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ANTI-DERMATOPHYTIC POTENTIAL OF *CINNAMOMUM TAMALA* LEAF ESSENTIAL OIL

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ABSTRACT

The antifungal drugs in common usage leads to failure in the treatment of mycosis due to the occurence of drug resistance. Hence, alternative natural anti-mycotic drugs are required which should be effective in the control of dermatophytosis in both animals and humans. The present study constitutes an initiative to explore the anti-dermatophytic potential of *Cinnamomun tamala* leaf essential oil against dermatophytes. Anti- dermatophytic activity against *Microsporum audouinii* and *Trichophyton mentagrophytes* were determined in terms of mycelial growth inhibition (MGI) and minimum inhibitory concentration (MIC). *C. tamala* essential oil showed lower values of MIC against both dermatophytes as compared to the standard antifungal chemicals. The essential oil also significantly inhibited the mycelial growth of both the fungi. Hence, *C. tamala* essential oil can be formulated into the antifungal ointments for topical applications in future.

KEYWORDS: **Dermatophytes, Indian bay leaf, Minimum Inhibitory Concentration, Mycelial Growth Inhibition, Mycosis**

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INTRODUCTION

Dermatophytic infections which are commonly known as "tinea" or ring worm infections, are highly prevalent skin infections in both humans and animals[5]. Distinct antifungal drugs are currently used for the treatment of cutaneous, fungal infections. However adverse side effects, narrow spectrum, low absorption, counter interaction and toxicity are key concerns for the present antifungals[8, 23]. *Cinnamomun tamala* Fr. Nees., (Indian bay leaf, tejpat or tejpatta), has aromatic leaves which are traditionally used for culinary purpose in Indian cuisine[12]. The Ayurvedic Pharmacoepia of India has characterized *C. tamala* leaves with antihelminthic , anti-inflammatory, diuretic and hypoglycaemic properties[21]. The antihelminthic property of methanolic extract of *C. tamala* bark extract has been found to be effective against *Pheretima posthuma* (earthworm)[1]. Antibacterial activity of volatile and oleoresins of *C.tamala* has been observed against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Shigella sonnei, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae* and *Streptococcus pyogenes*[7,6]. Remarkable antifungal activity of *C.tamala* essential oil and oleoresin has also been exhibited against *Aspergillus fumigatus, A.niger, A.oryzae, Candida albicans, Fusarium moniliforme, Rhizopus stolonifer and Penicillium* species[22, 14]. However, the anti-dermatophytic potential of *C.tamala* essential oil has not been explored till now.

MATERIAL AND METHODS

Essential Oils

Fresh mature leaves of *Cinnamomun tamala* were collected from the 'Demonstration Farm', Centre for Aromatic Plants (CAP), Selaqui, Dehradun. The leaves were crushed and thoroughly washed with distilled water twice. After draining the excess water, the leaves were dried in shade for 2 days on paper towel. The leaves were hydrodistilled for 4h in a Clevenger apparatus. Essential oil was collected after removal from the water oil surface and then dried over anhydrous sodium sulphate; the extracted oil was stored at 4 °C in a brown bottle until further use[3, 22, 11].

Preparation of Essential Oil Stock Solution

A stock solution (16%) of essential oil was prepared by diluting the essential oil in 10% DMSO and 0.5% Tween-80 in the aseptic conditions (stored at 4ºC, in dark)[15].

Dermatophyte cultures

The following microorganisms were used in the study:-

Microsporum audouinii MTCC 8197

Trichophyton mentagrophytes MTCC 8476

The cultures were obtained from Microbial Type Culture Collection, IMTech, Chandigarh. The routine subculturing was done on Saboraud's dextrose agar medium.

Screening of antifungal activity of essential oils against the dermatophytes

Preliminary screening of antifungal activity of essential oils against the dermatophytes was carried by disc diffusion method by poisoned food technique[4, 8, 14]. Briefly, mycelial discs of 5 mm diameter were 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 ºC and the observations were recorded on the seventh day. Percentage of mycelial growth inhibition (MGI %) was calculated according to the formula:

$$MGI\ \% = \frac{(dc - dt) \times 100}{dc}$$

where ,dc = fungal colony diameter in control sets, dt = fungal colony diameter in treatment sets. MGI % was determined on 7th, 10th and 15th day of incubation. The experiments were conducted in triplicates and the values of MGI % were expressed as average of three readings ± standard deviation (SD). Minimum Inhibitory Concentration (MIC, in µg/ml) of essential oil determined to be the lowest concentration which completely inhibited the fungal growth on 10 days of incubation.

Screening of antifungal activity of standard antifungal agents against the dermatophytes

The stock solutions of Amphotericin B (250 µg/ml), Fluconazole (128 µg/ml), Ketoconazole (200µg/ml) and Nystatin (200µg/ml) were prepared. MIC was also determined.

RESULTS

C.tamala essential oil showed higher inhibition in mycelial growth of*Microsporum audouinii* as compared with *Trichophyton mentagrophytes* (Table no.1 & Table no. 2). The antifungals namely Amphotericin B, Fluconazole, Ketoconazole and Nystatin also inhibited *Microsporum audiuoinii* and *Trichophyton mentagrophytes* significantly after 10 days of incubation (Table no 3). However, as MGI (%) of *C.tamala* essential oil and standard antifungal drugs were compared, both *Microsporum audouinii* and *Trichophyton mentagrophytes* were more inhibited by essential oil (Figure 1). MIC of different antifungal agents *C. tamala* essential oil, Amphotericin B, Fluconazole, Ketoconazole and Nystatin were determined to be 34.2 µg/ml, 125 µg/ml, 128 µg/ml, 200 µg/ml and 200 µg/ml against *M.audouinii* respectively whereas these values were determined to be 68.4 µg/ml, 250 µg/ml, 128 µg/ml, 200 µg/ml and 400 µg/ml (Figure 2). *C. tamala* essential oil showed comparatively lower values of MIC against both *T.mentagrophytes* (68.4 µg/ml) and *M.audouinii* (34.2 µg/ml) as compared to the antifungal agents. According to the present study, MIC of *C.tamala* was determined to be 0.06% and 0.03% against *T.mentagrophytes and M.audouinii* respectively. However, in a prior study MIC of *C. tamala* essential oil was determined to be quite higher against *A. niger* (MIC = 156%)[17]. MGI (%) of *C .tamala* essential oil was observed to be 46.75 at 0.01% and 59.17 at 0.03 % against *M.audouinii* and

T.mentagrophytes respectively, which is the foremost finding in this regard.

DISCUSSION

The results of the present study cannot be directly correlated with previous findings due to paucity of literature on the anti-dermatophytic effects of *C.tamala* essential oil. However, *Cinnamomum tamala* essential oil and oleoresins have been previously explored by a number of researchers for its antifungal properties. *C.tamala* essential oil has shown good antifungal activity as determined in terms of zone of inhibition against *Aspergillus niger, A. fumigatus, Candida albicans, Rhizopus stolonifer* and *Penicillium* spp[13]. Even complete inhibition has been observed at higher concentrations of *C.tamala* essential oil. The considerable differences in the values of mycelial inhibition and MIC can be attributed to the differences in methodologies and susceptibilties of individual fungal strains. Remarkable antifungal activity of *C.tamala* essential oil has been exhibited against *Fusarium moniliforme, Aspergillus niger, A. oryzae* and *A. solani*[8, 13]. Previous chemical studies have shown the prominence of monoterpenes in *C.tamala* essential oil, eugenol reported to be the main constituent[2]. The other monoterpenes such as trans-sabinene hydrate, (Z)-β-ocimene, myrcene, α-pinene and β-sabinene have also been detected. Cinnamaldehyde, cinnamyl acetate and terpenoid compounds have also been found[18, 10, 16]. Lysis of cell wall and plasma membrane, the change in permeability and release of intracellular constituents can be the possible mechanisms for anti-dermatophytic action of various essential oils suggested by different researchers[24, 25]. Mycelial growth inhibitory studies included in the present work have shown that *C.tamala* essential oil significantly inhibits the active growth of dermatophytes. Hence it can be used in the formulation of anti-dermatophytic medications. However, further studies are required to forfend the toxicity, inflammation and allergic reactions.

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Table 1

Mycelial growth of Microsporum audouinii and Trichophyton mentagrophytes by C. tamala essential oil.

Concentration (µg/ml)	Growth (mm) after 10days (*M.audouinii*)	Growth (mm) after 10 days (*T.mentagrophytes*)
285	-	-
136.8	-	-
68.4	-	-
34.2	-	40.75 ±3.385
11.4	26.95 ±0.36	37.85 ±3.915

Table 2

Comparison of mycelial growth inhibition (MGI%) of Microsporum audouinii & Trichophyton mentagrophytes of C.tamala essential oil.

Concentration (µg/ml)	Mycelial Growth Inhibition (MGI%) after 10 days (*M.audouinii*)	Mycelial Growth Inhibition (MGI%) after 10 days (*T.mentagrophytes*)
285	-	-
136.8	-	-
68.4	-	-
34.2	-	25.63 ± 1.8
11.4	33.45 ±1.9	30.93 ±2.1

Table 3

Comparison of Mycelial growth of Microsporum audouinii & Trichophyton mentagrophytes by Antifungal agents

Antifungal agents	Concentration (µg/ml)	Mycelial Growth (mm) after 10 days (*M.audouinii*)	Mycelial Growth (mm) after 10 days (*T.mentagrophytes*)
Amphotericin B	500	-	-
	250	-	-
	125	-	5.2
	62.5	4.8	6.4
	31.25	3.9	5.9
Fluconazole	256	-	-
	128	-	-
	64	4.9	1.9
	32	4.6	3.5
	16	4.4	3.2
Ketoconazole	400	-	-
	200	-	-
	100	1.9	11.2
	50	1.5	11.5
	25	2.1	10.9
Nystatin	400	-	-
	200	-	6.5
	100	6.1	6.2
	50	4.9	7.1
	25	5.2	7.3

Table 4
Comparison of mycelial growth inhibition (MGI%) of *Microsporum audouinii & Trichophyton mentagrophytes* by different Antifungal agents with their different concentrations after 10 days.

Antifungal agents	Concentration (µg/ml)	Mycelial Growth Inhibition (MGI%) after 10 days(*M.audouinii*)	Mycelial Growth Inhibition (MGI%) after 10 days (*T.mentagrophytes*)
Amphotericin B	125	-	25.7 ± 0.9
	62.5	12.7±0.9	8.5±0.5
	31.25	29.0 ± 1.9	15.7 ± 1.2
Fluconazole	64	23.0 ± 1.8	-
	32	19.2±1.1	6.12±0.8
	16	7.4±0.7	10.2±0.8
Ketoconazole	100	-	9.8 ± 1.2
	50	21±1.6	12.7±0.6
	25	10.5 ± 0.6	6.8 ± 1.3
Nystatin	200	-	9.7 ± 1.4
	100	12.9±0.7	13.8±1.1
	50	9.2 ± 1.7	1.3 ± 0.2
	25	3.7 ± 1.1	1.38 ± 0.3

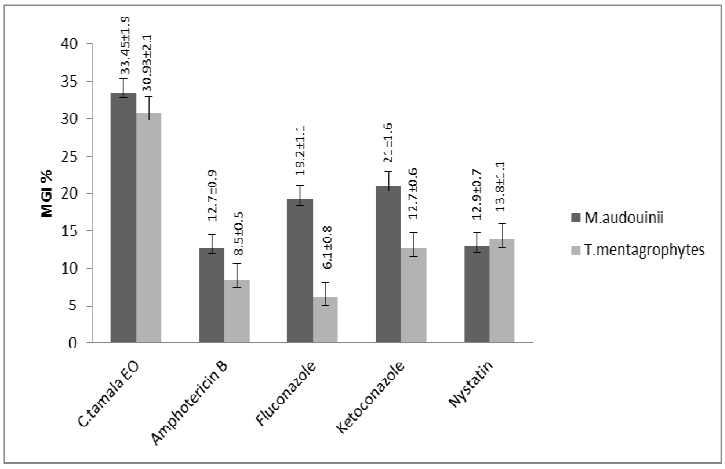

Figure 1
MGI% of Microsporum audouinii & Trichophyton mentagrophytes by subminimal concentration of different antifungal agents

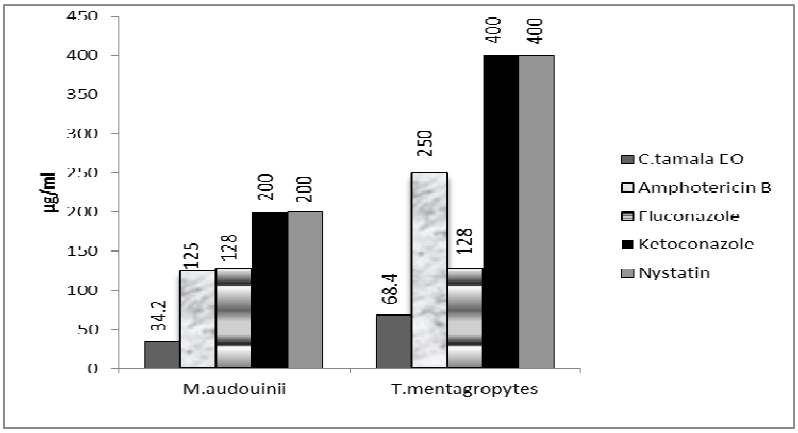

Figure 2
MIC of Microsporum audouinii & Trichophyton mentagrophytes of different antifungal agents after 10 days

CONCLUSION

The present study leads to the conclusion that *Cinnamomum tamala* essential oil has remarkable anti-dermatophytic activity, which suggests the development of formulations that can control both animal and human dermatophytic infections. However to gain further insight into its antifungal activity, extended studies are required.

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CONFLICTS OF INTEREST

We have no conflicts of interest.

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