Mycosporicidal activity of essential oils from selected herbals against isolates from HIV/AIDS patients

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Abstract

Spores are said to be more resistant to adverse conditions of both physical and chemical nature. Fungal spores are ubiquitous and are highly problematic that are involved in various opportunistic diseases, in safety food preparation, and microbial culture work etc. Essential oils from Cymbopogon citratus, Cymbopogon martinii, Cinnamomum zeylanicum, Rosmarinus officinalis, Mentha piperita, Pelarogonium graveolens, Vitex negundo were used to study their sporicidal activity against spores of fungal isolates such as Aspergillus niger, A.flavus, A.fumigatus, Mucor Spp, and Rhizopus Sp from HIV positive individuals. Different dilutions of those preparations were made and tested for their sporicidal activity against fungal spores from opportunistic pathogens. C. zeylanicum was highly effective in lower concentration (1:250) against all fungus. C.citratus, C. martini, R. officinalis, were effective at 1:500 concentration P.graveolens and V.negundo was at 1:1000. M. piperita was ineffective against all the test fungal spores even in higher concentration. The mycosporicidal activity is attributed to the components of essential oils, which mostly interferes with enzyme system of spores, which are needed for their viability. According to the results of this study, the essential oil may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of sporogenous fungal diseases in various patients, particularly, for HIV/AIDS patients.

Keywords: Sporicidal activity, opportunistic fungi, HIV/AIDS, essential oils, aromatherapy

INTRODUCTION

Before the widespread use of potent combination antiretroviral therapy (ART), opportunistic infections (OIs), which have been defined as infections that are more frequent or more severe because of immunosuppression in HIV-infected persons, were the principal cause of morbidity and mortality in this population. In the early 1990s, the use of chemoprophylaxis, immunization, and better strategies for managing acute OIs contributed to improved quality of life and improved survival (1). Although hospitalizations and deaths have decreased since the implementation of ART, OIs remain a leading cause of morbidity and mortality in HIV-infected persons.(2)(3)(4)(5)(6)(7)(8)(9)(10).

Sporadic activity, opportunistic fungi, HIV/AIDS, essential oils, aromatherapy

were found to be more resistant to toxicity of monoterpene, geraniol and its products than the vegetative cells(13). Spores are also highly resistant to mechanical forces. These characteristics apply to the products within the spores, as they do show tolerance to various chemical compounds and crucial conditions. The longevity that spore products have is the direct consequence of their higher resistance towards external factors (14). Fungal spores are known to have high catalytic activity and carry out bioconversion reactions. Spores of Aspergillus niger had glucose oxidase activity even after its storage for 3 months at -20 °C without any loss of enzyme activity (15).

Opportunistic infection by molds such as Penicillium marneffei and A. fumigatus is a common cause of illness and death among immunocompromised people, including people with AIDS. Health problems associated with high levels of airborne mold spores include allergic reactions, asthma episodes, irritations of the eye, nose and throat, infections, sinus congestion, and other respiratory problems. When inhaled by an immunocompromised individual, some mold spores may begin to grow on living tissue, attaching to cells along the respiratory tract and causing further problems. Generally, when this occurs, the illness is an epiphenomenon and not the primary pathology. A serious health threat from mold exposure for immunocompromised individuals is systemic fungal infection. Immunocompromised individuals exposed to high levels of mold, or individuals with chronic exposure may become infected. Sinuses and digestive tract infections are most common; lung and skin infections are also possible. Mycotoxins may or may not be produced by the...
invading mold. The most common form of hypersensitivity is caused by the direct exposure to inhaled mold spores that can be dead or alive or hyphal fragments which can lead to allergic asthma or allergic rhinitis. The most common effects are rhinorrhea (runny nose), watery eyes, coughing and asthma attacks. Another form of hypersensitivity is hypersensitivity pneumonitis. This is usually the direct result of inhaled spores or fragments in an occupational setting. It is predicted that about 5% of people have some airway symptoms due to allergic reactions to molds in their lifetimes (16). Certain molds excrete toxic compounds called mycotoxins, usually only under specific environmental conditions. Certain mycotoxins can be harmful or lethal to humans and animals when exposure is high enough (17) (18).

In this study, essential oils from Cymbopogon citratus, Cymbopogon martinii, Cinnamomum zeylanicum, Rosmarinus officinalis, Pelargonium graveolens, Mentha piperita, and Vitex negundo were used in different dilutions to test their sporicidal activity against spores of fungal isolates such as Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor spp, and Rhizopus spp from HIV/AIDS patients.

MATERIALS AND METHODS:

Essential oils

Seven essential oils such as Lemongrass oil (C. citratus-Graminae), Palmarosa oil (C. martindii-Graminae), Cinnamon bark oil (C. zeylanicum-Lauraceae), Rosemary oil (R. officinalis-Labiatae), Geranium oil (P. graveolens-Geraniaceae), Peppermint oil (M. piperita-Labiatae), and Chaste tree leaf oil (V. negundo-Lamiaceae) were obtained from Aromax Trading Co, Chennai, Tamilnadu, 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.

Test fungi

Microorganisms (Clinical fungal isolates from HIV infected) such as A. niger, A. flavus, A. fumigatus, Mucor spp, Rhizopus spp were used in different dilutions to test their sporicidal activity against spores of fungal isolates such as Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor spp, and Rhizopus spp from HIV/AIDS patients.

Table 1 - Qualitative chemical analysis of essential oils

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Alcohols</th>
<th>Terpenoids</th>
<th>Phenolics</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Aldehydes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbopogon citratus</td>
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<tr>
<td>Cymbopogon martinii</td>
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<tr>
<td>Cinnamomum zeylanicum</td>
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<td>+</td>
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<td>Rosmarinus officinalis</td>
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</tr>
<tr>
<td>Mentha piperita</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Pelargonium graveolens</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Vitex negundo</td>
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<td>+</td>
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</tr>
</tbody>
</table>

(+) Positive, (-) Negative

Qualitative chemical analysis of essential oils

The essential oils were subjected to qualitative chemical analysis for secondary metabolites, tannins, saponins, steroid, alkaloids, phenolics, terpenes and glycosides in accordance with (19)(20) with little modification and using simple qualitative methods of (21)(22).

Spore Germination Inhibition Assay

The fungi on sporulation, after 5-7 days at 27°C, pure culture of each fungus in agar plate was flooded with sterile distilled water, and the plate was gently swirled to dislodge the spores. The concentration of spores per ml was estimated for each fungus with haemocytometer slide (depth 0.1mm, 1/400mm²) under microscope (Labomed, India). Five hundred μl aliquots of magnetically stirred spores suspension were dispensed into sterile Petri dishes (diameter 60mm). Five milliliters portions of the solutions of essential oil in aqueous 0.01% Tween 20 were added to Petri dishes to provide 5.5 ml solutions containing 1000, 500, 250, 125, and 62.5 μg/ml of the essential oil.

Plates containing 500 μl of spore suspension in 5 ml of 0.01% Tween 20 was used as control (without essential oil). The plates were incubated at 27°C for 72 h and the numbers of germinated spores per treatment were counted in treated and control sets after the incubation period (23). The percentage inhibition of germination was computed using the expression:

\[ \text{% Inhibition of germination} = \frac{\text{IGc} - \text{IGt}}{\text{IGc}} \times 100 \]

where IGt is the number of germinated spores in treated sets and IGc is the number of germinated spores in the control for three replicates. The effects of the oil on fungus spore germination are shown in table 6.

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

Qualitative chemical analysis of essential oils

The results for qualitative chemical analysis of essential oils is presented in table No.1

Mycosporicidal activity

C. zeylanicum was highly effective in lower concentration (1:250) against all fungus. C. citratus, C. martindii, and R. officinalis,
were effective at 1:500 concentrations, \( P. graveolens \) and \( V. negundo \) were active against spores of the fungal isolates from HIV/AIDS cases. Cinnamon oil was more active compared to other oils among the 7 oils tested. Lemongrass, palmarosa, rosemary were found to be more than the moderate range. The others such as geranium, and chaste tree leaf oil were effective to the least level. As far as the chemical analysis, and antibacterial study, the following studies were comparable to the results with the present study.

**DISCUSSION**

All the selected essential oils except peppermint were found to be active against spores of the fungal isolates from HIV/AIDS cases. Cinnamon oil was more active compared to other oils among the 7 oils tested. Lemongrass, palmarosa, rosemary were found to be more than the moderate range. The others such as geranium, and chaste tree leaf oil were effective to the least level. As far as the chemical analysis, and antibacterial study, the following studies were comparable to the results with the present study.

Essential oils may possess antifungal activity and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread. Suppression on spore production by oil treatment could make a major contribution to limiting the spread of the pathogen by lowering the spore load in the storage atmosphere and on surfaces. The mechanism underlying the action of essential oil-enrichment on the switch between vegetative and reproductive phases of fungal development remains to be understood. The impacts of oils on sporulation may reflect effects of the volatiles emitted by oils on surface mycelial development (and thus the ‘platform’ to support spore production) and/or the perception/transduction of signals involved in the switch from vegetative to reproductive development (24).

Essential oils are complex volatile compounds produced in different plant parts, which are known to have various functions in plants including conferring pest and disease resistance (25). The complexity in essential oils is due to terpene hydrocarbons as well as their oxygenated derivatives, such as alcohols, aldehydes, ketones, acids and esters (26).
Interestingly, lemongrass oil showed higher activity than pure isolate (citral) as reported by (27). Lemongrass oil was only effective at 1000 ppm, whereas no inhibition observed for Rhizopus fungi (28). Indeed, antimicrobial activity and preservative of lemongrass oil are believed to be associated with phytochemical components of the lemongrass powder, like alkaloids, tannins and cardiac glycosides (29). Spore germination to be significantly (P0.05) reduced by lemongrass oil in C. coccodes, B. cinerea, C. herbarum and R. stolonifer with the impacts of oil dependent on different oil concentrations. The greater inhibition on spore germination was observed in C. herbarum (81%) and the least in R. stolonifer (2%). However, lemongrass oil (up to 100 ppm) accelerated spore germination for A. niger. Indeed, the greatest oil concentration (500 ppm) inhibited spore germination due to failure of spore production. Lemongrass oil-enriched PDA reduced germ tube length for C. coccodes, whereas no major differences were observed for B. cinerea, C. herbarum and R. stolonifer. However, oil treatments revealed no differences on fungal spore viability among the treatments when five essential oils (lemon-grass, cinnamon, rosemary, lavender and basil) were tested for antifungal activity against green mould (P. digitatum) implying that effects were fungistatic being in accordance with the previous studies (30)(31). Moreover, it was reported that lemongrass oil has not demonstrated fungicidal activity against P. digitatum and P. italicum when applied as a fumigant (32) contrasting the present results when examined in different pathogens probably due to variation of the treatment and/or pathogen per se.

This observation was similar with the earlier results when spores were permeabilized by freezing (33). Thus it showed that citral could help in permeabilization without hindering the enzyme activity. There is not much literature on the toxic effect of citral on the enzyme activity of spores. However, there are reports where citral is tested as antifungal and antibacterial agent (34) and (35). An interesting feature noted by (36) was that spores (10⁶) treated with citral (0.15 mg/ml) had 90% germination rate while untreated spores had only 70% germination rate. However, combination of pressure (350 MPA) and citral (0.75 mg/ml) treatment effectively inhibited and inactivated the spores of Colletotrichum gloeosporioides. Reports of (37) supported this fact, stating that the germination of Penicillium digitatum conidia was stimulated by compounds such as limonene, β-pinene, sabine, β-myrcene, etc. Thus the antimicrobial activity of terpenes is highly depended on their concentrations used. In this study where high concentrations of spores were used, the toxicity noticed was negligible even with high concentration of citral.

In HIV-infected children who are severely immunosuppressed or neutropenic, considerations for preventing exposure to Aspergillus might include excluding plants and flowers from rooms, avoiding food items such as nuts and spices that often are contaminated and minimizing application of non-sterile biomedical devices and adhesive tape (38)(39)(40)(41). Other hospital environmental measures that can help prevent aspergillosis outbreaks include placing suitable barriers between patient-care areas and construction sites; routinely cleaning showerheads, hot water faucets, and air-handling systems; repairing faulty air flow; confining patients to hospital rooms supplied with sterile laminar airflow; and installing high efficiency particulate air filters (42)(43)(44). Aspergillus spp. are ubiquitous molds that are widespread in soil and grow on plants and decomposing organic materials (45); they are infrequent pathogens in HIV-infected children. The most common species causing aspergillosis is A. fumigatus, followed by A. flavus (46)(47). Aspergillosis is rare but often lethal in pediatric AIDS patients; the estimated incidence of invasive aspergillosis in pediatric AIDS patients was 1.5%—3% before widespread use of HAART (48)(49)(50), and invasive aspergillosis is believed to be much less prevalent during the post-HAART era. (51)(52)(53)(54)(55).

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 mycosporicidal activity is attributed to the components of essential oils, which mostly interferes with enzyme system of spores, which are needed for their viability. The results of this study revealed that, the essential oil can be prepared in different forms of those essential oils may be suggested as a new potential source of natural antimicrobial for the prevention, treatment and control of opportunistic fungal diseases caused by aerosols with spores in various patients, particularly, for HIV/AIDS patients.

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