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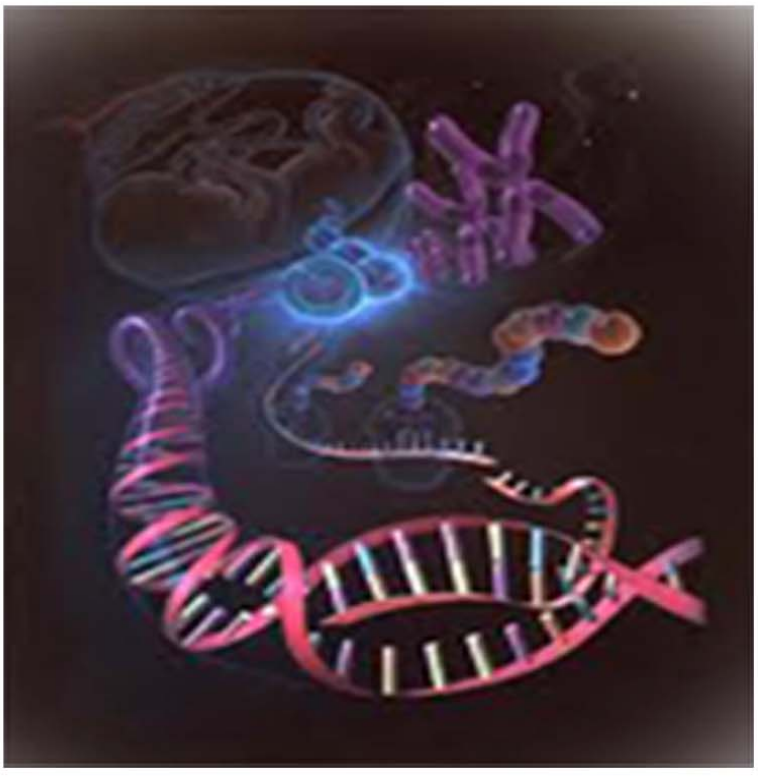

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Research Paper

ANTIFUNGAL SCREENING OF *CURCUMA LONGA* AND *CASSIA TORA* ON DERMATOPHYTES

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This study evaluated antifungal screening of *Curcuma longa L* and *Cassia tora L.* against two dermatophytes, rhizome and leaf of these plants were taken and extraction were made in different solvents like water, petroleum ether, chloroform and ethanol were tested against *Trichophyton mentagrophytes* MTCC No. 8476 and *Epidermophyton floccosum MTCC No*. 613. The preliminary screening by Poison Food Technique revealed that the ethanol extract of the *Curcuma longa* rhizome and chloroform extract of *Cassia tora* leaf showed excellent antimycotic activity against *Trichophyton mentagrophytes* and *Epidermophyton floccosum.* Petroleum ether extract of *Cassia tora* showed zone of 10 mm and 20 mm against *Trichophyton mentagrophytes* and *Epidermophyton floccosum.* The antibiogram profile indicated that Terbinafine has the maximum activity as is shown by its zone of inhibition viz., 40 mm and 32 mm for *Trichophyton mentagrophytes* and *Epidermophyton floccosum,* respectively. Fluconazole was found to be ineffective against the two-test fungi. Lowest MIC in case of ethanolic extract of *Curcuma longa* against *Trichophyton mentagrophytes* and *Epidermophyton floccosum* is 6.575 µg/ml and the chloroform extract of *Cassia tora* against *Trichophyton mentagrophytes* is 13.15 µg/ml.

Keywords: Antibiogram, Leaf extract, MIC, Rhizome extract

INTRODUCTION

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases (Sher, 2009). Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Recently, some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as antimicrobials such plant products would be biodegradable and safe to human health (Kumar *et al*., 2008; Wang *et al*., 2010). The acceptance of traditional medicine as an alternative form of

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health care hassled researchers to further investigate antimicrobial activity of medicinal plant. The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists' worldwide (Sofowora, 1982). World Health Organization corroborated this in its quest to bring primary health care to the people. The plant kingdom has for long time served prolific source of helpful drugs, food, additives, flavoring agents, colorants binders and lubricants, etc., as a matter of facts, it was estimated that about 25% of all prescribed medicines today are substances derived from plants (Bello *et al*., 2005).

For control of microbial infections and diseases, various synthetic drugs and chemical formulations have been used. But due to their indiscriminate use, microbes have developed wide resistance against these synthetic drugs such as broad-spectrum antibiotics. This resistance was developed after induction of new enzyme system in microbes which not only simplify drugs but also enhance drug threshold level in microbes. Therefore, to combat the problem of microbial infection and drug resistance new alternative of synthetic drugs have been explored, though antimicrobial activities of so many natural products have not been explored (Upadhyay *et al*., 2010). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed. It is on this basis that researchers keep on studying on medicinal plants in order to develop the best medicines for physiological uses (Usman and Osuji, 2007). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies (Okoro *et al*., 2010). *C. longa L*., botanically related to ginger belongs to the Zingiberaceae family is a perennial plant having ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in color. Turmeric rhizome is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant (Majumdar *et al*., 2000), antiinflammatory (Manimegalai *et al*., 2011), wound healing (Menon and Sudheer, 2007), anticancer (Nair *et al.,* 2005) and antibacterial activity (Pathak *et al*., 2010). It has been reported that 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 antifungal screening of rhizome extract of *Curcuma longa L* and leaf extract of *Cassia tora L.* and compared with recently used antifungal antibiotics, so that plant extracts can be used against skin aliments with higher efficacy and less side-effects.

MATERIALS AND METHODS

Plant material: Basing upon the local availability and medicinal values, leaf and rhizome of two plants were taken, i.e., *Curcuma longa L* and

Cassia tora L., were collected. The leaves and rhizomes were shade dried and brought to the Department of Microbiology, IGIPS, Bhubaneswar.

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

Preparation of leaf and rhizome extract: Leaves and rhizomes were powdered after drying 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 AOAC Method (AOAC, 1980) in different solvents like water, ethanol, chloroform and petroleum ether. For extraction 300 g each of the powdered leaves and rhizomes were taken with 1000 ml of different solvents (30%) separately.

Antifungal activity: Antifungal activity of blaw-leaves and rhizomes was determined using the Poison Food Technique. 5 ml of leaf extract was added to 95 ml of SDA media. The plates were left for 15 min to solidify. Then 4 mm diameter of Fungal colony punched with cork 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 and Griseofulvin.

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

Preliminary 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 Trease and Evans (1989). These tests were carried out in triplicate using various concentration of samples.

RESULTS AND DISCUSSION

The leaf and rhizome extract of the two 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 (i.e. >50%) 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 ethanol extract of *Curcuma longa* showed best antifungal activity (100% inhibition) against *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Table 1). This result coincides with the reports of Wuthi-udomlert *et al.* (2000), who had also reported the susceptibility of 29 clinical strains of dermatophytes by agar disc diffusion method to

Table 1: Screening for Antifungal Activity of Plant Extracts Against Two Test Fungi

Plant taken	Test fungi	Diameter of fungal growth (mm)							
		C	Water	C	Alcohol	C	Chloroform	C	Ether
Curcuma longa	T.m	20	15(25%)	26	0(100%)	19	6(68.4%)	30	25(16.6%)
	E.f	16	11(31.25%)	23	0(100%)	21	18(14.2%)	30	28(6.66%)
Cassia tora	T.m	20	17(15%)	26	9(65.4%)	19	-(100%)	30	10(66.6%)
	E.f	16	15(6.25%)	23	11(52.2%)	21	24(14.2%)	30	20(33.3%)

Note: ( ): % of inhibition. *T.m – Trichophyton mentagrophytes, E.f – Epidermophyton floccosum.*

ethanolic extract of *Curcuma longa* rhizome. Kumar *et al.* (2011), also reported the strong antifungal activity of ethanolic extract of *Curcuma longa* against 6 *Candida* sps. The chloroform extract of *Cassia tora* also showed best result against *Trichophyton mentagrophytes.* This result coincides with the reports of Onaolapo, Rai and Sokomba (1993), who reported the antimicrobial activities of the water, methanol, chloroform and ethanol extracts of *Cassia tora* and *C. occidentalis on Candida albicans, T. mentagrophytes ,Pseudomonas* spps., *E. coli*, etc. using both cup-plate and disc diffusion method. The ethanolic extract of *Curcuma longa* showed MIC value of 6.575 µg/ml against *Trichophyton mentagrophytes and Epidermophyton floccosum* (Table 3). The chloroform extract of *Cassia tora* showed MIC value of 13.15 µg/ml against *Trichophyton mentagrophytes (*Table 3). Terbinafine has maximum activity against the test fungi, followed by Clotrimazole and Ketoconazole. Fluconazole is ineffective towards the test fungi (Table 2). The phytochemical analysis suggests that the presence of flavonoids, cardiac glycosides and phenol in ethanolic extract of *Curcuma longa* and alkaloids, flavonoids, anthraquinone glycosides and terpenoids in the chloroform extract of *Cassia tora* (Table 4). These active compounds present in different extract of two plants may be responsible for inhibition of test fungi.

Table 2: Antifungal Activity of Selected Antibiotics Against Fungi

Antibiotics	Disc Content	Diameter of zone of inhibition (mm)	
		T. mentagrophytes	*E. floccosum*
Ter	30 µg	40	32
Cc	10 µg	31	30
F1	25 µg	-	-
Kt	15 µg	20	24
Gf	25 µg	10	-

Note: Ter- Terbinafine, Cc-Clotrimazole, F1-Fluconazole, Kt-Ketoconazole, Gf- Griseofulvin.

Table 3: MIC Results of Two Plant Extracts Against Test Fungi

Plants		MIC in μg/ml		
		Ethanol Extract	Chloroform Extract	Ether Extract
Curcuma longa	*T.m*	6.575	26.3	-
	E.f	6.575	-	-
Cassia tora	*T.m*	13.15	13.15	26.3
	E.f	52.6	-	-

Note: - not done.

Table 4: Phytochemical Screening Results

Phytochemical Screening		Plants Extracts	
		Curcuma longa (etnl)	*Cassia tora* (chl)
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)		–	+
Cardiac glycosides(Keller-Kiliani test)		+	_
Steroids and terpenoids (Libermann Burchard's test)		_	+(triterpenes)
Phenols (neutral ferric chloride solution)		+	–

Note: + present; – absent. chl - chloroform extracts, etnl - ethanol extracts.

CONCLUSION

The ethanolic extract of *Curcuma longa* showed excellent antifungal activity against *Trichophyton mentagrophytes* and *Epidermophyton floccosum* and chloroform extract of *Cassia tora* also gave best result against *Trichophyton mentagrophytes.* The aqueous, chloroform extract of *Curcuma longa* against *T. mentagrophytes* and *E. floccosum* and ether and ethanol extract of *Cassia tora* against *T.mentagrophytes* and *E. floccosum* showed moderate activity. The active constituents of the extracts can be found out for development of newer antifungal drugs.

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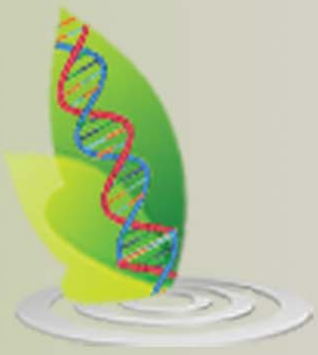

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