). All content is trapped in a film made of polyvinyl alcohol precipitated in acetic acid and depth-bound by the superficial epithelial layer of the EAC. The newly formed film is simply removed with a Hartmann forceps. A CEP result in a significant increase in disease-free intervals compared to standard treatments and provides a definitive cure for many patients(Fusconiet al., 2010). In the first phase, we treated patients affected by chronic external otitis with CEP or antibiotic/steroid treatment to compare the clinical and microbiological outcomes. In the second phase, we compared the microscopic findings observed in CEP samples of patients affected by COE's acute exacerbation (COEE) or by acute otitis externa (AOE) to demonstrate the role of biofilm in the pathogenesis of COE.

Although aromatherapy is a lesser known complementary therapy, it has much to offer nursing care ocular patients in particular for the control of resistant infections (Buckle, 1999). While the recognized definition states that "aromatherapy is the use of essential oils for therapeutic purposes" (Styles, 1997), the definition of clinical aromatherapy (as used in nursing) is more specific: "The use of essential oils for outcomes that are measurable" (Buckle, 2000). The definition of essential oils is also very specific: "Essential oils are the steam distillate of aromatic plants" (Tisserand&Balacs (1995). Other kinds of extracts that are not obtained by steam distillation are not essential oils. Extracts may contain residues of allergenic solvents. Lemongrass (*Cymbopogon 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. It was found that essential oils were effective against both acute and chronic infections in humans. He also found that concentrations that were insufficient to kill the pathogenic organism in a laboratory were effective in humans. The example given was an in vitro minimum inhibitory concentration (MIC) of 0.00025 g/mL as opposed to an in vivo concentration of 0.0000032 g/mL (Valnet et al.,1978). Geranium, cinnamon, and

peppermint were found by Viollon et al.,(1993) to be effective in vitro against *Candida*. Citral is the generic name for two different isomeric aldehydes (geranial and neral) that are found in many essential oils. Citral is thought to be the component most likely to be antifungal (Pattnaik et al., 1997). Onawunmi, (1989) found citral to have antifungal properties in dilutions as low as 0.005% to 0.008%.

Essential oils containing large amounts of citral are melissa, verbena, and lemongrass. Aldehydes are best avoided on a damaged mucous membrane, but they can be used diluted on the skin. A component of essential oils found by Beylier&Givaudan, (1979) to have anti-candida properties is citronellol. Citronellol is an alcohol and is the main constituent of lemon grass and *Eucalyptus citriadora*(60%-80%). Alcohol is safe to use on the skin and the mucous membrane. Pattnaik et al., (1996) reported that lemongrass, *Eucalyptus globulus*, palmarosa, and peppermint were the most effective essential oils tested against *Cryptococcus.* Basil and thyme were not included in this study. (Lemongrass was effective not only against *Cryptococcus* but against all 11 other fungi tested in low dilutions.)

The MIC for each of the four essential oils against *Cryptococcus* was 5 L/mL. In another article, Pattnaik et al., (1997) found that complete essential oils were more effective against *Cryptococcus* than the isolated, active component. There was one exception, lemongrass, which was equal to the isolated parts of citral and geranial (Larrondo& Calvo,1991) compared the topical and inhaled action of citral to the systemic effects of clotrimazole. Although the actual way essential oils work as fungicides is not completely clear, it appears that metabolism and growth of the fungus are inhibited, often with a breakdown in the lipid part of the membrane, resulting in increased permeability and/or rupture. Larrondo et al., (1995); Soliman et al., (1994) tested essential oil of rosemary. They investigated the essential oil distilled from two plants growing in different climatic conditions. They found that both rosemary essential oils were effective against *C. neoformans* in vitro and recommended that either essential oil could be an effective treatment in AIDS patients with cryptococcal meningitis and pneumonia. Although both types of rosemary were effective, the effectiveness could have been due to a different chemical component in each oil. Many of the essential oils used showed good fungistatic action. The best effects were from palmarosa, geranium, savory, sandalwood, thyme, marjoram, and lavender that appeared to agree with the findings of (Valnet et al., 1978). as discussed earlier in this article and could be related to the adaptogenic capacity of essential oils (and all plant medicines) to behave differently depending on the terrain they are in.(Lucini et al., 2006) indicated that mycelial growth inhibition is caused by the monoterpenes present in essential oils. These components would increase the concentration of lipidic peroxides such as hydroxyl, alkoxyl and alkoperoxyl radicals and so bring about cell death. For (Sharma and Tripathi, 2006), the EOs would act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and integrity of the hypha cell wall, resulting in its collapse and death of the mycelium.

Only few substances are known to inhibit human pathogenic fungi, which are often completely resistant to antibiotics, and most of them are relatively toxic. The increased incidence of therapeutic failure in the treatment of fungal infections and the

prevalence of opportunistic infections has renewed interest in the search for new antifungal agents, including those obtained from higher plants. Present results allow supposing that these natural compounds could be useful agents in the topical treatment of fungal infections. Medicinal plants have been used in developing countries as alternative treatments to health problems. Many plant extracts and essential oils isolated from plants have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants (Mart´ýnez et al., 1996). Brazil, Cuba, India, Jordan and Mexico are examples of countries that have a diverse flora and a rich tradition in the use of medicinal plants for both antibacterial and antifungal applications (Rehder et al.,2004).

Cryptococcus neoformans, a fungus which causes infection during the last stages of AIDS is inhibited both by Palmarosa oil and geraniol (Viollon et al., 1994). Potassium leakage from a different fungus, *C. albicans*, due to action of geraniol over a period of 2 h has been reported earlier (Bard et al., 1988).

Palmarosa oil led to changes in the composition of the yeast cell membrane, with more saturated and less unsaturated fatty acids in the membrane after exposure of *S. cerevisiae* cells to the oil. Some of the Palmarosa oil was lost by volatilization during incubation of the oil with the yeast cells. The actual concentration of the oil components affecting the yeast cells could not therefore be accurately determined (Anjali Prashar et al., 2003). Reports of some essential oils affecting membrane integrity include tea tree oil causing damage to membranes in *C. albicans* while other oils and their components have disrupted the permeability barrier of yeast cells (Cox et al.,1998). The fatty acid composition of microbial cell membranes affects their ability to survive in various environments (Ghfir et al., 1994). The ratio of saturated to unsaturated fatty acids can alter in response to environmental conditions (Odumeru et al., 1993).

Table.1 Qualitative chemical analysis essential oils

Essential oils	**Chemicals tested**					
	Alcohols	***Terpenoids***	***Phenolics***	***Flavonoids***	***Alkaloids***	***Aldehydes***
Cymbopogon citratus	+	+	+	+	+	+
Cymbopogon martini	+	+	+	+	+	+
Cinnamomum zeylanicum	+	+	+	+	+	+
Rosmarinus officinalis	+	+	+	+	+	+
Mentha piperita	+	+	-	+	+	-
Pelargonium graveolens	+	+	-	-	+	-
Vitex negundo	+	+	+	+	+	-

Table.2 Antifungal activity of essential oils against clinical isolates from HIV positive individual

Organisms	Essential oils/ Zone of Inhibition in mm							
	C.albicans	***C.tropicalis***	***Penicillium sps***	***A.niger***	***A.flavus***	***A.fumigatus***	***Mucor spp***	***Rhizopus spp***
Cymbopogon citratus	9.87±0.73	11.08±0.38	13.0±0.51	11.07±0.35	8.13±0.636	12.09±0.62	14.25±0.97	10.01±0.60
Cymbopogon martini	13.25±0.90	13.16±0.53	13.89±0.43	11.95±0.40	11.03±0.48	12.97±0.45	12.07±0.6	11.95±0.68
Cinnamomum zeylanicum	19.23±0.80	21.83±0.74	19.20±o.75	21.15±0.86	17.02±0.37	14.23±0.60	11.87±0.77	18.43±1.47
Rosmarinus officinalis	13.12±0.54	9.72±0.70	12.96±0.41	11.12±0.66	7.97±0.73	10.71±0.78	14.08±0.70	10.07±0.61
Mentha piperita	19.40±0.73	12.14±0.48	11.13±0.64	13.18±0.77	10.68±0.76	8.11±0.37	11.12±0.57	13.06±0.67
Pelargoniumgraveolens	13.13±0.46	13.17±0.31	9.95±0.50	9.03±0.58	12.0±0.48	13.12±0.37	17.51±0.96	18.04±0.55
Vitex negundo	12.09±0.59	8.99±0.47	10.09±0.42	13.03±0.81	11.08±0.71	9.18±0.57	12.06±0.58	13.09±0.43
Miconazole©	14.15±0.71	12.12±0.62	11.01±0.81	13.51±0.51	12.09±0.61	14.18±0.77	12.11±0.56	10.98±0.46

Fig.1 Antifungal activity of essential oils against clinical isolates from Otitis Externa cases

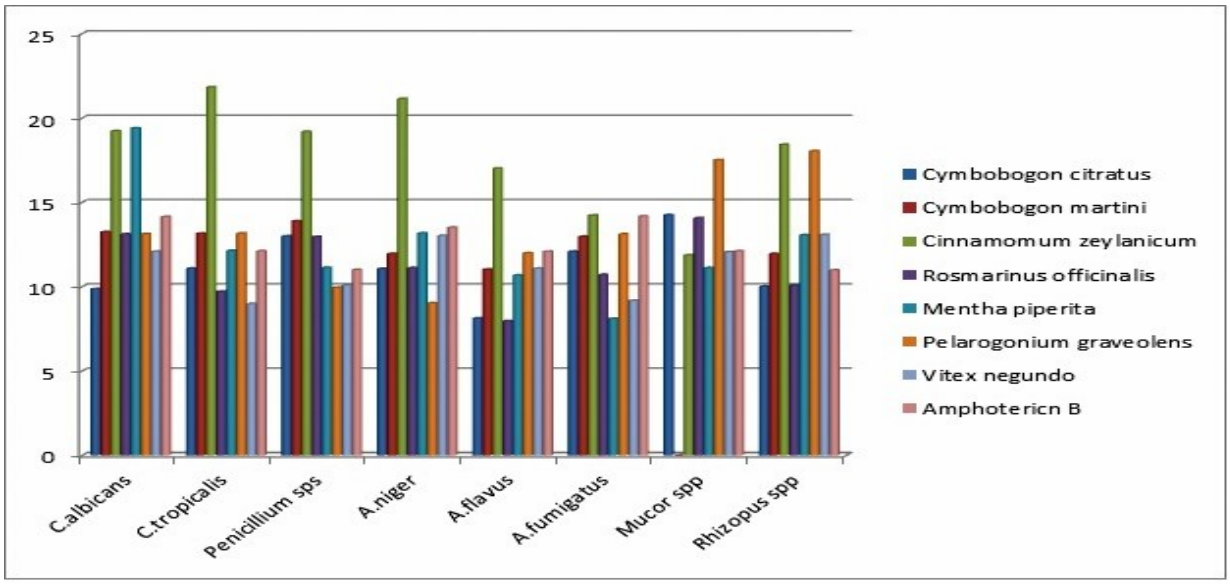


Table.3 MIC of essential oils against clinical fungal isolates from Otitis Externa cases

Organisms	*Cymbopogon citratus*	*Cymbopogon martini*	*Cinnamomum zeylanicum*	*Rosmarinus officinalis,*	*Mentha piperita*	*Pelargonium graveolens*	*Vitex negundo*	Miconazole (100 µg)
Candida albicans	250	250	15.6	125	1000	500	500	15.6
Candida tropicalis	250	250	15.6	125	1000	500	500	15.6
Cryptococcus neoformans	250	250	15.6	250	1000	500	500	15.6.
Aspergillus niger	500	500	31.25	500	1000	500	500	31.25
Aspergillus flavus	500	500	31.25	500	1000	500	500	31.25
Aspergillus fumigatus	500	500	31.25	500	1000	500	500	31.25
Mucor spp	500	500	31.25	500	1000	500	500	62.5
Rhizopus spp	500	500	31.25	500	1000	500	500	62.5

Fig.2 MIC of essential oils against clinical fungal isolates from Otitis Externa cases

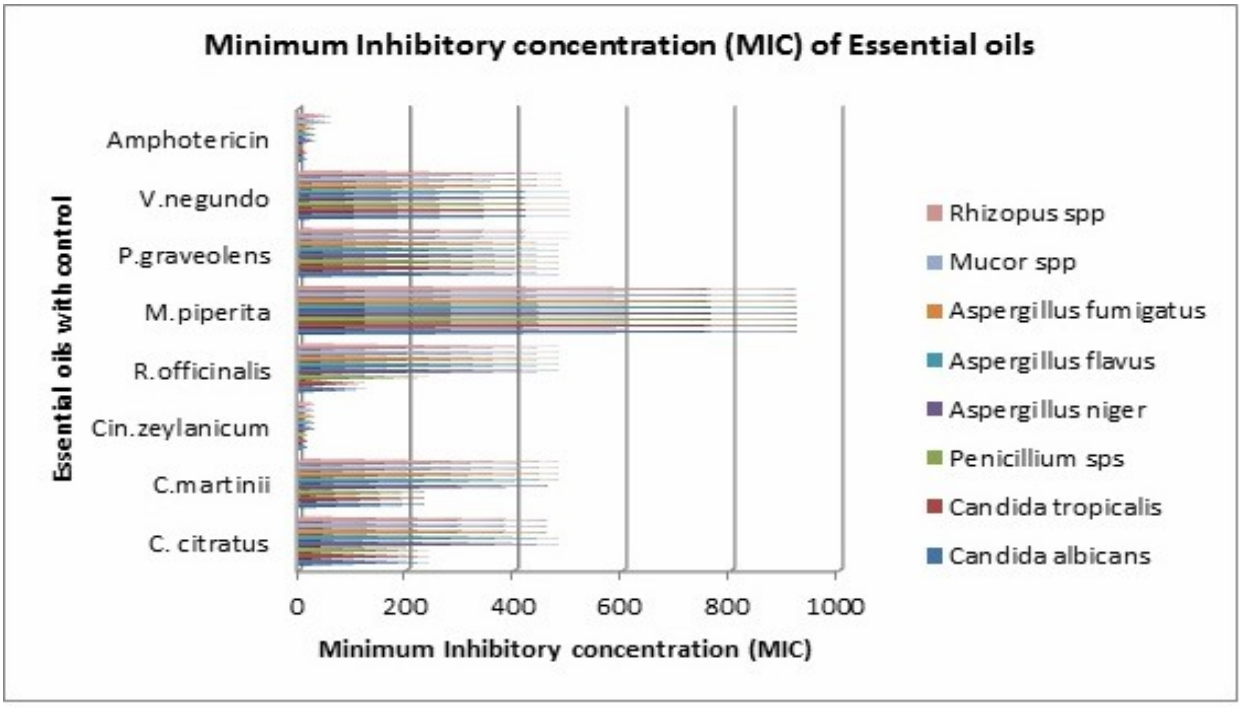
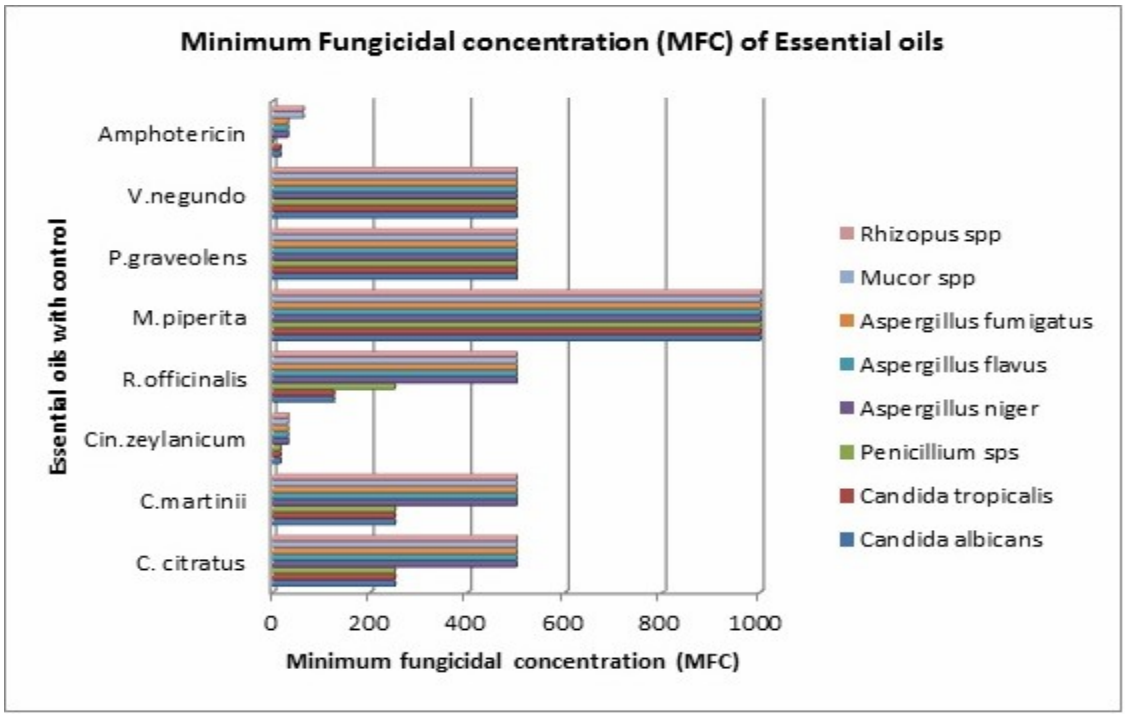


Table.3 MFC of essential oils against clinical fungal isolates from Otitis Externa cases

Organisms	*Cymbopogon citratus*	*Cymbopogon martini*	*Cinnamomum zeylanicum*	*Rosmarinus officinalis,*	*Mentha piperita*	*Pelargonium graveolens*	*Vitex negundo*	Miconazole (100 µg)
Candida albicans	250	250	15.6	125	1000	500	500	15.6
Candida tropicalis	250	250	15.6	125	1000	500	500	15.6
Cryptococcus neoformans	250	250	15.6	250	1000	500	500	15.6.
Aspergillus niger	500	500	31.25	500	1000	500	500	31.25
Aspergillus flavus	500	500	31.25	500	1000	500	500	31.25
Aspergillus fumigatus	500	500	31.25	500	1000	500	500	31.25
Mucor spp	500	500	31.25	500	1000	500	500	62.5
Rhizopus spp	500	500	31.25	500	1000	500	500	62.5

Fig.3 MFC of essential oils against clinical fungal isolates from Otitis Externa cases



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