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Anti-dermatophytic activity of some traditionally used medicinal plants of North Karnataka Region

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ABSTRACT

To find the potentiality of the following medicinal plants for their anti dermatophytic property. Agar and broth dilution methods, Leaf extracts of *Cassia occidentalis, Cassia tora, Lawsonia inermis, Xanthium strumarium* and *Caesalpinia bonducella* with various solvents viz., methanol, alcohol, acetone, acetone, petroleum ether, chloroform and ethyl acetate were evaluated for antidermatophytic activity against human pathogenic fungi. In agar and broth dilution methods, all extracts showed antifungal activity even at minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs). The most biologically active extract was found to be ethyl acetate leaf extract of C. bonducella, which inhibited all test fungi with time and dose dependent activity. This plant extract retarded the growth of all the organisms at 10000 µg/ml up to 30 days and beyond. It is further suggested for detail investigation of active constituents of the plants used in the present.

INTRODUCTION

Dermatophytosis is the most frequent superficial fungal infections in India. Clinical surveys have shown ringworm as one of the most common dermatomycoses caused by the species of *Epidermophyton, Microsporum* and *Trichophyton.* These fungal infections are not only causing primary diseases, but also responsible for various secondary ailments due to many predisposing factors. A widespread use of broad-spectrum antibiotics and immunosuppressive drugs for these ailments has led to an increase in the incidence of systemic fungal infections due to development of resistant strains of some of the fungi. In recent years, proliferation of new classes of drugs such as, and disability (Aravind *et al.,* 2002). allylamines (e.g terbinafine) and orally active triazoles (e.g. itraconazole), has been considered as the most effective in dermatophytosis therapy(Weitzman and Summerbell, 1995). Griseofulvin, the only antifungal drug which had been in use for many years for treatment of dermatophytoses (tinea capitis) is still the preferred drug, though it is reported that the fungus has already developed resistance to the said drug (Huang, 2004). The improved cure rate, reduced adverse effects, decreased drug interactions and lower cost of available topical agents make therapy with antifungal drugs a favourable choice to combat superficial fungal infections including dermatophytoses. In this context, the new antifungals of plant origin could be useful alternatives for the treatment of dermatophytoses, where a topical therapy is required. Keeping in view the reduced risk of side effects and lower cost, these natural compounds are preferred for human well being. Therefore, in recent years, there has been growing interest in search of suitable medicinal plants for skin ailments. The numerous ethnic tribes in India have developed their own herbal remedies for all their diseases including the skin ailments. Survey of ethno botanical literature in India, reveals *ca.* 269 plant species used by various ethnic groups for skin disorders in India (Jain, 1991).

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Table. 1: Ethnobotanical diversity of usage of plants (including skin diseases) included in the study.

Plants	Uses	Region	Reference
Caesalpinea bonducella	Intermittent Fever	Uttar Pradesh	Rajwar, 1983.
	Laxative	Maharashtra, Madhya Pradesh (MP), Andhra Pradesh (AP), Punjab, Haryana, Rajasthan, Gujarat.	Shah GL, 1984.
	Malaria	Do	Singh & Pandey, 1980
	Rheumatism	Sikkim, Bengal, B & O	Saxena & Dutta, 1975
	Skin diseases	Punjab, Haryana, Rajasthan, Gujarat	Singh & Pandey, 1980
Cassia occidentalis	Bone fracture	Kerala, Karnataka, Tamil Nadu, Goa	Apparanantham & Chelladurai, 1986
	Dysentery	Sikkim, Bengal, B&O	Tribedi *et al.*, 1985
	Eczema & ringworm	Maharashtra, MP, AP	Shah *et al*., 1983
	Skin diseases	Do	Malhotra & Moorthy, 1973
Cassia tora	Anthelmintic& antiseptic	Uttar Pradesh (UP)	Rajwar, 1983
	Cold	UP	Shah & Joshi, 1971
	Ringworm	Maharashtra, MP, AP	Bhalla *et al.*, 1982
	Scorpion bite	UP	Maheshwari *et al*., 1986
	Vermicide	Maharashtra, MP, AP	
Lawsonia inermis	Antifertility	Sikkim, Bengal, B & O	Sharma, 1981
	Headache, pain in muscles	Do	Jain & Tarafder, 1970
	Jaundice	Do	Gupta, 1981
Xanthium strumarium	Cancer wounds	Sikkim, Bengal, B & O	Pal & Jain, 1989
	Herpes & Malaria	Maharashtra, MP, AP	Puri, 1983
	Cures night blindness	Sikkim, Bengal, B & O	Tanaka, 1976
	Ringworm	UP	Maheshwari *et al*. 1987

Some of the dominant antidermatophytic plants traditionally used by the Adivasi tribes are species of *Ageratum, Aloe vera, Abrus, Acalypha, Aglaia, Andrographis, Azadirachta, Boswellia, Chenopodium, Cleome, Erythrina, Hypericum, Heliotropium, Limonia, Ocimum, Pongamia, Sesbania, Withania, Dryopteris, Cedrus, Centella asiatica, Butea* and a few more. In the present investigation five species *Caesalpinia bonducella* (L.) Flem., *Cassia tora* (L.), *Cassia occidentalis* (L.) (Caesalpiniaceae) *Lawsonia inermis* (L.) (Lythraceae), *Xanthium strumarium* (L.) (Asteraceae) which are extensively used by different tribes for skin ailments are studied (Table 1).

Cassia tora is used as a substitute for coffee by some groups and is believed to have naturant and anodyne action and is useful in skin diseases like ringworm and itch and psoriasis. There are reports on bioactivities such as antibacterial, antiplatelet aggregation, hepatoprotective; apart from many medicinal properties such as an antiseptic, antidiarrheal, antioxidant and antimutagen (Yen and Chung, 1999). Similarly there are reports also indicate that *Cassia* spp., particularly *Cassia alata* contain antimicrobial substances (Villasenor, 2002; Somchi, 2001). But, relatively little work has been done on the management of human pathogenic fungi using the *Cassia tora* despite its proven biological actions. Acharya and Chatterjee (1975) have reported the major antifungal component, chrysophanic acid-9-anthrone from defatted seeds of *C. tora*, said to be active against dermatophytes. There are also other reports on antifungal activities *C. tora* (Manas Dev Maji, 2005; Sushil Kumar Bagchi and Darokar, 1997). *Cassia occidentalis* is another important species in which antidermatophytic activity was established by Caceres *et al.*, 1993. Other known biological activities of *C. occidentalis* are: insecticidal, anthelmintic, antimalarial, antihepatotoxic, fertility regulating, antidiabetic, molluscicidal. *Xanthium strumarium* has again diverse medicinal uses.

The root is considered diuretic and the leaves taken internally and applied externally are given in cases of itch and other cutaneous diseases; while Gautam and Chavan, 2001; Gholamreza Amin *et al.,* 1999 reported antifungal activity of *X. strumarium*. *Lawsonia inermis* is also attributed with several traditional uses; whereas Aghel *et al.,* 2005; Krishnakishore and Pande, 2005; Shahidi , 2004 reported the antimicrobial activity.

In the present investigation basing on the available ethnobotanical reports, antidermatophytic activity of *Cassia occidentalis*, *Cassia tora*, *Lawsonia inermis*, *Xanthium strumarium* and *Caesalpinia bonducella* occurring in semi arid regions of North Karnataka is studied.

MATERIALS AND METHODS

Plant material

For the present investigation the leaves of *Cassia occidentalis* (*Senna occidentalis*), *Lawsonia inermis* (Henna), *Cassia tora*, *Xanthium strumarium* (cockle bur) and *Caesalpinia bonducella* (fever nut) growing in and around Gulbarga University, Gulbarga, Karnataka, India, were collected in June 2005. The voucher specimens of all the species bearing numbers HGUG-223, HGUG-517, HGUG-554, HGUG-66 and HGUG-208, respectively are deposited in herbarium of Gulbarga University, Gulbarga. The solvent extracts of leaves of all five species were obtained after subjecting to soxhlet extraction and were separately concentrated to dryness in a flash-evaporator under reduced pressure at 40^{o}C and preserved in refrigerator. Serial dilutions of the extracts were prepared (2500 to 40000 µg/ml) for bioassays.

Organisms

The isolates of dermatophytes viz., *Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum gypseum* and *Epidermophyton floccosum* used for

Table-2: Anti-dermatophytic activity of selected plants against skin pathogenic fungi (Agar dilution method).

Organism	Conc. µg/ml	Percent mycelial growth inhibition																										
		Cassia occidentalis					*Cassia tora*					*Lawsonia inermis*						*Xanthium indicum*						*Caesalpinia bonducella*			
		M	A	Ac	E	P	M	A	Ac	C	P	M	A	Ac	E	C	P	M	A	Ac	E	C	A	Ac	E	C	P
Trichophyton tonsurans	2500	41	35	55	61	58	82	82	85	88	82	58	70	55	58	85	47	70	53	41	55	82	17	35	26	82	11
	5000	70	70	100*	76	64	88	88	90	92	90	70	76	70	70	88	55	82	64	55	61	85	41	41	41	85	29
	10000	100*	100*	100	100*	100*	100	100*	100*	100*	100*	79	82	88	73	100*	73	100	91	76	85	100	44	64	55	100	55
	20000	100**	100**	100**	100**	100**	100	100	100	100	100	100*	100*	100*	100*	100	100*	100	100*	100*	100*	100*	85	100*	70	100	90
	40000	100	100	100	100	100	100*	100	100**	100	100	100**	100**	100**	100**	100**	100**	100	100	100	100	100	100*	100	100	100	100
Trichophyton mentagrophytes	2500	40	60	60	65	60	82	83	82	84	75	40	50	50	60	40	85	58	75	80	50	78	20	25	23	40	10
	5000	50	65	65	75	65	88	85	88	88	80	50	55	75	80	65	88	66	80	88	66	80	40	50	41	50	22
	10000	60	70	75	100*	100*	91	90	92	91	88	70	60	100*	100*	70	100	77	89	92	75	92	55	75	70	75	35
	20000	100*	100*	100*	100	100	100*	100*	100*	100*	100*	100*	100*	100	100	100*	100	100*	100*	100*	100*	100*	100*	100*	100***	100*	100**
	40000	100**	100**	100**	100**	100**	100	100	100	100	100	100**	100**	100**	100**	100**	100**	100**	100**	100**	100**	100**	100	100	100	100	100
Trichophyton rubrum	2500	0	20	20	20	20	75	80	72	88	75	10	0	26	0	10	20	61	78	89	72	75	80	75	50	30	0
	5000	20	30	30	30	30	80	95	85	95	82	33	10	33	47	13	40	75	86	92	85	88	100*	80	60	50	0
	10000	100*	40	50	100*	100*	90	100*	97	100*	100*	40	26	46	58	26	47	80	91	95	90	90	100	100*	100	60	100*
	20000	100	100*	100*	100	100	100*	100	100*	100	100	55	40	55	60	100*	55	100*	100*	100*	100*	100*	100	100	100***	100*	100
	40000	100**	100**	100**	100**	100**	100	100	100	100	100	70	47	100*	100*	100**	100*	100**	100**	100**	100**	100**	100**	100	100	100	100**
Microsporum gypseum	2500	0	20	0	0	10	75	81	89	88	72	10	30	30	60	60	20	30	22	25	20	20	60	40	0	20	20
	5000	50	100*	10	20	30	100*	100*	100*	100*	100*	40	40	40	70	70	40	42	40	30	55	46	75	50	80	50	40
	10000	100*	100	40	40	40	100	100	100	100	100	50	60	60	100*	100*	50	50	50	50	100*	100*	92	60	100***	100*	50
	20000	100**	100**	100*	100*	100*	100**	100	100	100	100	100*	100*	100*	100	100	100	100**	100*	100*	100	100	100*	100*	100	100	62
	40000	100	100	100	100	100	100	100	100**	100**	100**	100**	100	100	100	100**	100**	100**	100**	100**	100**	100**	100**	100	100	100**	100
Epidermophyton floccosum	2500	50	40	30	20	40	76	81	70	82	88	0	26	44	50	67	50	65	54	41	80	55	50	40	50	25	0
	5000	60	70	40	30	50	88	85	75	88	91	20	30	55	66	70	75	75	74	66	88	64	70	64	60	50	20
	10000	70	80	60	50	80	95	90	92	91	97	33	45	100*	100*	100*	100*	81	82	78	93	72	85	75	100*	55	100*
	20000	100*	100*	100*	100*	100*	100*	100*	100*	100*	100*	44	55	100	100	100	100	100*	100*	100*	100*	100*	100*	100*	100***	100*	100
	40000	100**	100**	100**	100**	100**	100	100	100	100	100	100*	100*	100**	100**	100**	100**	100**	100**	100**	100**	100**	100**	100**	100	100**	100**

M = methanol; A= alcohol; Ac = acetone; E = ethyl acetate; C = chloroform; P = petroleum ether
*= fungistatic; **= fungicidal; ***= fungistatic and fungicidal.

the present study were obtained from M. R. Medical College, Gulbarga and Vijayanagara Institute of Medical Sciences, Bellary, Karnataka. The organisms were maintained on Sabouraud Dextrose Agar (SDA) medium supplemented with chloramphenicol (50 mg/l) and streptomycin sulfate (500 mg/ml) and sub cultured on Potato Dextrose Agar (PDA) every 15 days to prevent pleomorphic transformations..

In vitro antifungal assay

Solid Agar Medium

In vitro antifungal activity of all the solvent extracts was done by poisoned food technique (Grover and Moore, 1962). Each experiment was done in triplicate. The inhibition of fungal growth was calculated by the formula:

$I = ((CF_c - CF_t) / CF_c)100$

Where I= percentage of inhibition: CF_c=growth in the control: and CF_t=growth in the treatment (Albuquerque *et al.*, 2006).

Broth Agar medium

One ml of sterile liquid Sabouraud medium was added to 11 sterile capped tubes, 1 ml of each solvent extract suspension was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube 9. 1 ml was discarded from tube 9. Fifty µl of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (without inoculum and drug) and inoculum control (no drug) tubes were prepared. The tubes were incubated at 30° C for 96 h. The fungal growth in each tube was evaluated visually depending upon the turbidity in the tubes.

MIC was defined as the drug concentration at which the turbidity of the medium remains same as the medium control. 10µl aliquot of cell suspension from the tube without observed growth of dermatophyte was inoculated on to Sabouraud dextrose agar, and MFC of test compound was determined as the lowest concentration of the agent at which no colonies were seen after 4 days at 30°C. Triplicate sets were maintained for each experiment.

RESULTS

In agar dilution method (Table-1), of the various solvent extracts, alcohol and acetone extracts of *C. occidentalis* inhibited *M. gypseum* and *T. tonsurans* at 5000 µg/ml each. At 2500 µg/ml, the percent inhibition was least for all fungi. Most susceptible organism was *M. gypseum* and resistant was *T. tonsurans*. Fungicidal activity was observed at 40000µg/ml for *T. mentagrophytes*, *T. rubrum* and *E. floccosum*, but the extract was fungistatic at varying concentrations for different organisms.

C. tora showed good antifungal activity against all the dermatophytes. Methanol, alcohol, acetone, chloroform and pet. ether extracts inhibited *M. gypseum* at 5000 µg/ml which was found susceptible to the extract. MIC's of all the solvent extracts were found to be 10000 µg/ml for *T. tonsurans*, 20000 µg/ml for *T. mentagrophytes*. *T. rubrum* was inhibited by alcohol and chloroform extracts at 10000 µg/ml, while remaining solvent extracts inhibited *T. rubrum* at 20000 µg/ml. Fungistatic and fungicidal action of *C. tora* with all solvent extracts differed with the organisms tested (Table- 1). Only *T. mentagrophytes* exhibited resistence.

Acetone, ethyl acetate, chloroform and pet. ether extracts of *L. inermis* retarded 100% growth of *T. rubrum* at 40000 µg/ml. *T. rubrum* exhibited susceptibility and *T. tonsurans* was found resistant, whereas all other solvent extracts retarded *ca.* 50% growth of *T. tonsurans*.

Xanthium strumarium extracted with methanol, alcohol, acetone, ethyl acetate and chloroform, potentially inhibited 100% growth of all organisms at 40000 µg/ml (fungicidal) and were found fungistatic at 20000 µg/ml for all fungi tested. Resistance was exhibited by *M. gypseum*, which grow at 10000 µg/ml. Susceptible organism was *T. rubrum*, which was inhibited by all the solvent extracts of *X. strumarium* at 10000 µg/ml (> 80% inhibition).

Of all the solvent extracts, ethyl acetate extract of *C. bonducella* proved to be the best inhibitor where 100% growth of all the test dermatophytes was retarded even at 5000 µg/ml. All the solvent extracts of *C. bonducella* were able to check the growth of fungi with varied MIC and MFC. Interestingly the ethyl acetate extract of *C. bonducella* was found to be fungicidal for all the organisms except *T. tonsurans* at 10000 µg/ml. This action was observed for 30 days and above incubation period, where no further growth was seen (Table-1).

The effect of various solvent extracts of *C. occidentalis*, *C. tora*, *L. inermis*, *X. strumarium* and *C. bonducella* were evaluated in broth medium (Table 2). Though, the results did not differ from agar dilution, the MIC varied to some extent (Table-2). MIC of *C. occidentalis*, *C. tora*, *L. inermis*, and *C. bonducella* was 10000 µg/ml and 20000 µg/ml for *X. strumarium*. MFC of *C. occidentalis*, *C. tora*, *L. inermis*, *X. strumarium* were more than 40000 µg/ml with all solvent extracts. However, ethyl acetate extract of *C. bonducella* exhibited no turbidity at 5000 µg/ml for all the organisms. Again *T. tonsurans* was found to be resistant while the remaining fungi exhibited susceptibility. The 100% growth of all the test fungi was retarded at 10000 µg/ml up to 30 days and above.

DISCUSSION

The emergence of antifungal resistant strains of various fungi such as, *Candida, Cryptococcus neoformans* has prompted researchers to develop new strategies for fighting fungal infection (Patterson *et al.*, 1999) which may be less toxic to humans. Besides these factors, the acquired resistance to certain antifungals has also occurred (Chee-Leok, 1994). Hence, search for new, cheaper antimycotics from natural sources is an urgent need. The data obtained in the present investigation proves the antifungal activity of the above species with varying MICs and MFCs. The response of dermatophytes to treatment with various plants extracts varied from organism to organism; nevertheless it was shown to be dose dependent as greater inhibition of growth was

observed as the concentrations of the extracts increased. The best MIC was obtained on dermatophytes with remarkable activity for ethyl acetate leaf extracts of *C. bonducella*. This is supported by the work of Farukh and Iqbal, 2003, where they revealed that, of the plants tested against bacteristatic fungi, a broad spectrum antimicrobial activity was detected among crude extracts of many plants including *C. bonducella* (seeds). The reports of Caceres *et al.*1991 are also in agreement with our findings where *C. occidentalis* exhibited antifungal activity against some other organisms like *E. floccosum, M. canis, T. rubrum, T. mentgrophytes* and *M. gypseum.*

In the present investigation, *L. inermis* exhibited antifungal activity against all the fungi as was also shown by Singh and Pandey, 1989, who screened barks of 30 plants species against *M. gypseum* and *T. mentagrophytes*, to find only *Lawsonia inermis* exhibiting absolute toxicity. The present findings demonstrated that various solvent leaf extracts of *C. tora. C. occidentalis, L. inermis, X. strumarium* and *C. bonducella* have concentration dependent antifungal activity against all the test differences in the antidermatophytic activity between the solvent extracts of *C. bonducella* might be due to the difference in the concentration of the phytocompounds of various secondary metabolites present in the extract as well as the extracting ability of the solvents. These could be the components that make *C. bonducella* free from herbivore attack and distasteful to some insects. Among the plants screened for antidermatophytic activity, ethyl acetate leaf extract of *C. bonducella* exhibited good antifungal activity. It was also observed that some solvent extracts (methanol, alcohol, acetone, ethyl acetate, pet. ether) of few plants (*C. tora, L. inermis*) could not inhibit completely or even 50% growth of the test dermatophytes.

This could suggest that probably certain phytochemicals exhibit their antimicrobial action only with other phytoconstituents in a synergistic way: in other words, used in the form of essential oils or decocts. As all the plants investigated in the present work are common in India, the recovery of their compounds is high and thus, these species may be exploited as potent herbal chemotherapeutics for dermatomycosis. Moreover, the antifungal action of the plants on the tested fungi responsible for the most mycoses, confirms their therapeutic potency and this appraisal may authenticate their use in traditional medicine by different tribes for certain skin infectious diseases. In view of the potentiality of the species it is further recommended for isolation, identification, and toxicity studies on the antifungal principles and their fractions for establishing cheaper, affordable and acceptable herbal products for future use.

Table - 3: Anti-dermatophytic activity of selected plants against skin pathogenic fungi (Broth dilution method).

Organism	Conc.	*Cassia occidentalis*					*Cassia tora*					*Lawsonia inermis*					
	µg/ml	M	A	Ac	E	P	M	A	Ac	C	P	M	A	Ac	E	C	P
Trichophyton tonsurans	2500	*	*	*	*	*	**	**	**	**	**	*	**	*	*	**	*
	5000	**	**	**	**	*	**	**	**	**	**	**	**	*	**	**	**
	10000	**	**	**	***	**	***	***	***	***	***	**	**	**	**	***	**
	20000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	40000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Trichophyton mentagrophytes	2500	*	*	*	*	*	**	*	**	**	**	*	*	*	*	*	**
	5000	*	*	*	*	*	**	*	**	**	**	*	*	**	**	*	**
	10000	**	*	*	**	***	**	**	**	**	**	**	*	***	***	**	***
	20000	***	**	**	***	***	***	***	***	***	***	***	**	***	***	***	***
	40000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Trichophyton rubrum	2500	-	*	*	*	*	**	**	**	**	**	*	-	*	-	*	*
	5000	*	*	*	*	*	**	**	**	**	**	*	*	*	*	*	*
	10000	**	*	*	***	***	**	***	**	***	***	*	*	*	*	*	*
	20000	***	***	***	***	***	***	***	***	***	***	*	*	*	*	***	*
	40000	***	***	***	***	***	***	***	***	***	***	**	*	***	***	***	***
Microsporum gypseum	2500	-	*	-	-	*	**	**	**	**	*	*	*	*	*	*	*
	5000	*	***	*	*	*	***	***	***	***	***	*	*	*	**	**	*
	10000	***	***	*	*	*	***	***	***	***	***	*	*	*	***	***	*
	20000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	40000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Epidermophyton floccosum	2500	*	*	*	*	*	**	**	**	**	**	-	*	*	*	*	*
	5000	*	*	*	*	*	**	**	**	**	**	*	*	*	*	*	**
	10000	**	**	*	*	**	**	**	**	**	**	*	*	***	***	***	***
	20000	***	***	***	***	***	***	***	***	***	***	*	*	***	***	***	***
	40000	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***

M = methanol; A= alcohol; Ac = acetone; E = ethyl acetate; C = chloroform; P = petroleum ether

*= fungistatic; **= fungicidal; ***= fungistatic and fungicidal.

Xanthium indicum					*Caesalpinia bonducella*				
M	A	Ac	E	C	A	Ac	E	C	P
**	*	*	*	**	*	*	*	**	*
**	*	*	*	**	*	*	*	**	*
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