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Original Research Article

Antifungal activity of various plant oils against yeast isolates from ICU patients

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ABSTRACT

Background: To evaluate the in-vitro antifungal activity of various essential plant oils against yeast species.
Methods: The present study was a prospective study conducted in Department of Microbiology along with Department of Pharmacology at Tertiary care teaching hospital. Various clinical samples from ICU patients were inoculated on Sabourauds Dextrose Agar (SDA) in accordance with the standard methods. Yeast isolates were recovered and identified as per standard recommended procedure. The antifungal activity of plant oils against these isolated yeasts was determined using disc diffusion method. The results were interpreted as <9mm-inactive, 9-12mm-partially active, 13-18mm-very active. Fluconazole and Itraconazole were taken as control drugs.
Results: The various yeast isolated were *C. albicans, C. tropicals, C. krusei, C. glabrata, C. gulliermondi, C. keyfr, Cryptococcus* and *Tricosporon.* Among essential plant oils, Cinnamon oil and clove oil showed high activity against all isolated yeast species. Olive oil showed least antifungal activity. Fluconazole was resistant in all yeast isolates, while Itraconazole was sensitive to all yeast isolates. Sensitivity of cinnamon and clove oil was statistically significant than Itraconazole.
Conclusions: To conclude present study shows the potential of essential plant oils as newer therapeutic alternatives to antifungal drugs. These may be used in combination with antifungal agents to overcome drug resistance, adverse effects and in shortening the long-term treatment with antifungal drugs. Thus, these essential plant oils can be used in future as antifungal agents in azoles resistant strains.

Keywords: Antifungal activity, Essential plant oils, Resistance, Yeast isolates

INTRODUCTION

Life threatening infections have increased worldwide in immunocompromised patients in developing countries and are becoming an important cause of morbidity and mortality.[1] Pathogenic microorganism like yeast from genus Candida have been recognized as a major agent of hospital acquired infections in humans though emergence of infections due to Non albicans Candida have been described in recent times.[2,3] Other yeast species like Cryptococcus and Tricosporon can also cause serious diseases in immunocompromised individuals.[4] Management of yeast infections can vary and are based on the anatomic location of the infection, the patient's underlying disease, immune status, patients risk factor for infection, the specific species responsible for infection and the susceptibility of the species to specific antifungal drugs.[5] Among antifungal agents Azole drugs and their derivatives continue to dominate as agents of choice for the treatment of these infections, either as topical application or oral dosage forms. These drugs though very widely acclaimed for their efficacy, can also cause

various side effects.[6,7] Drug like Fluconazole is fungistatic in nature and there is emergence of Fluconazole resistance among clinical isolates of Candida albicans.[8]

Thus, there is need for therapeutic alternatives against yeast infections which must be effective with lesser side effects. Essential plant oils are the aromatic oily liquids which are obtained from various plant parts and are known to have wide spectrum of antimicrobial activity.[9-11] Various essential plant oils have been tested for in vitro growth of *C. albicans.*[12] However, none of the study demonstrates their activity on other yeast species. Hence present study was done to know the activity of essential plant oils against various yeast species isolated from clinical samples. ICU patients were selected because they are more prone to develop fungal infections.

METHODS

The present study was an in vitro prospective study conducted in Department of Microbiology along with Department of Pharmacology.

The study was conducted after taking approval from Institutional ethical committee of Muzaffarnager Medical College and Hospital. Various clinical samples which include blood, urine, pus, BAL (bronchoalveolar lavage), throat swab, vaginal swab etc. from ICU patients were inoculated on Sabourauds dextrose agar (SDA) in accordance with the standard methods. Eight yeast isolates were recovered and identified on the basis of germ tube production, morphology on corn meal agar, HiChrome Candida agar (Hi media), urease test, carbohydrate fermentation tests and assimilation tests and other tests as per standard recommended procedure.[13] Yeast species isolated from various clinical samples were *C. albicans, C. tropicalis, C. krusei, C. glabrata, C. guilliermondi, C. kefyr, Tricosporon* and *Cryptococcus.* Antifungal susceptibility testing of the isolated yeast was done in accordance with the proposed guidelines for disc diffusion susceptibility methods by CLSI document M44-A. Fluconazole and Itraconazole were taken as control drugs. Essential plant oils like clove oil, cinnamon oil, eucalyptus oil, tea tree oil and olive oil were obtained from market. The antifungal activity of these oils against yeast was determined using disc diffusion method.[14] Essential plant oils were applied on filter paper (5µl disc, 6mm in diameter). Antimicrobial activity of essential oils was analyzed by observing the zone of inhibition. The results were interpreted as <9mm-inactive, 9-12mm-partially active, 13-18mm very active.[15,16]

RESULTS

Eight yeast species were isolated from various clinical samples obtained from ICU patients. The various yeast isolates were *C. albicans, C. tropicals, C. krusei, C. glabrata, C. gulliermondi, C. keyfr, Cryptococcus and Tricosporon.* Among Essential plant oils, Cinnamon oil and clove oil showed high activity against all isolated yeast species. Tea tree oil showed high activity against *C. glabrata, Cryptococcus* and *Tricosporon*, active against *C. albicans, C. tropicalis* and *C. gulliermondi*, partial activity against *C. krusei* and showed no activity against *C. kefyr*. Eucalyptus oil showed activity againt *C. tropicalis, C. glabrata, Cryptococcus* and *Tricosporon*, partial activity against *C. albicans* and no activity against *C. krusei, C. guilliermondi* and *C. kefyr*. Olive oil showed no activity against *C. glabrata, C. albicans, C. tropicalis, C. guilliermondia, C. kefyr, Trichosporon* and *Cryptococcus.* Fluconazole was resistant in all yeast isolates, while Itraconazole was sensitive to all yeast isolates. When sensitivity of various oils was compared with Itraconazole using unpaired t-test p-value of cinnamon and clove oil came out to be significant.

Table 1: Zone of inhibition (mm) of essential plant oils against various yeast isolates.

Yeast isolate	Cinn. Oil	Clove oil	T. Tree oil	Eucalypt. Oil	Olive oil	Fluconazole	Itraconazole
C.albicans	32	20	14	12	14	6	17
C.tropicalis	20	20	14	20	6	6	17
C.glabrata	36	36	36	36	28	7	13
C.guillimondi	40	28	14	8	6	6	18
C.krusei	32	25	10	8	6	6	28
C.keyfr	18	18	8	7	6	6	20
Tricosporon	36	36	36	20	10	6	26
Cryptococcus	36	36	36	36	6	7	22

DISCUSSION

Over the last decade there has been a rise in number of fungal infections at an alarming rate.[17,18] Among fungal infections, Candidemia has emerged as an alarming problem in healthcare setting causing increased morbidity and mortality.[19] Selected plant oils have been suggested to have potent antimicrobial activity against skin infections, insect bites, chicken pox, cold, flu, measles, sinus congestion, asthma, bronchitis, pneumonia,

tuberculosis and cholera due to their phenolic, alcoholic and terpenoid constituents.[5,6] In past two decades these essential oils have been tested for in-vivo and *in-vitro* antifungal activity and some were found to be potent antifungal agents. These essential plant oils attack on fungal cell membrane disrupts its structure causing leakage and cell death, stop the membrane synthesis and inhibit spore germination, fungal proliferation and cellular respiration.[20]

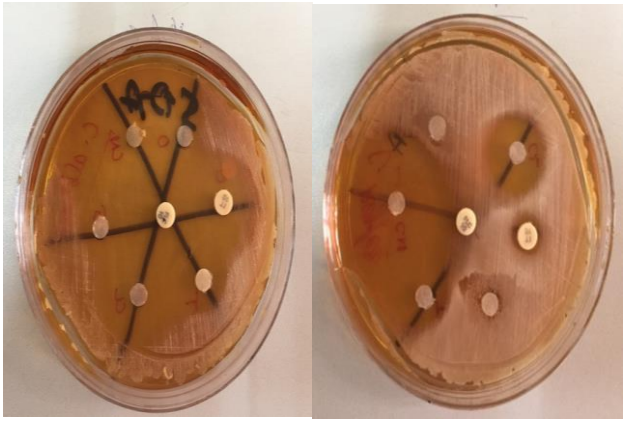

Figure 1: SDA plate of susceptibility to various essential plant oils and control drugs.

The present study was done to assess the antifungal properties of plant oils against various yeast isolates. Fluconazole and Itraconazole were chosen as controls in the study.

The Cinnamon oil and clove oil showed high activity against all isolates of yeast species. Results are in accordance with the other significant studies conducted which also showed cinnamon oil as most effective against *C. albicans.*[12] Our study also show cinnamon oil as most effective in other isolated yeast species also similar to the study published in Brazilian archives of Biology and technology which also noted that cinnamon oil inhibits all the strains of *C. albicans, C. tropicalis* and *C. krusei.*[21]

Clove oil showed high activity against all isolates of yeast species which is similar to study conducted by Amit kumar et al and Hammer et al which showed significant antifungal activity of clove oil against candida species isolated from blood stream infections.[22,23] Carvacrol found in clove oil is able to kill C.albicans by producing lesions in plasma membrane causing organism death.[24] Tea tree oil produced activity against all the species of yeast except for *C. keyfr* against which it was inactive. This is in accordance with study by Carson et al which showed activity of Tea Tree oil against all the yeast isolates.[25] In our study, we have found Eucalyptus oil showed activity against *C. tropicals, Tricosporon, C. glabrata, Cryptococcus* and parital activity against *C. albicans* and no activity against *C. keyfr, C. krusei and C. guillermondi*. However, Vishnu et al have mentioned eucalyptus oil as potent antifungal agent against *C. albicans* in their study.[26] Olive oil showed no antifungal activity against any of the isolated yeast species, but results of other study have demonstrated their marked antimicrobial activity against fluconazole Resistant *C. albicans* strain.[27] The studies conducted so far are mostly show susceptibility of plant oils against *C. albicans* species only and not against other non albicans group of yeast species which are emerging as major cause of infection. All yeast isolates were found to be resistant against Fuconazole. This could be due to its irrational or long- term use or infection acquisition by previously resistant strain. Moreover, samples were obtained from ICU patient, who are already immunocomprised. Our results are in accordance with studies conducted which states that resistance of yeast isolates against antifungal drugs was most commonly seen against Fluconazole.[28] Amit kumar et al have also reported Fluconazole resistance in five strains including *C. albicans, C. tropicalis and C. guillermondii*. Studies conducted by other workers have reported susceptibility profile of Candida isolated to Fluconazole and Itraconazole.[29] However in our study all the isolated yeast species were found to be resistant to Fluconazole and sensitive to Itraconazole similar to that of khan P et al.[28] The effect of plant oils could be due to the damage to the cell membrane of the fungus, causing leakage and fungal cell death due to stoppage of cell membrane synthesis, inhibition of spore germination, fungal proliferation and cellular respiration.[20]

CONCLUSION

To conclude present study shows the potential of essential plant oils as newer therapeutic alternatives to antifungal drugs and which may be used in combination with antifungal agents to overcome drug resistance and adverse effects and may help in shortening the long-term treatment with antifungal drugs and thus may reduce the morbidity and mortality due to fungal infections.

So, these can be used clinically as safe and more effective agents. Besides having antifungal properties these essential plant oils have other health benefits as antioxidant and anti-inflammatory agents. Thus, these essential plant oils can be used in future as antifungal agents in azoles resistant strains. However, further clinical studies are required.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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