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In vitro study of anti-dermatophytic activity of select plant extracts

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Abstract: *Dermatophytes are fungi that infest keratin-rich human tissues, such as skin, hair and nails. These fungal infestations, collectively known as dermatophytoses, are caused by various species of Microsporum, Epidermophyton and Trichophyton and constitute an important public health problem. Dermatophytoses generally respond well to topical antifungal therapy. However, topical therapy may not be suitable for extensive infestations. The emergence of drug resistance has sparked a hunt for new anti-fungal agents. Extracts of easily available plants, such as, Ocimum tenuiflorum Linn. (Holy Basil), Cynodon dactylon Pers. (Bahama grass) and Cassia fistula Linn. (Indian Laburnum) were selected for the present study. The results indicate that the extracts of Ocimum tenuiflorum (at 10, 15 and 20% concentrations) and Cynodon dactylon (20% concentration) inhibited the growth of Trichophyton rubrum and Trichophyton verrucosum. However, at 15% concentration, Cynodon dactylon inhibited the growth of Trichophyton verrucosum only. Extract of the bark of Cassia fistula (20% concentration) inhibited the growth of Trichophyton verrucosum and was not found to inhibit growth of Trichophyton rubrum. The results of this in vitro study reveal that extracts of these plants exhibit anti-fungal properties and are prospective alternative inexpensive remedies for dermatophytoses.*

Keywords: Dermatophytes, Dermatophytoses, Anti-fungal agents, Plant extracts

I. Introduction

The superficial fungal infections are known to affect humans since ancient times. Materia Indica [1] has reported topical application of plant extracts as remedies for superficial fungal infections. Fungal infestations caused by Trichophyton, Microsporum and Epidermophyton species have been reported to respond to topical application using extracts of Ocimum tenuiflorum (Holy Basil; Hindi & Marathi: tulsi), Cynodon dactylon (Bahama grass; Hindi: doob ghaas; Marathi: durva) and Cassia fistula (Indian Laburnum; Hindi: amaltas; Marathi: bahava) against skin infections. Hexane, dichloromethane and butanol fractions of Wedelia paludosa (Creeping daisy; Creeping ox-eye) have been reported to exhibit activity against Epidermophyton floccosum, Trichophyton rubrum and Trichophyton mentagrophytes with minimal inhibitory concentrations. An aqueous ethanol extract of leaves of Chromolaena odorata (Hindi: Tivra gandha) and some of its fractions were examined for their antifungal properties by dilution methods on solids and liquid media, using yeast and filamentous fungi [2]. Anti-fungal activity of the essential oil and aqueous extract of the Tagetes erecta (Mexican marigold) against Fusarium oxysporum and Trichophyton mentagrophytes [3] and mycotoxic activity of Ricinus communis (Castor bean plant; Hindi: Arandi; Marathi: Erandi) Euphorbia hirta (Asthma weed; Hindi: Dudhi; Marathi: Dudnali/ Govardhan), Putranjiva roxbhurghii (Lucky bean tree; Hindi: Putijia; Marathi: Jivanputra) against ringworm causing fungi like Trichophyton mentagrophytea, Microporum gypseum and Epidermophyton flocossum [4] have been reported in literature.

II. Objectives

The purpose of this study was to determine the *in vitro* effect of crude plant extracts on the growth of dermatophytes and compare these effects with that of known antifungal agents.

III. Materials And Methods

3.1. Materials: *Ocimum tenuiflorum* and *Cynodon dactylon* were obtained from the campus of St. Xavier's College, Mumbai, India. *Cassia fistula* was collected from Vikhroli , Mumbai. These plants were authenticated by the Blatter Herbarium (Reference Centre at Department of Botany, St. Xavier's College, Mumbai, India).

3.2. Preparation of plant extracts: For fresh plant extract, 2 g of fresh plant material (leaves / whole plant) was crushed in 10 ml of 50% ethanol, filtered through Whatman No. 1 filter paper and the filtrate was passed through Whatman No. 42 filter paper and finally through Millipore filter aseptically and used as crude

extract (200mg / ml). For dry plant extract, fresh plant material (leaves / whole plant) was dried in an oven at 60º C till the constant weight was obtained. The material was then powdered using a grinder. The powder was soaked overnight in 50% aq ethanol. It was then filtered through Whatman No.1 filter paper and the filtrate was passed through Whatman No. 42 filter paper and finally passed through Millipore filter aseptically and was used as crude extract. [5]

3.3. Preparation of inoculums: The 20 laboratory isolates selected were common fungal pathogens (14 and 6 isolates of *Trichophyton rubrum* and *Trichophyton verrucosum*, respectively). These isolates were cultured on corn meal agar plates (Becton Dickinson) at 30°C for 7 to 15 days for sporulation. Stock inoculum suspensions were obtained from each strain by covering the fungal colonies with 5.0 ml of sterile saline and gently rubbing the colonies with the tip of a transfer pipette. The resulting conidial suspensions were used as inoculums. [6]

3.4. Antifungal activity test: The antifungal activity of the extracts was tested as per published protocol [7] using disposable Petri plates (9.00 cm diameter), partitioned into 4 quarters. The negative control comprised 5 ml of Sabouraud Dextrose Agar (SDA) supplemented with Chloramphenicol and Gentamicin + 0.25 ml of spore suspension. The negative solvent control consisted of 5.0 ml of SDA medium supplemented with Chloramphenicol and Gentamicin + 0.1 ml of 50 % ethanol + 0.25 ml of spore suspension. The positive control (Griseofulvin control) contained 5.0 ml of SDA medium supplemented with Chloramphenicol and Gentamicin + 0.1ml of Griseofulvin (25 mg/ ml) + 0.25 ml of spore suspension Clotrimazole discs (6 mm diameter coated with 1 µg). Crude plant extracts comprised – [1] 5.0 ml of SDA medium supplemented with Chloramphenicol and Gentamicin + 0.1ml of fresh plant extract of leaves of *Ocimum tenuiflorum* (200 mg/ ml)+ 0.25 ml of spore suspension; [2] 5.0 ml of SDA medium supplemented with Chloramphenicol and Gentamicin + 0.1ml of fresh whole plant extract of *Cynodon dactylon* (200 mg/ ml) + 0.5 ml of spore suspension, and [3] 5.0 ml of SDA medium supplemented with Chloramphenicol and Gentamicin + 0.1ml of fresh plant extract of bark of *Cassia fistula* (200 mg/ ml) + 0.25 ml of spore suspension. All the plates were incubated in the dark at 27±2ºC for 6 days. The antifungal activity of each plant was tested at 10, 15 and 20% concentrations as preliminary experiments with 5% extract of fresh plants did not exhibit inhibition of fungal growth. The antifungal activity of the dry plant extract was not tested due time constraints. The inhibition of growth (if any) by extract was compared with the solvent control and that of Griseofulvin and Clotrimazole.

IV. Results And Discussion

4.1. Leaf extracts of Ocimum tenuiflorum (Holy Basil): The results indicate that the leaf extracts of *Ocimum tenuiflorum* (at 10, 15 and 20% concentrations) inhibited the growth of both *Trichophyton rubrum* and *Trichophyton verrucosum*. (Fig. 1 and Table 1)

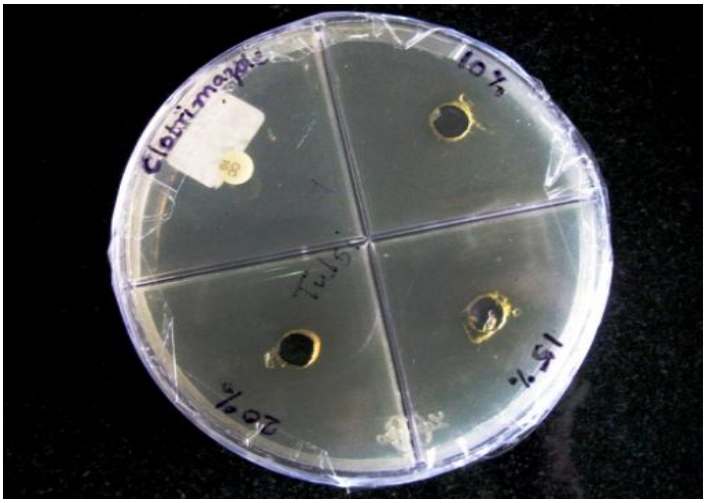

Fig. 1: Anti-fungal activity of leaf extracts of *Ocimum tenuiflorum* (Holy Basil) against *Trichophyton verrucosum*

Table 1: Anti-fungal activity of leaf extracts of *Ocimum tenuiflorum* (Holy Basil)

Organism	Ethanol control	Antibiotic control	Leaf extract		
			10%	15%	20%
Trichophyton verrucosum	No growth	No growth	No growth	No growth	No growth
Trichophyton rubrum	No growth	No growth	No growth	No growth	No growth

4.2. Leaf extracts of Cynodon dactylon (Bahama grass): At 15% concentration, leaf extract of Cynodon dactylon inhibited the growth of Trichophyton verrucosum only. But at 20% concentration, this extract inhibited the growth of both Trichophyton rubrum and Trichophyton verrucosum. (Table 2 and Fig. 2)

Table 2: Anti-fungal activity of leaf extracts of *Cynodon dactylon* (Bahama grass)

Organism	Ethanol control	Antibiotic control	Leaf extract		
			10%	15%	20%
Trichophyton verrucosum	No growth	No growth	Less growth	No growth	No growth
Trichophyton rubrum	Less growth	No growth	Less growth	Less growth	No growth

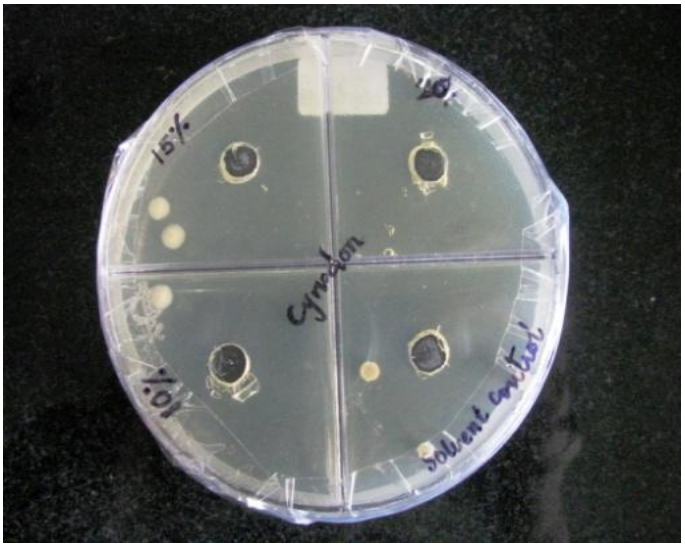

Fig. 2: Anti-fungal activity of leaf extracts of *Cynodon dactylon* (Bahama grass) against *Trichophyton rubrum*

4.3. Bark extracts of *Cassia fistula* (Indian Laburnum): Extract of the bark of *Cassia fistula* (20% concentration) inhibited the growth of *Trichophyton verrucosum* and was not found to inhibit growth of *Trichophyton rubrum*. (Fig. 3 & 4 and Table 3) The extract of another plant from the same genus viz. *Cassia alata* (Ringworm shrub; Hindi: Dadmurdan; Marathi: Dadamardana) has been shown to inhibit the growth of *Trichophyton* species by other researchers. [7]

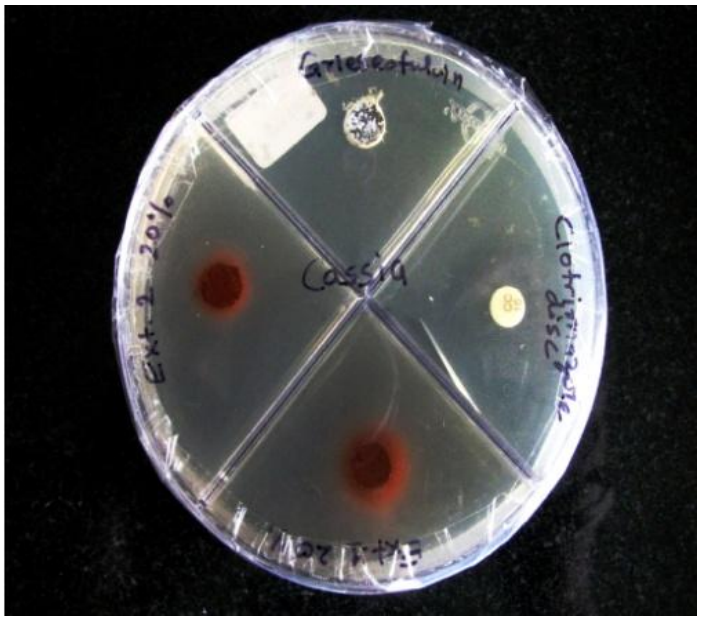

Fig. 3: Anti-fungal activity of bark extracts of *Cassia fistula* (Indian Laburnum) against *Trichophyton verrucosum*

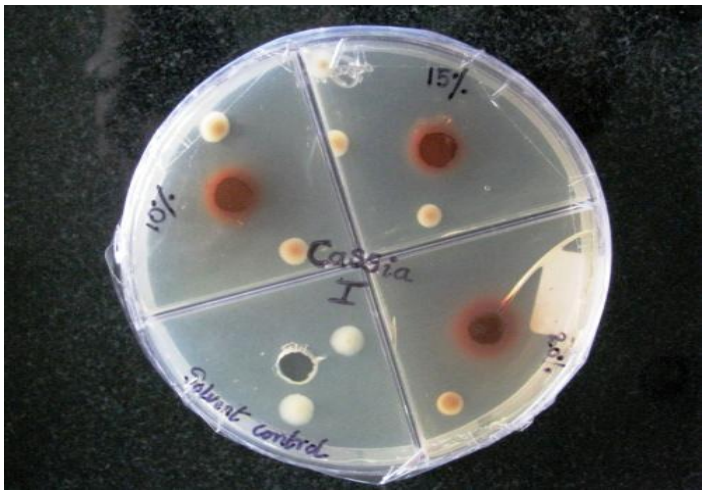

Fig. 4: Anti-fungal activity of bark extracts of *Cassia fistula* (Indian Laburnum) against *Trichophyton rubrum*

Table 3: Anti-fungal activity of bark extracts of *Cassia fistula* (Indian Laburnum)

Organism	Ethanol control	Antibiotic control	Leaf extract		
			10%	15%	20%
Trichophyton verrucosum	Less growth	No growth	Less growth	Less growth	No growth
Trichophyton rubrum	More growth	No growth	More growth	More growth	Less growth

4.4. Limitations: Contamination of the SDA plates during the experiments led to increase in the duration of the study. Due to time constraints, plant extracts of easily available plants were selected for the present study.

V. Conclusion

The leaf extracts of Ocimum tenuiflorum (Holy Basil), Cynodon dactylon (Bahama grass) and bark extract of Cassia fistula (Indian Laburnum) have anti-fungal properties and seem to be prospective, alternative, inexpensive, anti-fungal remedies for dermatophytoses.

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