

Influence of Essential Oils on Health Risk Causing Fungi

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Abstract

In the present study two undiluted essential oils derived from *Eucalyptus citriodora* and *Cymbopogon citratus* fresh leaves were tested *in vitro* against five dermatophytes viz. *Trichophyton tonsurans*, *T. rubrum*, *T. mentagrophytes*, *Microsporum fulvum* and *M. gypseum*. Among five test pathogens, *T. mentagrophytes* was recorded as the most susceptible revealing highest zone of inhibition (ZI) 15.08 mm and *M. fulvum* was observed as the most resistant with recorded lowest ZI (8.83 mm) towards *C. citratus* oil. Both the oils showed higher antifungal activity as compared to a standard antibiotic, miconazole at 300 µg/ml concentration.

Key words : Essential oils, Dermatophytes, Antifungal property, Zone of inhibition.

Superficial fungal infection on different body parts or intertriginous areas of man and animals by a group of health risk causing pathogenic fungi is known as dermatomycosis and the causal organisms are known as dermatophytes. The target areas of dermatophytes are skin, hair and nails consisting of fibrous protein (keratin). This disease is not fatal but highly contagious and one can easily get infected in contact with an infected person. A 29-year-old man presented with an itching, scaly lesion on the prepuce of ten days duration denied having sexual exposure. It was noted that his wife had a ringworm infection of the groin and anterior abdomen. The culture was grown and found as *T. rubrum* (1). An outbreak in a child care center in Denmark was reported, 98 individuals were examined clinically and mycologically, *Microsporum audouinii* was cultured from 12 people (2). Infections may come even from contaminated dust and soil samples. A report said 330 dust and soil samples collected from different sites at 13 elementary schools and seven public parks in which seven species of keratinophilic fungi were identified by hair-baiting techniques (3). A 3-months-old girl reported tinea capities and the fungal strain was identified as *T. rubrum* (4). Although, there are a number of antidermatophytic ointments/medicines available but they may have major disadvantages of having toxicity and side effects. A study established that a 43-year-old woman with interdigital tinea pedis and onychomycosis caused by *Trichophyton rubrum* was treated with milk containing 1% ciclopiroxolamine

but the patient had a history of hepatitis. After 14 days application of this cream, erythematous-squamous lesions appear in the interdigital spaces spreading to the toes and upper surface of the feet. Patch tests revealed intense positivity for ciclopiroxolamine 1% and Micoxolamina milk (5). In this context, extensive work on herbal alternatives is being carried out throughout the world for replacing chemotherapeutic agents. Scientists are emphasizing on the exploitation of botanicals for inhibiting the pathogenic micro-organisms responsible for many human and animal ailments.

An *in vitro* study was conducted by 'disc diffusion agar plate method' technique which authenticated that *C. martini*, *C. nardus*, *Mantha arvensis* leaves and *E. citriodora* bark essential oils were effective against *Microsporum gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. simii* and *T. tonsurans* responsible for tinea capitis as compared to a standard reference, griseofulvin 500 ppm. *M. arvensis* oil showed higher efficacy against *T. tonsurans* and revealed zone of inhibition as 25 mm (6). Antifungal activity by mycelial growth method was studied with *Achillea atrata* L. essential oil against 18 pathogenic fungi including six dermatophyte species and the oil exhibited strong inhibitory action, wherein MIC values were recorded as 2.0—8.0 µl/ml (7). Undiluted *E. citriodora* matured seed oil revealed almost 1.7 times greater zone of inhibition against a pathogenic fungi *Candida albicans* in comparison with two standard antibiotics by 'disc

Table 1. Efficacy of two essential oils against dermatophytes. *Mean of six replications (excluding 5 mm dia disc).

Test dermatophytes	*Zone of inhibition per μ l at 96 h incubation			
	<i>E. citriodora</i> oil (mm)	<i>C. citratus</i> oil (mm)	Absorption per disc (μ l)	Miconazole, 300 μ g/ml cons (mm)
Trichophyton tonsurans	14.33	14.75	2	7.17
Trichophyton rubrum	9.75	11.58	2	4.75
Trichophyton mentagrophytes	9.83	15.08	2	4.92
Microsporum fulvum	10.08	8.83	2	4.17
Microsporum gypseum	10.25	10.83	2	4.50

diffusion' method (8). Antifungal activity of *Thymus capitellatus* oil was studied against five dermatophytic clinical strains and MIC values were recorded as 0.32—1.25 μ l/ml (9). Considering the immense and unexplored biotic wealth of Eastern India, present experiment was conducted to study the effect of undiluted essential oils from *Eucalyptus citriodora* (leaf) and *Cymbopogon citratus* (leaf) against five dermatophytes.

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Methods

Test dermatophytes viz. *Trichophyton tonsurans* (*Tt*), *T. rubrum* (*Tr*), *T. mentagrophytes* (*Tm*), *Microsporum fulvum* (*Mf*) and *M. gypseum* (*Mg*) for the experiment were collected from School of Tropical Medicine, Kolkata.

E. citriodora and *C. citratus* leaves were collected from Tezpur (Assam) area and this place is located at 79 meter height from mean sea level (MSL).

Essential oils were extracted from the fresh plant materials by using Clevanger's apparatus. Commercial uses of *E. citriodora* oil is employed in soap and perfumery industry containing citronellal (65—80%), citronellol (15—20%) and esters (10). On the other hand *C. citratus* oil is chiefly used in India for flavouring soups and curries. An infusion of the grass is sometimes taken as a refreshing beverage and this use gives it the name 'green tea', the principal constituent is citral 54—87% (11). In the present study, antimycotic activity of the oil was assayed by 'disc diffusion method', Twenty-five ml sterilized Sabouraud's Dextrose Agar (SDA) medium was taken in each sterile petriplates (90 mm diameter) aseptically and 0.2 ml of 96 h old broth cultures of the test pathogens were evenly distributed on the surface of the settled medium with the help of glass spreader. A standard antibiotic miconazole at a concentration of 300 μ g/ml was used for comparative study. Five mm diameter sterilized Whatman filter paper No.1 discs were moistened with same quantity (2 μ l) of undiluted oils and antibiotic was placed over the surface of the culture containing petriplates. Distilled water moistened discs were also used for control in the same procedure and incubated at 28 C for 96 hours. Antimycotic activity of the oils was determined by measuring the zone of inhibition (ZI).

Results and Discussion

Table 1 shows that two tested essential oils were antifungal varying in degree of their activity depending on the nature of their effective compounds and capacity of diffusion into agar medium.

E. citriodora oil exhibited the highest zone of inhibition (ZI) per μ l against *Tt* (14.33 mm) followed by *Mg* (10.25 mm), *Mf* (10.08 mm), *Tm* (9.83 mm) and *Tr* (9.75 mm). *C. citratus* oil revealed more effectiveness against *Tm* (15.08 mm) followed by *Tt* (14.75 mm), *Tr* (11.58 mm), *Mg* (10.33 mm) and *Mf* (8.83 mm). Out of five test pathogens only *Mf* showed higher ZI (10.08 mm) under the influence of *E. citriodora* oil than *C. citratus* oil (8.83 mm). Between these two oils, *C. citratus* oil was recorded as more potent than *E. citriodora* oil and as far as test pathogens are concerned, *Tm* was found as more susceptible wherein higher ZI (15.08 mm) was recorded and *Mf* was more resistant which recorded lower ZI (8.83 mm) towards

C. citratus oil. Both oils have shown higher antifungal property and established double or more than double ZI values against standard antibiotic, miconazole at 300 µg/ml concentrations.

Today, there is an increasing interest in the use of botanicals because of the necessity for finding herbal alternatives also preventing pollution and degradation of the environment. Thus the present study may help in developing suitable herbal formulation against pathogenic fungi responsible for health risk.

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