

Ocular infection –Fungal isolates and their sensitivity to essential oils of selected herbals



Zoology

KEYWORDS : Antifungal activity, opportunistic fungi, pathogenic fungi, ocular fungal infections, essential oils, aromatherapy.

* P.Sivamani	Microlabs, Institute of Research and Technology, Arcot, Vellore, Tamilnadu, India-632 503. * Corresponding Author
B.Asha Nancy	Microlabs, Institute of Research and Technology, Arcot, Vellore, Tamilnadu, India-632 503
M. Mary Queen	Microlabs, Institute of Research and Technology, Arcot, Vellore, Tamilnadu, India-632 503
R.V.Kalaimathi	Postgraduate & Research Department of Zoology, Arulmigu Palaniandavar College of Arts and Culture, Palani- 624601, Tamilnadu, India.
C.Kandeepan	Postgraduate & Research Department of Zoology, Arulmigu Palaniandavar College of Arts and Culture, Palani- 624601, Tamilnadu, India.

ABSTRACT

Ocular microbiology remains an applied science. Many pathogens and opportunistic pathogenic agents are increasingly encountered in ocular infections. Fungi isolated from ocular infections 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 all forms such as, yeastlike 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 (500 µg/ml), when compared with the reference drug. The MIC of Vitex negundo was effective but to the least level (500 µg/ml), 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 ocular patients.

INTRODUCTION

Infection of the eye leads to conjunctivitis, keratitis, endophthalmitis and other infections which are responsible for increased incidence of morbidity and blindness worldwide (Chirambo et al., 1986; Juarez-Verdayes et al., 2006). Fungal keratitis (keratomycosis) is a fungal infection of the cornea. It primarily affects the corneal epithelium and stroma, although the endothelium and anterior chamber of the eye may get involved in more severe disease. It is primarily seen in tropical climates and is rare in temperate areas. Its incidence is between 6%–20% of all microbial keratitis cases depending on the geographic location. (Liesegang & Forster, 1980; Gopinathan et al., 2009). Traditionally, it is considered a disease of rural areas and is frequently caused by trauma with vegetative material. However, the major risk factor in developed countries is contact lens use at this time. Its incidence has been reported to be increasing due to widespread use of contact lenses, especially bandage contact lenses, and topical steroid usage. (Iyer et al., 2006; Gower et al., 2010). While tropical climates show a preponderance of filamentous fungi, temperate climates show higher percentages of yeast infections (Liesegang & Forster, 1980; Gopinathan et al., 2002; Ritterband et al., 2006).

The only commercially available antifungal drug in the United States is Natamycin (also called Pimaricin) available as a 5% suspension. In other parts of the world where keratomycosis is seen much more frequently such as India, additional antifungal agents such as Fluconazole and Miconazole are available. However, various other drugs can be compounded into eye drops (by compounding pharmacies) and are effective. The most commonly used drugs are Voriconazole (1%), (Loh et al., 2009; Jurkunas et al., 2007) Amphotericin B (0.15%), Fluconazole, and Miconazole. Antiseptics such as Chlorhexidine 0.2% and Povidone iodine (5%) have also been advocated as cheap and easily available alternatives but are not as effective. (Panda et al.,

2003). Systemic antifungal medications have been advocated as adjunctive therapy in severe cases, especially ulcers with anterior chamber reaction but there have been no controlled studies showing a clear benefit of adding systemic antifungals. (Thakar, 1994; Agarwal et al., 2001). Therapy should be aggressive and most authors advocate dual therapy to avoid the risk of resistance. Typically, the topical antifungals are given every hour initially. The duration of treatment is from 3–4 weeks on average. If efficacy is not noted within a week or there is worsening, consideration of fibrinoid aqueous should preferably be referred to a cornea specialist within a day. Paracentral ulcers may be managed in the community if smaller and without evidence of anterior chamber penetration. However, if no response is noted within a week, the physician should consider referring the patient to a specialist.

Fungal organisms can penetrate through the corneal stroma without perforation of the cornea resulting in an infectious hypopyon or endothelial plaque. The problem is that the majority of antifungal medications have very poor penetration especially in the face of an intact epithelium. Unlike bacterial keratitis, the corneal epithelium overlying a stromal fungal infection can heal despite the presence of active infection once treatment is initiated and should not, by itself, be used as a guide to successful therapy. Fungal keratitis should be suspected in cases of keratitis that do not respond to antibacterial agents especially in cases of vegetative trauma or extended wear contact lens usage. These cases should be scraped and sent for KOH or Gomorimethenamine silver stains as well as culture on Sabouraud's Dextrose agar. Suppurative keratitis can cause corneal opacity and perforation, which leads to severe visual loss and is the second most common cause for blindness in developing countries (Upadhyay et al., 1991; Ashaye and Aimola, 2008). The etiological cause for suppurative keratitis may vary at different geographical locations (Leck et al., 2002). Different types of fungi that are

one of the important etiological agents also affect cornea orbit and other ocular structures. Fungal infection is a life threatening condition which needs early diagnosis and treatment to save the patients' eye. In some cases when medical treatment fails early surgical debridement is resorted (Thomas, 2003). The study has been conducted to detect various types of eye infections and the different trends of bacterial as well as its fungal etiology.

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-carcinogenic 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 antimicrobial activity was shown by *C. martinii* followed by *C. citratus*, *Eucalyptus globulus* and *C. zeylanicum*. Aggarwal et al., (2000) reported antimicrobial 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 antimicrobial activity of cinnamon bark due to presence of cinnamaldehyde is well known (Viollon and Chaumont, 1994). Similarly, *in vitro* antimicrobial activity of *C. zeylanicum* (bark) against human pathogenic fungi and commensal bacteria was studied by Chaumont, (2003) and Matan et al., (2006). The oils of *M. spicata*, *Azadirachta indica*, *Eugenia caryophyllata*, *Withania somnifera* and *Zingiber officinale* exhibited moderate activity. The essential oil of mint was found to have strong antimicrobial 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, coumarins 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, Rose-

mary oil, Geranium oil, Peppermint oil, and Chaste tree leaf oil on the growth of fungus commonly associated with ocular infection cases.

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.

Collection of Specimens

The sample collection was made by the ophthalmologist base on the procedures for diagnosis of ocular infections by K. Lilytherese and H.N. Madhavan, L&T Microbiology Research centre, Vision research foundation, Chennai.

Eyelid margin specimen

For this no topical anesthetic is needed. Sterile cotton tipped swab or calcium alginate swab moistened in Brain heart infusion broth (BHIB) was rubbed over the lid margin. Swab was inoculated directly on Blood agar (BA) Preferably a single petri dish/Chocolate agar (CA) medium was used for each specimen, Sabouraud's Dextrose agar (SDA) from each eyelid. If pus is present, swabs were used for its collection and BA, CA, MA, SDA and BHIB were directly inoculated and at least three smears were made using fresh sterile swab. All the medium were incorporated with antibacterial agent to suppress bacterial growth

Collection of Conjunctival material (Conjunctival Swab)

Sterile moistened cotton swab or calcium alginate swab was used. Bacterial culture medium such as BHIB or normal saline was used for moistening the swab. Patient was requested to look up, the lower eye lid was pulled down using thumb with an absorbing tissue paper and moistened swab was rubbed over the lower conjunctival sac from medial to lateral side and back again. The procedure was often slightly painful. Sterile plastic (soft) microbiological loop was used for collection of material. Collection of tears alone was avoided. Swab was directly inoculated onto blood agar (aerobic incubation) chocolate agar (5-10% CO₂) and SDA. On solid media main inoculum only was made and further streaking was done in the laboratory.

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; Erdemoglu et al., 2003). Each fungal isolate was suspended in Sabouraud's Dextrose (Himedia, India) broth and diluted to approximately 10⁵ 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 10⁶ CFU/ml). This suspension was used as the inoculum for the test in the agar plates.

Fungal suspensions (3µl) were inoculate using a automatic micropipette (Transasia), and plates (diameter: 25 cm) were incubated at 37°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

Isolates

Based on morphological and cultural characters, isolates were identified as *Candida albicans*, *Candida tropicalis*, *Candida kefyri*, *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 like fungi and molds. *C. citratus* was highly active against *Mucor spp*, *Candida kefyri* and least against *A.flavus spp*. *C.martini* was highly active against *C.albicans*, *C.tropicalis*, *Candida kefyri* and least against *A.flavus*. *C.zeylanicum* was highly active against *Candida kefyri*, *Aniger*, *C. albicans*, and least against *Mucor spp*. *R. officinalis* was highly active against *Mucor spp*, *C.albicans* and least against *A.flavus*. *M.piperita* was highly active against *Rhizopus spp*, *C.albicans* and least against *A.fumigatus*. *P. graveolens* was highly active against *Rhizopus spp*, *Mucor spp*, and least against *Aniger*. *V.negundo* was found to be inactive against all test organisms. All fungi were found to be sensitive to all test essential oils except to *Vitex negundo* 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.albicans*, *C.tropicalis* and *Candida kefyri* and it was 500 µg/ml, for *Aniger*, *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 drug Amphotericin -B. The minimal inhibitory concentration of *C. martini* was similar to *C. citratus*. The minimal inhibitory concentration of *C.zeylanicum* was 15.6 µg/ml against *C.albicans*, *C.tropicalis*, and *Candida kefyri* and 31.25 µg/ml against *Aniger*, *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 *Candida kefyri* and 500 µg/ml for *Aniger*, *A.flavus*, *A.fumigatus*, *Mucor spp* and *Rhizopus spp*. The results showed that they were active against *C.albicans* and *C.tropicalis* effective; moderate to *Candida kefyri* 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 also 1000 µg/ml for all the test fungi. The minimal inhibitory concentration of *Vitex negundo* was not studied since it has not produced positive results in agar well diffusion test. 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.

DISCUSSION

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 ineffective in a 2.5% cream as four other commercial creams against string worm 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 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 0.000032 g/mL (Valne 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). Onawumi, (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 citriodora* (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 geraniol (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 alkoxyperoxyl 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 (Martinez 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).

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 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 found to be 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 ocular infection patients after further deep analysis on their toxicity.

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Table 1. Antifungal activity of essential oils against clinical isolates from Ocular infection cases

Organisms	Essential oils/ Zone of Inhibition in mm						
	C.albicans	C.tropicalis	A.niger	A.flavus	A.fumigatus	Mucor spp	Rhizopus spp
Cymbopogon citratus	9.07±0.63	11.08±0.39	11.07±0.36	8.13±0.66	12.09±0.61	14.25±0.97	10.01±0.61
Cymbopogon martinii	13.25±0.89	13.16±0.53	11.95±0.40	11.03±0.47	12.97±0.55	12.07±0.61	11.95±0.67
Cinnamomum zeylanicum	18.23±0.79	20.83±0.74	18.15±0.86	17.02±0.32	14.23±0.62	11.87±0.70	17.43±1.46
Rosmarinus officinalis	13.12±0.67	9.72±0.70	11.12±0.66	7.97±0.72	10.71±0.76	14.08±0.71	10.07±0.60
Mentha piperita	9.40±0.37	7.14±0.48	9.18±0.77	8.68±0.71	8.11±0.27	7.12±0.47	8.06±0.66
Pelargonium graveolens	9.13±0.56	8.17±0.31	9.03±0.58	10.0±0.41	10.12±0.47	13.51±0.86	11.04±0.56
Vitex negundo	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amphotericin B ©	14.15±0.67	12.12±0.62	13.51±0.51	12.09±0.60	14.18±0.65	12.11±0.46	10.98±0.44

Fig 1. Antifungal activity of essential oils against clinical isolates from Ocular infection cases

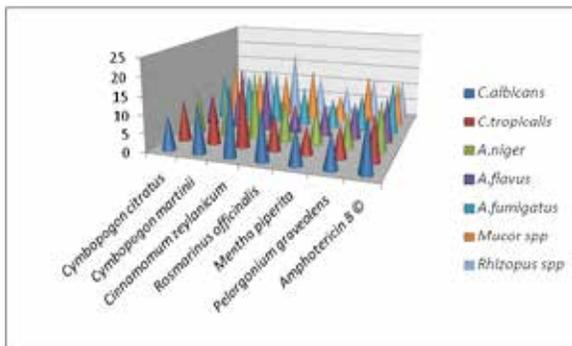


Table 2. MIC of essential oils against clinical fungal isolates from Ocular infection cases

Essential oils -MIC (µg/mL)								
Organisms	Cymbopogon citratus	Cymbopogon martini	Cinnamomum zeylanicum	Rosmarinus officinalis	Mentha piperita	Pelargonium graveolens	Vitex negundo	Amphotericin (100 µg)
Candida albicans	250	250	31.25	125	1000	1000	ND	15.6
Candida tropicalis	250	250	31.25	125	1000	1000	ND	15.6
Aspergillus niger	500	500	31.25	500	1000	1000	ND	31.25
Aspergillus flavus	500	500	62.50	500	1000	1000	ND	31.25
Aspergillus fumigatus	500	500	62.50	500	1000	1000	ND	31.25
Mucor spp	500	500	62.50	500	1000	1000	ND	62.50
Rhizopus spp	500	500	62.50	500	1000	1000	ND	62.50

ND – NOT DONE

Fig 2. MIC of essential oils against clinical fungal isolates from Ocular infection cases

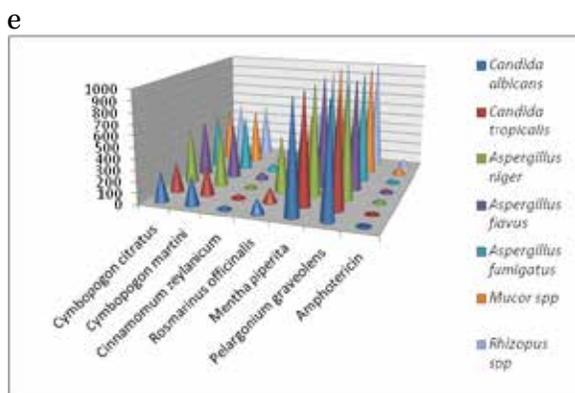
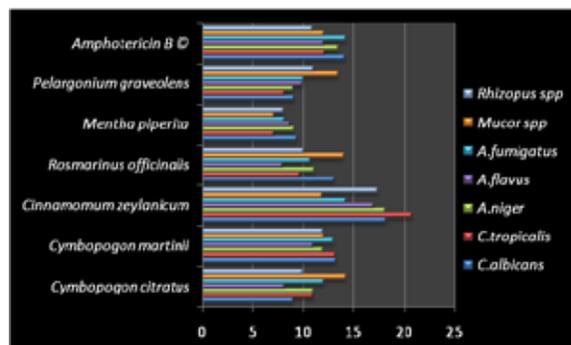


Table 3. MFC of essential oils against clinical fungal isolates from Ocular infection cases

Essential oils-MFC (µg/mL)								
Organisms	Cymbopogon citratus	Cymbopogon martini	Cinnamomum zeylanicum	Rosmarinus officinalis	Mentha piperita	Pelargonium graveolens	Vitex negundo	Amphotericin (100 µg)
Candida albicans	250	250	31.25	125	1000	1000	ND	15.6
Candida tropicalis	250	250	31.25	125	1000	1000	ND	15.6
Aspergillus niger	500	500	31.25	500	1000	1000	ND	31.25
Aspergillus flavus	500	500	62.50	500	1000	1000	ND	31.25
Aspergillus fumigatus	500	500	62.50	500	1000	1000	ND	31.25
Mucor spp	500	500	62.50	500	1000	1000	ND	62.50
Rhizopus spp	500	500	62.50	500	1000	1000	ND	62.50

ND – NOT DONE

Fig 3. MFC of essential oils against clinical fungal isolates from Ocular infection cases



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