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DETERMINATION OF ANTIDERMATOPHYTIC EFFICACY OF FLAVONOIDS FROM *ABRUS PRECATORIUS*

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ABSTRACT

Introduction: Plants represent untapped reservoir of novel compounds, hence dominate indigenous system of medicine. Exploration of herbal drug is directly related to its traditional utilization in alternative medicine. Dermatophytosis/ ringworm is caused by keratinophilic fungi and is a common skin problem in country like India where climatic conditions favors the infection. Selected plant *Abrus precatorius* is being used to treat various skin infections in folklore medicine. In the present study efforts are made to identify new sources with antidermatophytic potential from selected plant. **Material and methods:** Shade dried parts of the plant were subjected to flavonoid extraction by following well-established protocols and tested against test dermatophytes to screen their antidermatophytic nature. Minimum inhibitory concentration and minimum fungicidal concentration (MIC and MFC) was also determined along with total activity (TA). **Results:** Remarkable inhibition zone (IZ) and activity index along with low values of MIC and MFC were recorded for the active extracts. Same values of MIC and MFC were found for most of the extracts which indicated their fungicidal nature. *Trichophyton mentagrophytes, T. rubrum* and *E. floccosum* were observed more susceptible than *M. canis* and *M. gypseum*. Free and bound flavonoids were recorded to be highly antidermatophytic against all test pathogens. Greater values of total activity of flavonoid extracts also indicated their potent antidermatophytic nature. **Conclusion:** Present study recommends chemical exploration of flavonoid extracts of stem and leaves and

preclinical evaluation of the same. Potential antidermtophytic compounds may be used as a topical ointment and/ or hand sanitizer/ wash.

Keywords: *Abrus precatorius*, flavonoids, dermatophytosis/tinea/ringworm, dermatophytes

INTRODUCTION

Medicinal plants are an indispensable part of the traditional medicine practiced all over the world because of low cost, easy access and ancestral experience **[1]**. During the past decade, traditional system of medicine has become increasingly important as they are considered to be safe and have long lasting effect. In many developing countries a large part of population relies on herbal medicines. Phytomedicines have maintained popularity for historical and cultural reasons and have attracted attention as an alternative therapy **[2, 3**]. According to 'World Health Organization' (WHO) more than 80% of the world's population relies on traditional herbal medicine for their primary health needs **[4, 5]**. Even as we entered into the century with its exciting prospects of gene therapy, herbal medicine remains one of the most common forms of the therapy available to the world's population. Medicinal plants dominate indigenous/ alternative system of medicine and are common elements in Ayurveda, Chinese, Homeopathy, Naturopathy, oriental and Native American medicines. Several plant species are used by many ethnic groups for the treatment of various ailments from minor infections to dysentery, skin diseases, asthma, malaria **[6]**. About $3/4^{th}$ of the plant derived drugs correlated directly with their traditional uses. It is estimated that at least 12000 different secondary metabolites have been isolated from plants which constitute less than 10% of the total secondary metabolites. One hundred and nineteen secondary metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% pharmacologically active plant derived compounds were discovered **[7, 8]**.

Dermatophytosis/ Tinea/ ringworm is one of the most infectious diseases of humans, caused by invasion of stratum corneum by dermatophytic fungi viz. *Trichophyton spp, Microsporum spp, Epidermophyton spp* **[9]**. It remains a common global public health problem especially in tropical countries such as India where, moist climatic conditions facilitate the fungal infections **[10]**. Dermatophytic infections are not confined only to humans but also infect animals as well. Although mycotic infections caused by dermatophytes are not fatal, but are unpleasant and are responsible for erratic behavior of patients.

Although many antifungal synthetic drugs viz. imidazole, butanafine, terbinafine etc. are available for treating dermatophytosis **[11]** but due to resistant dermatophytic strains, treatment fails and adverse side effects appear. In the search of possible source of alternative and effective antifungal drugs, plants and their products have gained much importance, as green medicine is believed to be cheaper and safer with least or no side effects. In the present study efforts have been made to identify new source (plants) or compounds of plant origin with antidermatophytic activity.

MATERIAL AND METHODS

Selected plant:

Abrus precatorius of family Fabaceae was selected for the present investigation. Selected plant is well known for their medicinal properties especially in skin diseases in folklore medicine. *A. precatorius* is considered as laxative, expectorant and aphrodisia and is used in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/leukemia). It is a nerve stimulant and is used in vata disorders like joint pains and paralysis. The paste of seeds is applied locally in alopecia and skin diseases. It has also been mentioned as a contraceptive in Ayurveda **[12]**.

Selected plant was collected from various localities of Jaipur and its nearby areas and sample specimens were submitted to Herbarium, Department of Botany, University of Rajasthan. Different parts of selected plant were separately collected; shade dried and finely powdered using grinder. Each powdered sample was kept in an air tight polythene bags, labeled properly, for further use.

Test microorganisms:

Pathogenic microorganisms selected for study include five dermatophytes *Trichophyton mentagrophytes* (MTCC 7687), *Trichophyton rubrum* (MTCC 296), *Epidermopyton floccossum* (MTCC 7880), *Microsporum canis* (MTCC 3270), *Microsporum gypseum* (MTCC 284).

Selected microorganisms were procured from IMTECH, Chandigarh, India. Fungal strains were kept on 'Sabouraud Dextrose Agar' medium (Peptone 10 g; Dextrose 20 g; Agar 20 g in 1000 ml of distilled water; pH adjusted to 6.8 - 7.0) at 27 ± 2^0 C.

Preliminary Detection of Phytochemicals/Secondary Metabolites

Phytochemical screening procedures were carried out on the aqueous extracts and on the powdered plant samples using standard procedures to detect the presence of alkaloids and flavonoid in the plant parts **[13, 14]**.

Extraction of Flavonoids

Different parts of selected plants were subjected to flavonoid extraction, following well established protocol **[15]**. Hundred grams of finely powdered sample of each plant part was Soxhlet extracted with 80% hot methanol on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether, and ethyl acetate. Petroleum ether fractions were discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids, respectively. Ethyl acetate fraction of each of the sample was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h. Resulting mixture was filtered and the filtrate was extracted with ethyl acetate which was then washed with distilled water to neutrality. Ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried *in vaccuo*, weighed and stored at 4°C in air tight glass vials for further use.

Antimicrobial screening of extracts

Disc diffusion assay (DDA) was performed for antimicrobial screening **[16, 17]**. SD agar base plates were seeded with the standard size of fungal inoculum (1×10^6 CFU/ml). Sterile filter paper discs (6 mm in diameter) were impregnated with 100 µl of each of the extract (10 mg/ml concentration) to give a final concentration of 1 mg/disc, left to dry *in vaccuo* to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates. Each extract was tested in triplicate along with terbinafine (1 mg/disc) as standard. The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 27± 2°C for 5-7 days. Zone of inhibition (IZ) or depressed growth of microorganisms was measured and the 'Activity Index' (AI) for each extract was calculated.

$$\text{Activity Index(AI)} = \frac{\text{Inhibition Zone of the Sample}}{\text{Inhibition Zone of the Standard}}$$

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MBC/MFC)

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against test pathogens in disc diffusion assay. Broth microdilution method **[18]** was followed for determination of MIC values. Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two fold serial dilution. Thereafter 100 µl inoculum of standard size was added to each well. Fungal suspensions were used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 27±

2°C for 5-7 days for dermatophytic fungi. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms. The minimum fungicidal concentration (MFC) was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MFC.

Total activity (TA)

Total activity (TA) is a measure of the amount extracted from the plant in relation to the MIC of the extract/fraction/ compound isolated. Total activity is the volume at which test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g **[19]**. In mathematical terms it can be expressed as:

$$\text{Total Activity (TA)} = \frac{\text{Amount extracted from 1g plant material (mg / g.d.w)}}{\text{MIC of the extract (mg / ml)}}$$

RESULTS

Significant amount of total flavonoids were extracted from root, stem and leaf of the plant. Amount of free flavonoids was obtained more than bound flavonoids from almost all plant parts **(Figure 1)**. Remarkable inhibition zone (IZ) and activity index (AI) were recorded for flavonoids from stem and leaves of the plant **(Table 1)**. Low values of MIC and MFC were obtained for the active extracts and values were in accordance with the IZ values. Most of the extracts were found to be fungicidal (i.e. same values of MIC and MFC) which indicated capacity of extracts to completely kill the dermatophytes even at very low concentration. *Trichophyton mentagrophytes, T. rubrum* and *E. floccosum* were observed more susceptible than *M. canis* and *M. gypseum*. Free and bound flavonoids from the leaf and stem were found with excellent antidermatophytic potential against all tested dermatophytes. Total activity was also calculated co-relates antimicrobial activity with amount of extract isolated from the plant. Bound flavonoids of stem showed maximum values of TA against all tested pathogens **(Figure 2)**.

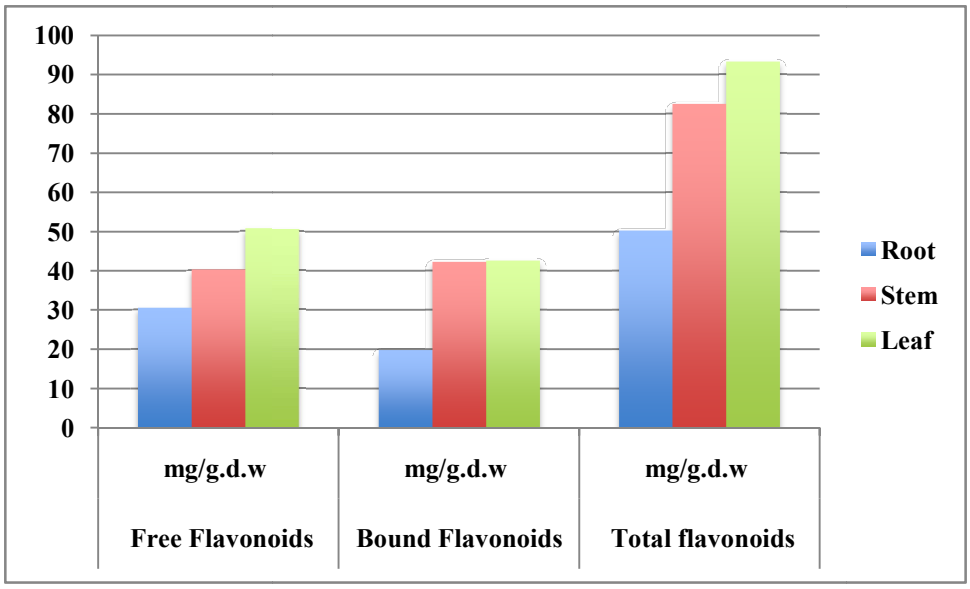

Figure 1: Quantity of total flavonoids of *Abrus precatorius*

Table 1: Antidermatophytic activity of flavonoids of *Abrus precatorius*

Plant part	Extract	Test microorganism									
		T. mentagrophytes		*T. rubrum*		*E. floccosum*		*M. canis*		*M. gypseum*	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Root	RE_1	10	0.22	9	0.19	9	0.25	-	-	-	-
	RE_2	11	0.24	8	0.17	8	0.22	-	-	-	-
Stem	SE_1	25	0.55	21	0.44	22	0.62	24	0.48	20	0.41
	SE_2	27	0.60	25	0.53	26	0.74	27	0.54	26	0.54
Leaf	LE_1	20	0.44	22	0.46	26	0.74	20	0.40	19	0.39
	LE_2	22	0.48	23	0.48	25	0.71	28	0.56	23	0.47

IZ = Inhibition zone in mm (mean value; including 6 mm diameter of disc), AI = Activity Index (IZ developed by extract/IZ developed by standard), - No activity, E_1 = Free flavonoids, E_2 = Bound flavonoids, IZ of standard drug terbinafine against *T. mentagrophytes* (45mm), *T. rubrum* (47 mm), *E. floccosum* (35 mm), *M.canis* (50 mm) and *M. gypseum* (48 mm)

Table 2: MIC and MFC values of flavonoids of *Abrus precatorius*

Plant part	Extract	Test microorganisms (mg/ml)									
		T. mentagrophytes		*T. rubrum*		*E. floccosum*		*M. canis*		*M. gypseum*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
Root	RE_1	1.25	1.25	1.25	2.5	1.25	1.25	-	-	-	-
	RE_2	1.25	1.25	1.25	1.25	1.25	1.25	-	-	-	-
Stem	SE_1	0.312	0.312	0.312	0.312	0.312	0.625	0.312	0.312	0.625	0.625
	SE_2	0.156	0.156	0.156	0.312	0.156	0.156	0.078	0.078	0.156	0.156
Leaf	LE_1	0.312	0.312	0.312	0.625	0.078	0.625	0.625	0.625	0.625	1.25
	LE_2	0.312	0.312	0.312	0.312	0.078	0.625	0.312	0.312	0.312	0.312

E_1 = Free flavonoids; E_2 = Bound flavonoids; MIC= Minimum Inhibitory Concentration (mg/ml), MBC/MFC= Minimum Bactericidal/Fungicidal Concentration (mg/ml)

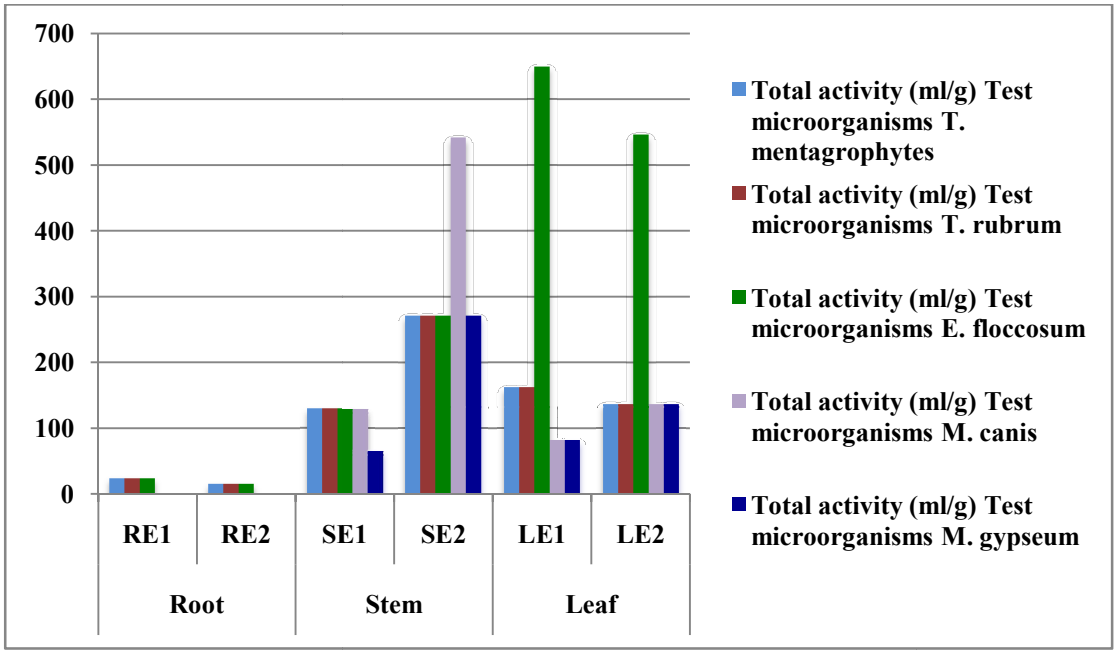

Figure 2: Total activity of the active extracts of *A. precatorius*

CONCLUSION

Medicinal plants are being explored to find new herbal antidermatophytic compounds/principle **[20-22]**. Selected plant is chemically explored by several researchers but systematic scientific studies are still lacking to relate bioactivity with the active compound and most of the work is done on crude extract of the plant **[23-30]**. Identification and isolation of active principle is indispensible in order to establish a phytochemical as a drug. Therefore research towards this direction is always needed.

In the view of the fact that deep-seated infection remains in latent phase, disease reoccur time to time when its favorable climatic conditions come. Socio-economic conditions of poor population (where disease prevails) also exacerbate the situation due to high cost of drugs. Therefore cost effective, easily available drugs are always in need. Present investigation was aimed to explore herbal potential source/s of antidermatophytic nature and recommends chemical exploration of flavonoid extracts of stem and leaves and preclinical evaluation of the same. Potential antidermtophytic compounds may be used as a topical ointment and/ or hand sanitizer/ wash.

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