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Efficacy and Environmental Safety of *Cymbopogon citratus* oil against Tinea pedis

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ABSTRACT

Tinea pedis is one of the common and contagious fungal disease in human being that effects skin on the feet. It is commonly called athlete's foot and caused by mainly members of fungal genera *Trichophyton* and *Microsporum*. Lemon grass (*Cymbopogon citrates* DC) possess pharmacological properties that are used for medical purposes worldwide. Lemon grass oil was extracted from the leaves by steam distillation using Clevenger apparatus. During antifungal screening of the essential oil, it exhibited the strong activity, completely inhibiting the mycelia growth of common infecting fungi *viz. Trichophyton rubrum, T. mentagrophytes* at concentration 0.1µl/ml and *Micosporum canis* at 0.2µl/ml. The essential oil was found to be fungicidal at a concentration 0.2µl/ml against *Trichophyton rubrum* and *T. mentarophytes* and 0.3µl/ml against *Microsporum canis*. The fungicidal activity of the oil was found no decrease in activity up to 24 months of storage. The oil also showed a broad fungi toxic spectrum, inhibiting the mycelia growth of other dermatophytic fungi. Moreover, it did not exhibit any adverse effect on mammalian skin up to 5% concentration. As such, the oil has a potential use as an effective herbal chemotherapeutic after undergoing successful clinical trials.

KEY WORDS- Tinea pedis ,*Cymbopogon citratus*, Essential oil, *Trichophyton*, *Microsporum*

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INTRODUCTION

Tinea pedis is one of the common and contagious fungal disease in human being that effects skin on the feet. It is commonly seen in athletes hence called athlete's foot. Athlete's foot fungus may infect any part of the foot, but most often grows between the toes. Fungal infection in man and animal are common in tropical and sub tropical countries due to prevailing moisture, over population, poor hygienic living conditions and temperature regimes.[1-3] The same fungus may also affect the nails or the hand.[4] The infections occur in both healthy and immune compromised persons. Tinea pedis is caused by a number of different fungi. The *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum canis* are commonly involved in such infection.[5,6] These pathogenic bio-agents are able to invades human keratinized tissues, causing lesions in the skin and other body parts.[7,8] Infections are difficult to control effectively, and mostly synthetic antifungals (azoles,allylamines and morpholine derivates) are used to treat these infections which are largely non-renewable petro-products that are non-biodegradable and cause adverse effects and residual toxicity.

Thus, in a meaningful search for new treatments with better and cheaper substitutes, plant resources are the natural choice. Plant derived essential oils are natural compounds that show potential effects against bacteria and fungi[9]. Essential oils as antimicrobial agents have two main advantages firstly, most essential oils are safer for consumers; secondly, there is low risk of the micro-organisms developing resistance to essential oils.[10] Thus it can used to be an effective source of chemotherapeutic agents without undesirable side effects and with strong fungicidal activity.[11-15]

The objective of this work was to evaluate the antifungal efficacy of plants derived essential oils against three widely spread pathogenic fungal strains that cause athlete's foot in humans. There fore, they can be used as a natural therapy to inhibit fungal pathogens causing infections.[5, 16] My present investigation result on the *Cymbopogon citratus* (DC) essential oil a member of Poaceae is very effective against Tinea pedis causing fungi.

EXPERIMENTAL SECTION

Plant *Cymbopogon citratus* (lemon grass) collected during the month of August from the botanical garden of D.A-V. College Kanpur UP India. The essential oil was extracted from the aerial part (leaves) of *Cymbopogon citratus* by hydro distillation using a Clevenger apparatus.[17] A clear light-yellow-colored oily layer was obtained on the top of the aqueous distillate, which was separated from the latter and dried over anhydrous sodium sulphate. The physicochemical properties of oil are given in (table-1).

In *in vitro* studies, the minimum inhibitory concentrations (MICs) of the oil against test pathogens were determined following the poisoned food technique[18] with slight modification.[19] The

requisite quantity of the oil samples were mixed in acetone (2% of the required quantity of the medium) and then added in pre-sterilized sabouraud dextrose agar (SDA) medium, pH 5.6. In control sets, sterilized water (in place of the oil) and acetone were used in the medium in appropriate amounts. Mycelial discs of 5mm diameter, cut out from the periphery of 7 day old cultures, were aseptically inoculated upside-down on the agar surface of the medium. Inoculated Petri plates were incubated at 27 ± 1^0C and the observations were recorded on the seventh day. Percentage of mycelial growth inhibition (MGI) was calculated according to the formula:

$$MGI= (dc - dt) \times 100/dc$$

Where, dc = fungal colony diameter in control sets.

dt = fungal colony diameter in treatment sets.

The minimum fungi static /fungicidal concentrations of the oil at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston.[20] This was done by reinoculated the inhibited fungal discs at MICs on SDA medium. Observations were recorded after 7 days of incubation. Fungal growth on the seventh day indicated a fungi static nature, while the absence of fungal growth denoted fungicidal action of the oil. The effect of inoculums density (increased progressively up to 30 discs in multiples of five, each of 5 mm diameter) of the test pathogens on MICs of the oil was determined following the procedure outlined by Dikshit and Dixit.[21] The effect of physical factors viz. temperature and expiry of toxicity during storage of the oil, was evaluated according to Shahi *et al.*[19] Five lots of oil were kept in small vials, each containing 5 ml oil; these were exposed to different temperatures (30, 50 and 70^0C) in an incubator for 1 h. Antifungal activity was then tested at MICs by the poisoned food technique.[18] Expiry of toxicity of the oil was determined by storing the oil at room temperature and testing antifungal activity at MICs at regular intervals of 60 days up to 24 months, following the poisoned food technique.[18]

The minimum killing time (MKT) of the oil was determined by the mycelial disc killing technique (MDKT) of Shahi *etal.*[19] Two treatment sets were maintained, one with pure oil (PO) and the other with the minimum fungicidal concentrations (MFCs) of the oil. The treatment set using MFCs of the oil was prepared by mixing the required quantity of the oil samples in acetone (5% of the total quantity of the treatment solution) and then adding this to the appropriate quantity of distilled water. Simultaneously, controls were maintained using sterilized water (in place of the oil) and acetone, adding into the distilled water in appropriate quantities.

Mycelial discs of 5 mm diameter, cut out from the periphery of 7 day-old cultures of the test pathogens, were aseptically placed in the culture tubes of different treatment and control sets. These mycelial discs were taken out of the tubes at different time intervals and washed immediately in the washing solution (containing acetone: sterilized distilled water, ratio 1:2) to remove the treatment

solution. These washed mycelial discs were aseptically transferred upside-down to the SDA medium (pH 5.6) in the Petri plates. The same procedure was followed with the control sets. The inoculated Petri plates were incubated at 27±1^{0}C and the observations recorded as an average value of five replicates on the seventh day. The percentage of fungal growth inhibition (FGI) was calculated by the formula of Shahi *et al*.[19]All the experiments were repeated twice, each containing five replicates, and the data presented here are based on their mean values (table-5). To determined the maximum tolerable concentrations (MTCs) and long-term toxicity for irritant activity, if any, of the oil by their topical application on human skin and nails, we followed the patch test method as described by Shahi *et al*.[19]

People of both sexes aged 10-30 years were selected randomly and a group of 30 individuals of each sex was constituted. Circular areas of 5 cm^2 on upper hairy and lower glabrous surface of the palms, nail and 3 cm^2 of neck region of each individual were first washed with distilled water followed by 70% ethyl alcohol and then allowed to dry for 5 min. Five drops of the graded concentrations of testing solution were applied to each individual separately for 3 weeks. The volunteers were not allowed to wash the applied area. Qualitative observations were recorded afterwards at intervals of 24 h up to 3 weeks.

Table-1. Physicochemical properties of *Cymbopogon citratus* oil.

Properties studied	Observations
Plant height (cm)	155–180
Oil yield (%)	0.4
Colour	Light yellow
Specific gravity at 29.5 ◦C	0.9050 -0.9475
Refractive index at 20 ◦C	1.4721-1.4861
Optical rotation	- 0.33 ◦
Acid value	6.99
Ester value	35.15
Citral (%)	68–80
Solubility	90% alcohl

Table-2. Minimum fungistatic concentration(s) and minimum fungicidal concentration (c) of the oil *Cymbopogon citratus* against dominant tinea pedis causing fungi.

Concentration (µl/ml)	Mycelial growth inhibition (MGI) (%)		
	Trichophyton rubrum	***Trichophyton mentagrophytes***	***Microsporum canis***
1.0	100[c]	100[c]	100[c]
0.9	100[c]	100[c]	100[c]
0.8	100[c]	100[c]	100[c]
0.7	100[c]	100[c]	100[c]
0.6	100[c]	100[c]	100[c]
0.5	100[c]	100[c]	100[c]
0.4	100[c]	100[c]	100[c]
0.3	100[c]	100[c]	100[c]
0.2	100[c]	100[c]	100[s]
0.1	100[s]	100[s]	80

[c] Fungicidal, [s] Static

Table -3 Minimum killing time(MKT) of the oil *Cymbopogon citrtus* against test pathogens:

Minimum killing time (MKT)	Mycelial growth inhibition (MGI) (%)					
	Trichophyton rubrum		*Trichophyton mentagrophytes*		*Microsporum canis*	
	PO	*MCCs*	PO	*MCCs*	PO	*MCCs*
120m	100	100	100	100	100	100
80m	100	100	100	100	100	100
70m	100	100	100	100	100	100
60m	100	100	100	100	100	100
50m	100	100	100	100	100	80
40m	100	88	100	63	100	45
30m	100	78	100	50	100	28
60s	100	-	100	-	100	-
30s	100	-	100	-	100	-
20s	100	-	100	-	100	-
10s	100	-	100	-	98	-
5s	93	-	89	-	76	-
1s	58	-	52	-	35	-

PO (pure oil), MCCs (minimum fungicidal concentration)

RESULTS AND DISCUSSION

The essential oil was extracted from the leaves of *Cymbopogon citratus* by hydro distillation using Clevenger apparatus.[17] A clear light-yellow-colored oil on hydro distillation, yielded 0.4 % essential oil. The physicochemical properties of the oil are shown in (Table-1). The minimum inhibitory concentration (MICs) of *Cymbopogon citratus* essential oil as a fungi static was found to be 0.1µl/ml against *Trichophyton rubrum* and *Trichophyton mentagrophytes,and* .2µl/ml against *Micrsporum canis* (Table-2). The minimum fungicidal concentration (MCCs) was found *0.2*µl/ml against *Trichophyton rubrum* and *Trichophyton mentagrophytes and 0.3*µl/ml against *Microsporum canis.* The oil inhibited heavy doses of inocula which exhibited 100% mycelial growth at their respective fungicidal concentrations. The activity of the oil did not expire even up to 24 months storage and persisted up to 70^0C. The pure oil killed the fungi in just 10 seconds (s) while at its minimum fungicidal concentration it required 50 minutes (m) against *Trichophyton rubrum* and *Trichophyton mentagrophytes* and 60 minutes (m) against *Microsporus canis* (Table-3). The oil also exhibited a broad range of antifungal activity, inhibiting some other fungi, e.g. *Aspergillus flavus, A. fumigates, A.niger, Candida albicans, Malassezia, Epidermophyton floccosum, M. gypseum, M.*

nanum, T. violaceum in range of 0.3- 1.2 µl/ml concentration. When tested for irritant activity and long-term toxicity on human skin and nails, the oil did not show any irritation or adverse effect at 5% concentration up to 3 weeks.

CONCLUSION

The essential oil of *Cymbopogon citratus* exhibiting strong toxicity against the test fungi causing athlete's foot known as tenia pedis. The oil also appears to possess wide range of antifungal activity. The findings suggest that *C. citratus* may be used for the cure of tenia pedis diseases in animals and human beings after suitable clinical trials.

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