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ASSESSMENT OF ANTI-DERMATOPHYTIC ACTIVITY OF SOME MEDICINAL PLANTS

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Anti-dermatophytic activity of 7 medicinal plant materials have been studied using the isolates of *Trichophyton rubrum* and *Trichophyton mentragrophytes* obtained from patients having skin diseases. Water and ethanol extracts were prepared and their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined. Ethanol extracts of plants exhibited more activity than water extracts. Among the plants tested *Costus speciosus* and *Calandula officinalis* showed the highest antidermatophytic activity of 0.24 mg ml^{-1} and 0.27 mg ml^{-1} respectively. Other plant extracts exhibited moderate antidermatophytic activity.

Keywords : Anti-dermatophytic activity; Ethanol extracts; Skin disease; *Trichophyton rubrum.*

Introduction

Due to poor sanitary conditions and to the climate, often characterized by very hot temperature, high humidity and over population, the skin infections of deep and superficial wounds are common in tropical developing countries like India. Therapy with synthetic antibiotics is not always possible due to their high cost. In the past decades, the incidence of dermatophytosis has risen dramatically. Among the infectious diseases, diseases caused by fungal infections account for a larger proportion of health problems in humans particularly among children and women[1]. Skin infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide[2]. Herbal medicines are an important part of the culture and traditions of Indian continent. Today, most of the population in urban, as well as small rural communities is reliant on herbal medicines for their health care needs. Apart from their cultural significance, this is because herbal medicines are generally more accessible and affordable[3]. As a consequence, there is an increasing trend, worldwide, to integrate traditional medicine with primary health care.

A number of studies have been carried out reporting garlic as an antifungal agent[4,5]. The essential oil of *Ocimum* spp. has been reported to have antimicrobial and antidermatophytic properties[6]. Turmeric oil was found to inhibit dermatophytes *in vivo*[7]. *Psoralea corylifolia* seed essential oil showed moderate antifungal activity[8]. 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*[9]. Ibrahim studied the inhibitory activity against dermatophytes using ethanol extracts of *Cassia alata* leaves[10]. Since antimycotic activities of plants remain largely unexplored, interest has grown in studying antifungal activity from plant sources. Anticandidal activities of 20 household Indian medicinal plants and / or plant products have been reported using 30 *Candida albicans* isolates obtained from vaginal samples of patients with candidiasis[11]. This paper reports the *in vitro* antifungal activity of the medicinal plants collected from the Botanical Garden, Karnatak University, Dharwad, India against two dermatophytes such as *Trichophyton rubrum* and *Trichophyton mentagrophytes.*

Material and Methods

Plant material : The plants used in this study are 1) *Clitoria ternatea* (Leguminaceae), 2) *Costus speciosus* (Zingiberaceae), 3) *Chlorophytum borivillianum* (Liliaceae) 4) *Calendula officinalis* (Asteraceae) 5) *Punica granatum* 6) *Gymnema sylvestre* (Asclepiadaceae) 7) *Vinca rosea* (=*Catheranthus roseus*) (Apocynaceae) and were collected from the Botanical Garden, Karnatak University, Dharwad, India.

Aqueous and alcoholic extracts : For aqueous extracts, fresh plant material (leaves, roots, flower and rhizome) were washed, macerated (g ml^{-1}), 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 alcoholic supernatants were evaporated separately under pressure in a vaccum evaporator at 45°C. The final product in each case was dissolved in 5% DMSO to obtain a concentration of 300 mg ml^{-1}, stored in Eppendorff tubes and refrigerated

at 4°C[12].

Inoculum preparation :Trichophyton rubrum and *Trichophyton mentagrophytes* were collected from the local hospital and were tested. Dermatophyte inoculum was prepared by scraping the infected skin with a sterile scalpel and macerating in a 10 ml sterile distilled water. The scraped samples were inoculated on Sabouraud dextrose agar (SDA) with antibiotics and incubated at room temperature (30 ± 2° C) for 2 months. The developed colonies were examined and identified by slide culture according to their macro and micro morphological features[13]. Inoculums standardization was done using a standard procedure[13].

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 33 ± 2°C for 20 days. The MIC was the lowest concentration of the extract that did not permit any visible growth. For MFC determination[14], 21 days old incubated (MIC) suspension were subcultured in SDA plates using an inoculum size of 1 ml, and were incubated at room temperatures for 20 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 25-0.4 μg ml^{-1} in the broth were tested for their antifungal activity against the isolates of *T. rubrum* and 4 isolates of *T. mentagrophytes* using two fold serial dilution technique[15].

Results and Discussion

Antidermatophytic activites of aqueous and ethanolic extracts of medicinal plants against the isolates of *T. rubrum* and *T. mentagrophytes* are presented in the 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 the tested organisms (isolates) showed similar response to all plant extracts. Among the plant extracts, *C. speciosus* and *C. officinalis* showed the maximum fungicidal activity at 0.24 mg ml^{-1} and 0.27 mg ml^{-1} against both *T. rubrum* and *T. mentagrophytes* when compared with other plants. Rest of the plant extracts exhibited moderate antidermatophytic activities, having their MFC at 0.30 and 0.52 or 2.26 to 0.95 mg ml^{-1}. In this study ethanol extracts possessed more potent antidermatophytic activities. This may be due to increased solubility of active principle(s) in ethanol. Alade and Irobi[3] showed that the alcoholic extract of *Acalypha wilkesiana* had antifungal activities *in vitro* with MFC of 1, 16 and 32 mg ml^{-1} on *T. mentagrophytes*, *T. rubrum* and *C. albicans* respectively[3, 15]. Antifungal effect of a mixture of sulphurous compounds from the steam distillate of fresh matured leaves of *A. indica* was reported active against *T. mentagrophytes*[15]. Further Vaijayanthimala *et al.*[11] reported the anti-dermatophytic activities of 23 Indian medicinal plant materials have been studied using the five isolates of

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

Plants	Parts* used	*T.rubrum* Water extract (mg ml^{-1}) MIC	*T.rubrum* Water extract (mg ml^{-1}) MFC	*T.rubrum* Ethanol extract (mg ml^{-1}) MIC	*T.rubrum* Ethanol extract (mg ml^{-1}) MFC	*T.menta* Water extract (mg ml^{-1}) MIC	*T.menta* Water extract (mg ml^{-1}) MFC	*T.menta* Ethanol extract (mg ml^{-1}) MIC	*T.menta* Ethanol extract (mg ml^{-1}) MFC
Clitoria ternatea	R	0.30	0.30	0.26	0.26	0.30	0.30	0.26	0.26
Costus speciosus	Rhz	0.24	0.24	0.21	0.21	0.24	0.24	0.21	0.21
Chlorophytum borivillianum	Rf	0.33	0.33	0.30	0.30	0.33	0.33	0.30	0.30
Calendula officinalis	Fla	0.27	0.27	0.25	0.25	6.27	6.27	2.25	2.25
Punica granatum	Lfa	0.30	0.30	0.27	0.27	0.30	0.30	1.80	1.80
Gymnema sylvestre	Lfa	0.52	0.52	0.40	0.40	0.52	0.52	0.2	0.2
Vinca rosea	R	9.3	9.3	4.0	4.0	7.1	7.1	0.95	0.95

*Fla-Flower, Lfa-leaf, Rf-dried fasciculated storage roots, Rhz-rhizome, R-root

T. rubrum and four isolates of T. *mentagrophytes*. Ethanol extracts of plants exhibited more activity than water extracts. Among 23 plants garlic showed least MIC and MFC. In addition they have also reported that ethanolic extracts of 11 plants showed less than 11.5 mg ml^{-1} MFC against *T. rubrum* and 9 plants showed less than 11.5 mg ml^{-1} MFC against *T. mentagrophytes*[11,15].

In the present study the aqueous and ethanolic extracts obtained from the fasciculate storage roots of *C. borivillianum* showed remarkable antifungal activity of 0.33 mg ml^{-1} (Table 1). The fasciculate storage roots reputed to have aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the Ayuverdic system of medicine in India[15]. Whereas *C. officinalis* plant extracts from flower exhibited a strong antifungal activity of 0.27 mg ml^{-1} against dermatophytes (Table1). In a folk medicine the flowers have been used as external anti-inflammatory agents against suppurative processes since 12th century[16]. Aqueous extracts from flowers have diuretic, sudorific and detergent properties. Additionally it is also used in the treatment of various skin tumors, dermatological lesions, ulcer and swellings. The anifungal activity is mainly due to the presence of two series of oleanllic acid glycosides and glucuronides. The free oleanolic acid has anti-viral properties while its glycosides are haemolytic, fungistatic as well as allelopathic agents[16]. On the other hand the leaf extracts of *G. sylvestre* also showed high antifungal activity with the ethanolic extracts as compared to aqueous extracts. This might be due to the presence of acidic glycosides and anthroquinones, which have antidiabetic, antisweetner and anti-inflammatory activites[17]. Our results are in agreement with the literature. Further the rhizome of *C. speciosus* also exhibited antifungal activity might be due to the presence of antihelimintic compound diosgenin[18-20]. In the Indian folk medicine the rhizome was extensively used for curing catarrhal fever, coughs, skin diseases and snake bites. In our present study the aqueous and ethanolic extracts of roots of *C. ternatea* showed antifungal activity (Table 1) which might be due to the presence of an alkaloid *Clitorin*[21-24]. The extracts of *P. granatum* as well as *V. rosea* showed moderate antifungal activity (Table 1). The results of this study indicate that all the plants analyzed posses principles of antidermatophytic activity, but varied in their quantum.

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