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Research Article

IN-VITRO ANTIFUNGAL EFFICACY STUDY OF PLANT LEAF EXTRACTS AGAINST THREE *DERMATOPHYTES*

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ABSTRACT
The study evaluated in-vitro antifungal activity of *Azadirachta indica L., Cassia tora L.* and *Lawsonia inermis L.* against three human pathogenic fungi, leaves of these plants were taken and extraction were made in different solvents like water, ethanol, chloroform, benzene and petroleum ether and were tested against *Trichophyton rubrum* MTCC No. 296, *Trichophyton mentagrophytes* MTCC No. 8476 and *Epidermophyton floccosum MTCC No*. 613. The preliminary screening by Food poisoning method revealed that the chloroform extract of the three plants showed excellent antimycotic activity against *Trichophyton mentagrophytes* and *Trichophyton rubrum* .Benzene extract of *Cassia tora* showed zone of 41mm against *Epidermatophyton floccosum.* The antibiogram profile indicated that Terbinafine has the maximum activity as is shown by its zone of inhibition viz., 55mm, 45mm and 40 mm for *Trichophyton mentagrophytes, Trichophyton rubrum* and *Epidermatophyton floccosum* respectively. Fluconazole was found to be ineffective against the three test fungi. Lowest MIC value in case of *Trichophyton mentagrophytes* and *Trichophyton rubrum is* obtained from chloroform extract of *Cassia tora* & ether extract of *Lawsonia inermis* i.e.13.15µg/ml. Lowest MIC in case of *Epidermophyton floccosum* is obtained from ethanolic extract of *Azadirachta indica* i.e. 6.575µg/ml.

Key Words: *Antibiogram, Leaf Extracts, MIC*

INTRODUCTION
Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses (Valeria *et al.,* 1996 and Amer *et al.,* 2006). Dermatophytoses are one of the most frequent skin diseases of human (Tsang *et al.,* 1996). The disease is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are *Epidermophyton*, *Trichophyton* and *Microsporum*. A few antifungal compounds are available and licensed for use in human being treatment. The use of systemic drugs is limited to treat man due to their high toxicity and problems of residues in products intended for human consumption (Araujo *et al.,* 2009). Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option includes antifungal agents (Aly, 1997 and Agwa *et al.,* 2000), but recently the use of some natural plant products has been emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late 19th century. These natural plants involve garlic, lemon grass, datura, acacia, a triplex, ginger, black seed, neem, basil, eucalyptus, alfalfa and basil (Aly *et al.,* 2000 and Aly and Bafiel, 2008). They are safe to human and the ecosystem than the chemical antifungal compounds, and can easily be used by the public (Shelef *et al.,* 1980 and Shelef, 1983). Plant extract has been used traditionally to treat a number of infectious diseases caused by bacteria and fungi (Soylu *et al.,* 2005; Yoshida *et al.,* 2005; Nejad and Deokule, 2009). A number of reports are available in vitro and in vivo efficacy of plant extract against human pathogens causing fungal infections (Ali, 1999 and Natarajan *et al.,* 2003). Venugopal and Venugopal (1995) reported the activity of plant extracts against 88 clinical isolates of dermatophytes which includes *Trichophyton rubrum, T*

mentagraphytes, and Epidermophyton floccosum by agar dilution technique. While Vlietinck *et al.,* (1995) reported clinical findings of Rwandese medicinal plants (267 plants extracts) used by traditional healers to treat microbial infections and found 60% of these extracts were active against dermatophytes. It has been reported that Neem (*Azadirachta indica L.*) (Family: Meliaceae) is used in leprosy, skin disorders, hair disorders, eye disorders, piles, urinary disorders. Leaf extract is antihelmenthic. Mehendi (*Lawsonia inermis L.*) (Family: Lythraceae) has antihelmenthic, antifungal, anti-inflammatory activities. Leaves are prophylactic against skin diseases. Cassia (*Cassia tora L.*) (Family**:** Leguminosae) is used in treating constipation, common cold, fevers, intestinal disorders, skin disorders etc. Cassia leaves possess antimicrobial properties (Kirtikar and Basu, 1935).

Keeping this in view, the present study was designed to evaluate the in vitro anti-dermatophytic activity of leaf extracts of *Azadirachta indica L., Cassia tora L. and Lawsonia inermis L.* and compared with recently used antifungal antibiotics.

MATERIALS AND METHODS

Plant Material

Basing upon the local availability and medicinal values, leaves of three plants were taken i.e. *Azadirachta indica L., Cassia tora L., and Lawsonia inermis L.* were collected. The leaves were shade dried and brought to the Department of Microbiology,OUAT, Bhubaneswar.

Fungal Cultures

Three fungal pathogen used were procured from Institute of Microbial Type Culture Collection, Chandigarh (IMTECH) viz., *Trichophyton mentagrophytes* MTCC No.8476 , *Trichophyton rubrum* MTCC No.296 and *Epidermophyton floccosum* MTCC No.613 , and are maintained in Sabouraud Dextrose Agar.

Preparation of Leaf Extract

Leaves were powdered after drying of leaves at 37ºC for 3 to 5 days. Exposure to sunlight was avoided to prevent the loss of active compounds. Extraction of plant products were prepared by A.O.A.C. Method (AOAC, 1980) in different solvents like water, ethanol, chloroform, benzene and petroleum ether. For extraction 300 grams each of the powdered leaves were taken with 1000ml of different solvents (30%).

Antifungal Activity

Antifungal activity of blaw-leaves was determined using the food poisoning method. 5ml of leaf extract was added to 95 ml of SDA media. The plates were left for 15mins to solidify. Then 4mm diameter of fungal colony punched with corn borer was placed onto plates containing media with leaf extract in aseptic condition. Plates were made in duplicate and incubated for 3-5 days at 28ºC. Reading were taken after 3-5 days by measuring zone of inhibition and values less than control were considered as active extracts against fungus.

Comparison of the Efficacy of Selected Antibiotics and Plant Extracts

The relative efficacy of some commonly used antifungal antibiotics was compared with plant extract discs by employing the filter paper Disc diffusion method (Loo *et al.,* 1945). The antifungal antibiotics used were Terbinafine, Clotrimazole, Fluconazole, Ketoconazole, Miconazole and Griseofulvin.

Estimation of Minimum Inhibitory Concentration (MIC)

MIC of the effective plant extracts were determined by Tube dilution Method (Cruicshank *et al*., 1975).

Priliminary Phytochemical Analysis

Qualitative phytochemical test for the identification of alkaloids, flavonoids, steroids, terpenoids, carbohydrates, glycosides, amino acids and tannins were carried out for extracts by the method described by Harborne (1998) and Sazada *et al.,* (2009). These tests were carried out in triplicate using various concentrations of samples.

RESULTS

Table 1: In-vitro antifungal activity of plant extracts against three test fungi

Plant taken	Test fungi	C	Water	C	Alcohol	C	Chloroform	C	Benzene	C	Ether
							Diameter of fungal growth (mm)				
	T.m	18	15 (16.66%)	-	-	19	- (100%)	12	11(8.33%)	18	13(33.3%)
	T.r	16	15(6.25%)	-	-	<10	- (100%)	12	12(0%)	14	12(14.3%)
Lawsonia inermis	E.f	16	11(31.25%)	-	-	21	18(14.2%)	30	15(50%)	30	27(10%)
	T.m	18	15(16.66%)	-	-	19	- (100%)	12	9(25%)	18	12(33.3%)
	T.r	16	15(6.25%)	-	-	<10	- (100%)	12	9(25%)	14	14(0%)
Cassia tora	E.f	16	15(6.25%)	-	-	21	24(14.2%)	30	41(36.6%)	30	10(66.6%)
	T.m	18	18(0%)	-	-	19	- (100%)	12	12(0%)	18	13(27.7%)
	T.r	16	18(12.5%)	-	-	<10	- (100%)	12	10(16.6%)	14	11(21.4%)
Azadiracht -a indica	E.f	16	13(18.75%)	-	6(100%)	21	23(9.5%)	30	25(16.6%)	30	15(50%)

( ): % of inhibition/stimulation. T.m – Trichophyton mentagrophytes, T.r –Trichophyton rubrum, E.f –Epidermophyton floccosum.

Table 2: Antifungal activity of selected antibiotics against fungi

Antibiotics	Disc Content	Diameter of zone of inhibition (mm)		
		T.mentagrophytes	***T. rubrum***	***E. floccosum***
Ter	30µg	55	45	40
Cc	10µg	31	29	30
F1	25µg	-	-	-
Kt	15µg	26	25	26
Mi	10µg	**15**	**15**	**16**
Gf	25µg	12	**10**	**-**

Cc-Clotrimazole, F1-Fluconazole, Kt-Ketoconazole, Mi- Micoconazole, Gf- Griseofulvin, Ter- Terbinafine.

Table 3: MIC results of three plant extracts against test fungi

Plants		MIC in µg/ml			
		Ethanol extract	**Chloroform extract**	**Benzene extract**	**Ether extract**
Lawsonia inermis	*T.m*	-	13.15	-	-
	T.r	-	13.15	-	-
	E.f	-	-	26.3	-
Cassia tora	*T.m*	-	13.15	-	26.3
	T.r	-	6.575	-	-
	E.f	-	-	-	13.15
Azadirachta indica	*T.m*	-	6.575	-	52.6
	T.r	-	13.15	-	-
	E.f	13.15	-	-	13.15

Not done T.m – Trichophyton mentagrophytes, T.r –Trichophyton rubrum, E.f –Epidermophyton floccosum.

Table 4: Phytochemical screening results

Phytochemical screening		Plants			
		***Lawsonia inermis*(chl)**	***Cassia tora* (chl)**	***Azadirachta indica***	
				(chl)	**(etnl)**
Alkaloid	**Dragendroff's test**	+	_	+	_
	Mayer's test	-	_	_	_
	Wagner's test	-	+	_	_
	Hager's test	_	+	_	_
Amino acids (Millon's test)		_	_	_	_
Carbohydrate (Molisch test)		_	+	_	_
Flavonoids (Alkaline reagent test)		+	+	+	_
Tannins (Ferric chloride test)		_	_	_	_
Anthraquinone glycosides(Borntrager's test)		_	+	+	_
Steroids and terpenoids (Libermann Burchard's test)		_	+(triterpenes)	+(steroids)	_

+: positive - : negative chl- chloroform extracts, etnl-ethanol extracts.

DISCUSSION

The leaf extracts of the three plants used in this study were preliminary screened against the test fungi by food poisoning method. The relative efficacy of the commonly used antifungal antibiotics was compared with plant extract discs. The MIC value of those extracts which gave positive results during preliminary screening were determined by Tube dilution method.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The chloroform extract of *Lawsonia inermis, Azadirachta indica* and *Cassia tora* showed best antifungal activity against *Trichophyton rubrum* and *Trichophyton mentagrophytes*. This result coincides with the reports of Onaolapo *et al.*, (1993) who had also reported the susceptibility of *Trichophyton mentagrophytes* to chloroform extract of *Cassia tora* and *Cassia occidentalis*. The ethanolic extract of *Azadirachta indica* showed good antifungal activity against *Epidermophyton floccosum.* The chloroform extract of *Azadirachta indica* shows MIC value 6.575µg/ml against *Trichophyton mentagrophytes* and *Cassia tora* shows MIC value 6.575µg/ml against *Trichophyton rubrum.* The chloroform extract of *Lawsonia inermis* shows MIC value of 13.15µg/ml against *Trichophyton rubrum* and *Trichophyton mentagrophytes*. The ethanolic extract of *Azadirachta indica* shows MIC value of 13.15µg/ml against *E.floccosum*. Terbinafine has maximum activity against the test fungi, followed by Clotrimazole and Ketoconazole. Fluconazole is ineffective towards the test fungi. The phytochemical analysis suggests that the presence of flavonoids, anthraquinone glycosides, steroids & terpenoids. These active compounds present in different extracts of three plants may be responsible for inhibition of test fungi.

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