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SCREENING OF PLANTS FOR ANTI DERMATOPHYTE ACTIVITY

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ABSTRACT

Mycotic infections of skin are caused by dermatophytes. Screening of plants for anti dermatophyte activity was carried out based on the literature search done. Native plants of Maharashtra (India) were screened for anti dermatophyte activity. Various plant parts from different regions were collected and then extracted with three different solvents viz. alcohol, hydro-alcohol and aqueous. The obtained extracts were subjected for anti dermatophyte activity using agar-well diffusion technique. Three different concentrations of extract were checked for activity. Two species of dermatophytes, viz. - *Trichophyton* and *Microsporum* were used in the screening assay. Out of the twenty-eight plants screened by agar diffusion method, seven were found to be active with different activity profile. Methanol extract was the most active extract. *Pterospermum suberifolium, Trachyspermum ammi, Peltaphorum pterocarpum, Ixora coccinia, Persicaria glabra, Terminallia elliptica* and *Cicca acida* showed activity at different concentrations against the two species of dermatophytes. The data obtained can be used for further studying the anti dermatophyte potential of active plants.

INTRODUCTION: Majority of superficial infections are caused by a closely related group of keratinophilic fungi called dermatophytes, which cause ringworm infection or Tinea infection [1].

Dermatophytes are aerobic, keratinolytic fungi which derive their nutrients from keratin. These particular fungi grow within the layers of the stratum corneum, but do not penetrate to the deeper skin layers. Dermatophytoses constitute an important public health problem as yet unresolved caused by the species *Epidermophyton, Microsporum* and *Trichophyton* [1].

Mycotic infections are probably the most common cause of skin disease in developing countries of tropical regions. These infections are frequent not only in underdeveloped countries but also in elderly and immunocompromised patients in the developed nations worldwide [2]. In the last two decades the incidence of infections caused by dermatophytes and other fungi has increased considerably [3].

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value [4]. The drugs used against dermatophytoses exhibit several side effects and have limited efficacy. Therefore, there is a distinct need for the discovery of new safer and more effective antifungal agents [4, 5, 6] . The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increased attention.

Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [7]. India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants [8, 9, 10] .

The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging to both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs [11].

This paper presents the data on screening of various plants belonging to different families for their anti dermatophyte potential. Some of the plants screened are known for their antiinfective potential and here are being used to assess their activity against dermatophytes.

MATERIALS AND METHODS:

Preparation of plant material and extraction: Plant materials were collected and dried before use. The dried plant materials were ground to form a powder using a mechanical grinder. The powdered plant materials were individually extracted using methanol, aqueous methanol and water as solvents. The extracts were filtered and vacuum-dried under reduced pressure. The extracts were tested against dermatophytes at three different concentrations viz. 20, 10 and 5 mg/ml.

Microorganisms: The agar-well diffusion method was used as a preliminary test to find out if the plant extracts were active. The test organisms used were the dermatophyte strains (clinical isolates) of *Trichophyton mentagrophytes* and *Microsporum gypseum*. Sabourauds Dextrose agar was used as a culture media according to the manufacturer's direction. The dermatophyte cultures were aseptically inoculated on Sabourauds agar plate and incubated at 28^{o}C for 72 hours. The growth obtained was used as inoculum for the bioassay.

Bioassay: For bioassays, the growth from plates was suspended in sterile 0.85% normal saline and transmittance was adjusted to 80-85 % at 530 nm using a spectrophotometer. This yielded approximately $1.5x10^{8}$ cells/ml. Predecided volume of culture was mixed with molten Sabourauds agar and poured in sterile plates. The medium was allowed to set and wells of 6 mm diameter and about 2 cm apart were punctured in the culture media using sterile cork borers.

As a precaution for not missing any trace amounts of antimicrobials, a relatively high concentration of 20 mg/ml of each extract along with lower concentrations (10 and 5 mg/ml) were prepared and administered 50 microlitres in each well using a micropipette. The samples were allowed to diffuse from the wells into the agar medium by leaving the plates in the laminar flow for one hour. After the incubation period of 72 hours at 28^{o}C, bioactivity was determined by the measurement of the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls had solvent without test compounds. The results were compared with a standard azole i.e., Ketoconazole disc.

RESULTS AND DISCUSSION: The demand of plant based/herbal medicines is increasing day by day as they come with minimum or no side effects in the era of developed resistance against synthetic antibiotics. Plants belonging to different families, from the region of Maharashtra (India) were screened for their anti-infective properties against dermatophytes. Total 28 plants were extracted with three different solvents, viz. methanol, hydro-methanol and aqueous. **Table 1** gives the details of plants studied, in terms of their botanical name and family.

The prepared extracts were tested against the dermatophytes species, *Trichophyton mentagrophytes* and *Microsporum gypseum*. Out of the 28 plants tested, seven plants were active against the tested species. Methanol extract was the most active extract amongst these seven active plants. The activity data is listed in **table 2**; which shows the plant extracts activity in terms of diameter of zone of inhibition values against the tested species of dermatophytes.

TABLE 1: PLANTS STUDIED FOR ANTI DERMATOPHYTE ACTIVITY

Plant Name	Family
Adiantum philippense	Adiantaceae
Berberis tinctoria	Berberidaceae
Caesalpinia bonduc	Caesalpiniaceae
Cassytha filiformis	Lauraceae
Cicca acida	Euphorbiacea
Dendrophthoe falcata	Loranthaceae
Dryopetes roxburghii	Euphorbiaceae
Haldina cordifolia	Rubiaceae
Hygrophila schulli	Acanthaceae
Ixora brachiata	Rubiaceae
Ixora coccinia	Rubiaceae
Lannea coromandelica (Stem)	Anacardiaceae
Lannea coromandelica (Twigs)	Anacardiaceae
Mahonia leschenaultii	Berberidaceae
Oxystelma esculanta	Asclepiadaceae
Pedalium murex	Pedaliaceae
Peltophorum pterocarpum	Fabaceae
Persicaria glabra	Polygonaceae
Phyllanthus reticulata	Euphorbiaceae
Pseudospermum acerifolium (Leaves)	Malvaceae
Pseudospermum acerifolium (Twig)	Malvaceae
Pterospermum suberifolium	Sterculiaceae
Schleichera trijuga	Sapindaceae
Terminallia elliptica	Combretaceae
Trachyspermum ammi	Apiaceae
Trewia nudiflora	Euphorbiaceae
Viscum articulatum	Viscaceae
Wendlandia heynei	Rubiaceae

TABLE 2: ACTIVITY PROFILE OF ACTIVE PLANTS

Plant Name	Extract	Conc. (mg/ml)	Diameter of zone of inhibition (mm)	
			Trichophyton mentagrophytes	***Microsporum gypseum***
Trachyspermum ammi	Methanol	5	15	13
		10	18	16
		20	24	22
Pterospermum suberifolium	Methanol	5	12	11
		10	16	14
		20	20	19
Peltophorum pterocarpum	Methanol	5	18	16
		10	24	23
		20	26	24
	HM	5	20	18
		10	24	25
		20	28	29
Ixora coccinia	Methanol	5	16	16
		10	20	21
		20	27	26
	HM	5	12	12
		10	14	14
		20	18	18
	Aqueous	5	12	12
		10	14	14
		20	19	18
Terminallia elliptica	Methanol	5	11	11
		10	12	11
		20	13	14
	HM	5	11	13
		10	12	13
		20	13	13
	Aqueous	5	9	-
		10	10	11
		20	12	13
Persicaria glabra	Methanol	5	9	9
		10	9	9
		20	13	12
	HM	5	12	11
		10	14	14

		20	16	16
	Aqueous	5	12	11
		10	14	14
		20	16	15
Cicca acida	Methanol	5	12	11
		10	15	12
		20	17	14
	HM	5	12	10
		10	15	11
		20	17	15
Ketoconazole	(10µg/disc)		22	26

Key: - no zone of inhibition, HM: Hydro-methanol extract

Pterospermum suberifolium, Trachyspermum ammi, Peltaphorum pterocarpum and *Ixora coccinia* were most active with the inhibition zone diameters of greater than 20 mm followed by *Persicaria glabra, Terminallia elliptica* and *Cicca acida*. (Table-2). In the case of *P.glabra*, *T.elliptica* and *I.coccinia* all the three extracts viz. methanolic, hydroalcoholic and aqueous were found to be active.

T. ammi is studied for its anti microbial potential; Rosina Khan et al s work shows its activity against multidrug resistant microbes; the positive inhibition zones obtained against dermatophytes, in this study confirms its antimicrobial nature [12]. *P. suberifolium* is listed as a cytotoxic plant in a study carried out by George *et al*., [13], its anti dermatophytes activity is demonstrated over here. *P. pterocarpum* is studied for its anti bacterial and antifungal activity [14], we got good anti dermatophyte activity for this plant with all its three extracts showing inhibition zones greater than 20mm.

I. coccinia showed very good anti-dermatophyte activity with all its extracts being active and inhibition zones greater than 20 mm. Anti microbial activity has been found for this plant in the study carried out by J. Annapurna *et al*., [15]. All the three extracts studied for *T. elliptica* were active against the dermatophytes species. Previously anti oxidant and antimicrobial activity has been reported for *Terminalia* species [16, 17]. The anti dermatophyte activity of *T. elliptica* throws light on its ethno botanical use for skin diseases and other microbe related ailments. Phytochemical study has been carried out on *P. glabra* along with the mention of its anti microbial activity [18]; here anti dermatophyte activity was observed for all its three extracts which further substantiate its activity spectrum. The anti dermatophyte activity of *C.acida* obtained in this study substantiates its ethanomedicinal use in skin diseases previously reported [19]. Further phytochemical studies including isolation of active compound/s from these plants will help to get plant based anti dermatophytes agent.

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