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Anti-dermatophytic activity of some Indian medicinal plants

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Abstract

Objective: Anti-dermatophytic activities of 23 South Indian medicinal plant materials have been studied using five isolates of *Trichophyton rubrum* and four isolates of *Trichophyton mentagrophytes* obtained from the patients of Raja Muthiah Medical College & Hospital, India and compared. Materials and methods: Water and ethanol extracts were prepared and their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined. Results and conclusion: Ethanol extracts of plants exhibited more activity than water extracts. Among 23 plants garlic showed least MIC & MFC. In addition, ethanolic extracts of 11 plants showed less than 11.5 mg/ml MFC against *T. rubrum* and 9 plants showed less than 11.5 mg/ml MFC against *T. mentagrophytes.*

Key words: Dermatophytosis, *Trichophyton rubrum, Trichophyton mentagrophytes,* medicinal plants.

1. Introduction

In the past decade, the incidence of dermatophytosis has risen dramatically. The humid weather, over population and poor hygienic conditions are ideally suited for the growth of dermatophytes and these factors are more important in a tropical country like India. Among the infectious diseases, diseases caused by fungal infections account for a larger proportion of health problems in humans particularly among women and children [1]. A number of studies have been carried out reporting garlic as an antifungal agent [2,3]. Turmeric oil was found to inhibit dermatophytes *in vivo* [4]. Ibrahim studied the inhibitory activity against dermatophytes using ethanol extracts of *Cassia alata* leaves [5].

The ethanol extract of the whole plant of *Lawsonia inermis* showed antifungal activity against *Trichophyton mentagrophytes, Candida albicans, Candida neoformans, Aspergillus niger* and *Microsporum canis* [6]. The essential oil of *Ocimum* spp. has been reported to have

antimicrobial and antidermatophytic properties [7]. *Psoralea corylifolia* seed essential oil showed moderate antifungal activity [8].

Since antimycotic activity of plants remain largely unexplored, interest has grown in studying antifungal activity from plant sources. Anticandidal activities of 20 household South Indian medicinal plants and/or plant products have been reported using 30 *Candida albicans* isolates obtained from vaginal samples of patients with candidiasis [9].

In this report we have investigated the fungicidal and fungistatic effect of 23 medicinal plants against *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

2. Materials and methods

2.1 Plant material

The plants were collected from the Annamalai University campus or purchased from local Siddha medical shop. The taxonomic identification of the plants was established by the Department of Botany, Annamalai University. Vouchers are located in the Herbarium of Department of Botany, Annamalai University.

2.2 Aqueous and alcoholic extracts

For aqueous extracts, fresh leaves were washed, macerated (g/ml), extracted with sterile distilled water and filtered through cheese cloth. For alcoholic extracts, shade dried plant materials were powdered, suspended in ethanol and kept at 4°C for 2 days with intermittent shaking.

The aqueous filtrate and the alcoholic supernatant were evaporated separately under pressure in a vacuum evaporator at 45°C. The final product in each case was dissolved in 5% DMSO to obtain a concentration of 300 mg/ml, stored in Eppendorff tubes and refrigerated at 4°C [10].

2.3 Inoculum preparation

T. rubrum and *T. mentagrophytes* were isolated from 9 patients, who came to Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar. Five isolates of *T. rubrum* and four isolates of *T. mentagrophytes* were tested. Dermatophyte inoculum was prepared by scraping the infected skin with a sterile scalpel and macerating in 10 mL sterile saline.

The scraped samples were inoculated on Sabouraud dextrose agar (SDA) with antibiotics and incubated at room temperature (35 ± 2°C) for 2 months. The developed colonies were examined and identified by slide culture according to their macro and micro morphological features [11]. Inoculum standardization was done using a standard procedure [12].

2.4 In vitro antidermatophytic assay : MIC determination

Plant extracts were serially diluted using Sabouraud dextrose broth (SDB) by a two fold "serial dilution technique". Twenty microlitre of inoculum was added. SDB containing only 20 ml of inoculum served as positive control, SDB alone served as negative control. The tubes were incubated at (35 ± 2°C) for 20 days.

The MIC was the lowest concentration of the extract that did not permit any visible growth. For MFC determination [13], 21 days old incubated (MIC) suspension were subcultured in SDA plates using an inoculum size of 1 ml, and were incubated at room temperature for 21 days. The MFC was recorded as the lowest concentration that prevented the growth of any fungal colony on the solid media.

Miconazole was used as control and the drug at doses ranging from 24-0.375 µg/ml in the broth were tested for their antifungal activity against the 5 isolates of *T. rubrum* and 4 isolates of *T. mentagrophytes* using two fold serial dilution technique.

3. Results

Antidermatophytic activities of aqueous and ethanolic extracts of 23 South Indian medicinal

Table 1.
MIC and MFC of water and ethanol extracts of various plants against *T. rubrum* and *T. mentagrophytes*

Family / Species name	Parts used	Common name	*T. rubrum*				*T. mentagrophytes*			
			Water extract mg/ml		Ethanol extract mg/ml		Water extract mg/ml		Ethanol extract mg/ml	
			MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Liliaceae										
1. *Allium sativum*	L.	C Garlic	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
2. *Allium schoenoprasum L.*	C	E.garlic	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
3. *Allium cepa var. cepa L.*	B	Onion	4.6	4.6	2.3	2.3	18.7	18.7	2.3	2.3
4. *Allium cepa var. aggregatum L.*	B	M.onion	18.7	18.7	9.3	9.3	18.7	18.7	9.3	9.3
Euphorbiaceae										
5. *Acalypha indica L.*	L	Acalypha	9.3	9.3	2.3	2.3	9.3	9.3	2.3	2.3
Meliaceae										
6. *Azadirachta indica* A. Juss.	L	Neem	9.3	9.3	2.3	2.3	9.3	9.3	2.3	2.3
7. *Azadirachta indica* A. Juss.	S	Neem	4.6	4.6	0.57	0.57	2.3	2.3	0.57	0.57
Theaceae										
8. *Camellia sinensis* L. O. Ktze.	L	Tea	2.3	2.3	1.15	1.15	18.7	18.7	2.3	2.3
Solanaceae										
9. *Capsicum annuum* L.	F(d)	Red chilli	9.3	9.3	2.3	2.3	9.3	9.3	2.3	2.3
10. *Capsicum annuum* L.	F(g)	Green chilli	4.6	4.6	1.15	1.15	9.3	9.3	1.15	1.15
Caesalpinaceae										
11. *Cassia alata* L.	L	Dadmurdan	9.3	9.3	2.3	2.3	18.7	18.7	2.3	2.3
12. *Cassia fistula* L.	L	Laburnum	9.3	9.3	2.3	2.3	18.7	18.7	2.3	2.3
13. *Cassia occidentalis* L.	L	Negro coffee	4.6	4.6	1.15	1.15	4.6	4.6	1.15	1.15
Rubiaceae										
14. *Coffea arabica* L.	S	Coffee	9.3	9.3	0.57	0.57	9.3	9.3	1.15	1.15
Apiaceae										
15. *Cuminum cyminum* L.	S	Cumin	9.3	9.3	1.15	1.15	9.3	9.3	1.15	1.15
Zingiberaceae										
16. *Curcuma longa* L.	R	Turmeric	4.6	4.6	0.57	0.57	9.3	9.3	1.15	1.15
Poaceae										
17. *Cynodon dactylon* (L.) Pers.	L	Grass	9.3	9.3	1.15	1.15	9.3	9.3	1.15	1.15
Lythraceae										
18. *Lawsonia inermis* L.	L	Henna	9.3	9.3	1.15	1.15	4.6	4.6	2.3	2.3
Rutaceae										
19. *Murraya koenigii* L. Spreng	L	Curry leaf	18.7	18.7	9.3	9.3	18.7	18.7	9.3	9.3
Labiatae										
20. *Ocimum sanctum* L.	L	Tulsi	4.6	4.6	2.3	2.3	9.3	9.3	2.3	2.3
Piperaceae										
21. *Piper betle* L.	L	Betel	9.3	9.3	1.15	1.15	9.3	9.3	1.15	1.15
Fabaceae										
22. *Psoralea corylifolia* L.	S	Bauchi	2.3	2.3	0.57	0.57	2.3	2.3	0.57	0.57
Verbinaceae										
23. *Vitex negundo* L.	L	Vitex	4.6	4.6	2.3	2.3	9.3	9.3	2.3	2.3

C - clove, B - bulb, L - leaf, S - seed, F(d) - fruit (dry), F(g) - fruit (green), R - rhizome - E. garlic - Elephant garlic, M. onion - Multiple onion.

plants against 9 isolates of *T. rubrum* and *T. mentagrophytes* are presented in Table 1. It shows the comparison of water and ethanol extracts against dermatophytes. Whether water or ethanol extract, the MIC of the plant extract is sufficient for fungicidal activity. All tested organisms (isolates) showed similar response to all plant extracts. *A. sativum* and *A. schoenoprasum* showed maximum fungicidal activity at 0.28 mg/ml against both *T. rubrum* and *T. mentagrophytes* when compared with other plants.

Eleven plants exhibited moderate antidermatophytic activities, having their MFC at 0.57 or 1.15 mg/ml against *T. rubrum* while nine plants exhibited activities against *T. mentagrophytes*. All other plant extracts showed lower activities.

4. Discussion

In this study, *A. sativum* and *A. schoenoprasum* the plants chosen for comparison, showed the highest activity. In general our investigation revealed (except the above two) that ethanol extracts rather than aqueous extracts possessed more potent antidermatophytic activities. This may be due to increased solubility of active principle(s) in ethanol.

Alade and Irobi showed that the alcoholic extract of *Acalypha wilkesiana* had antifungal activities *in vitro* with MFC of 1, 16 and 32 mg/ml on *T. mentagrophytes*, *T. rubrum* and *C. albicans* respectively [10]. In our investigation, ethanolic extract of the leaf of *A. indica* (another species) exhibited MFC of 2.3 mg/ml for both *T. rubrum* and *T. mentagrophytes.* Antifungal effect of a mixture of sulphurous compounds from the steam distillate of fresh matured leaves of *A.indica* was reported active agains *T. mentagrophytes* [14]. Tea from the *C. sinensis* species of the Theaceae family is one of the most widely consumed beverages in the world. Leaf extracts are reported to contain saponin [15] and theoflavin [16] with antibacterial activity. No report is available on antifungal activity.

Our experiment showed that ethanolic extract of roasted tea leaf powder had antifungal activity against *T. rubrum* and *T. mentagrophytes*. *Capsicum annum* (chilli) fruit, both fresh and dried, are used as spices in Indian food. Green chilli exhibited more antifungal activity than red chilli in our investigation.

Ibrahim *et al.* studied the ethanol extract of *C. alata* leaves against *Microsporum gypseum*, *T. rubrum* and *T. mentagrophytes* and found an MFC of 125 mg/ml [5]. In our experiment, the MFC of ethanol extract is 2.3 mg/ml which is less than the above reference showing that *C. alata* is more potent than it has been reported.

The acetone extract of root bark and stem bark of *C. fistula* had the maximum activity against *T. rubrum*, *M. tonsurans* and *T. megninii*. The root bark had maximum activity, 100 mg of it being more potent than 16 mg of griseofulvin *in vitro*. The activity might be due to the presence of flavonoids [17]. Antifungal activities of flavonoid compounds from the root of *C. fistula* have been reported [18].

No report is available on the antifungal activity of leaf extract. Ethanol extract of leaves in our study exhibited 2.3 mg/ml as MFC, showing higher antifungal activity than the root bark. The leaves of *C. occidentalis* possess anti-mycotic activity towards ringworm genera [19]. No report is available on dermatophytes. In our study, ethanolic extracts of *Cassia occidentalis* showed antidermatophytic activity of 1.15 mg/ml as MFC.

Coffee prepared from the roasted powdered seed of *Coffea arabica* is drunk all over the world. In our work, ethanolic extract of roasted powdered seed showed MFC of 0.57 mg/ml against *T. rubrum* and 1.15 mg/ml

against *T. menta-grophytes*. Cumin seed is a spice commonly added in South Indian food. Ethanolic extract of seeds of *Cuminum cyminum* showed an MFC of 1.15 mg/ml against both the species studied in our work. *Curcuma longa* (turmeric) is also a commonly used spice and an yellow substance called curcumin is present in it.

Powdered rhizome is used as colouring agents in foods, drugs and cosmetics. Oil from *C. longa* proved to be more effective against dermatophytes than curcumin. However, both turmeric oil and curcumin were found to be ineffective against yeast [4]. In our study, *C.longa* rhizome ethanolic extract showed more activity against *T. rubrum* (0.57 mg/ml) than *T. mentagrophytes* (1.15 mg/ml).

Leaves of *Cynodon dactylon* is used for worshipping purpose by Tamilians. Juice of the plant is used to apply on fresh cuts and wounds. The alcoholic extract of the entire plant of *C. dactylon* was found to have antiviral activity against *Vaccinia virus* [20]. No work has been carried out on the antifungal activity of this plant. The ethanolic extract of leaves showed a MFC of 1.15 mg/ml against both the species studied.

The leaf extract of *Lawsonia inermis* showed antifungal activity against *C. albicans*, *Microsporum gypseum, T. mentagrophytes, Helminthosporium spp*. [21,22]. Ethanolic extract of leaves of *Lawsonia inermis* showed more activity against *T. rubrum* (1.15) mg/ml) than *T. mentagrophytes* (2.3 mg/ml) under our experimental conditions.

Murraya koenigii leaves are extensively used as a flavouring agents in curries and chutneys. The ethanolic extract of the roots and the whole plant excluding roots did not show antifungal activity against *C. albicans, C. neoformans. T. mentagrophytes, Microsporum canis* and *Aspergillus niger* [20].

In our study also ethanol extract of the leaf showed the least activity. Essential oil from *O. sanctum* leaf exhibited maximum activity against *Aspergillus niger, A. flavus, Fusarium oxysporum* and *Penicillium spp*. [23]. The ethanolic extract of the leaves showed fungitoxic activity against *Rhizoctonia solans* [24]. The essential oil from *O. canum, O. gratissimum, O. trichoden* and *O. urticifolium* have been reported to have antimicrobial and antidermatophytic properties [7].

Our experiment with ethanolic extract of leaves showed an activity of 2.3 mg/ml against *T. rubrum* and *T. mentagrophytes*. The antidermatophytic properties found in other species are found in essential oil, however in this study the activity is in the ethanolic extract of leaves.

Fresh leaves of *Piper betle* are chewed with betel nut and other adjuncts in most parts of India. The leaf extract revealed antifungal activity against *Alternaria alternata* [25]. In our investigation, ethanol extract of leaves showed MFC of l.15 mg/ml against both species.

The essential oil of *Psoralea corylifolia* showed moderate antifungal activity against *Aspergillus niger, A. fumigatus, A. flavus* and *Rhizopus stolonifer.* Petroleum ether extract showed mild activity against *Microsporum gypseum* and inactive against *C. albicans* [8, 26, 27].

In our study, ethanolic extract of seed showed an activity of 0.57 mg/ml against *T. rubrum* and *T. mentagrophytes*. Though no report is available on the antifungal activity of *Vitex negundo,* our work showed an activity of 2.3 mg/ml with the ethanol extract of leaf.

MIC and MFC are same for each plant extract either for *T. rubrum* or *T. mentagrophytes*. The antidermatophytic activity of various medicinal plants may be due to the presence of secondary metabolites such as coumarin, quinones, flavonoids, phenols and tannins and

their glycosides [28]. Adetumbi [29] proposed that blockage of lipid synthesis activity to be an important feature of the antifungal activity of garlic.

The results of our study indicate that all the plants analysed posses principles of antidermatophytic activity, but varied in their quantum. Among 23 plants studied, garlic showed least MFC and apart from garlic, ethanolic extracts of 11 plants showed less than 11.5 mg/ml MFC against *T. rubrum* and 9 plants showed less than 11.5 mg/ml MFC against *T. mentagrophytes.*

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