



ANTIFUNGAL PROPERTIES OF WEED PLANT (*C.TORA*) ON DERMATOPHYTOSIS

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ABSTRACT

Dermatophytosis or ringworm is a clinical condition caused by fungal infection of the skin in humans, pets and domesticated animals. The dermatophytes are groups of closely related filamentous fungi that infect only superficial keratinized tissues- the skin, hairs and nails. They cause a variety of clinical conditions, collectively known as dermatophytes, popularly called tinea or ring worm. Dermatophytes have been classified into three genera-Trichophyton, microsporum and epidermophyton. All are known to cause infection in man and animals¹. The objective of this current study is to evaluate the effects of *Cassia tora* extracts on the growth of ring worm fungi in vitro. Crude extracts of lipid and alkaloids compounds from leaves of *Cassia tora* with the help of petroleum ether and methanol were investigated for their antifungal activities on ring worm or dermatophytes basically trichophyton and epidermophyton. Both the extracts of lipids and alkaloids have given the results on the basis of well diffusion method. In this experiment we observed that alkaloid compounds are more effective than lipid compounds. These types of fungus infect only keratinized tissues, so protein extract may not be effective for them. Five different fungal growth sample collected from two patients (P1 & P2) has shown different size of zone of inhibition in different concentration of alkaloid and lipid compounds (100%, 75%, 50% and 25%). From P1 we got four different fungal colonies (1, 2, 3 and 4) and from P2, only single colony obtained. In the test of alkaloids, in P1, colony 1 did not give any result while colony 2 has shown 9 mm, 7 mm, 6 mm and 4 mm zone of inhibition in 100%, 75%, 50% and 25% conc. respectively. Same like colony 3 and 4 also shown good result, in colony 3 we observed 6 mm, 5 mm and 3-3 mm and in colony 4, 6 mm, 5 mm, 5 mm and 3 mm in 100%, 75%, 50% and 25% respectively. But in P-2, colonies did not give any results. In the test of lipid compounds, in P1, colony 1 has shown zone of inhibition of 8 mm on 100% conc. lipid compounds and on 50% and 25% shown 4mm, while 75% give 5 mm. Same like this, colony 2 shown the zone of inhibition of 11 mm, 10 mm, 5 mm on 100%, 75%, 50% and 25%, respectively. And colony 3 & 4 did not give any result. In P2, colony has shown 4 mm and 3 mm in 100% and 75% respectively, but 50% and 25% conc. did not given any results. Effectiveness of the plant extract can be concluded as -

Alkaloids > Lipids

Key words: Dermatophytes, *Cassia tora*, Anthrophilic, Zoophilic, Geophilic, Chitin.

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INTRODUCTION

Cassia Tora L., (*Cassia obtusifolia* L.), caesalpiaceae, is a wild crop and grows in most parts of India as a weed, extensively used in traditional medicine in tropical and warm subtropical countries. *Cassia tora* commonly found in waste grounds and secondary forest. Decoctions of parts of *Cassia tora* are used as an analgesic, anticonvulsant, antipyretic, antifungal, antihelminth, diuretic, expectorant, laxative, purgative, treatment of glaucoma and hypertension, treatment of skin disease, ringworm and itch¹. According to ayurveda the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiogenic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders³. Chemical components of *Cassia tora* are anthraquinones, chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides. Naphthopyrones, rubrofusarin, norrubrofusarin, rubrofusarin, entriobioside. Toralactone, torachryson. Roots contain², 3,5-trihydroxy-6-7-dimethoxy-2-methylanthroquinone and beta-sitosterol. While seeds contain naphtho-alpha-pyrone, toralactone, chrysophanol, physcion, emodin, rubrofusarin, chrysophonic acid-9-anthrone. Emodin, tricontan-1-ol, stigmasterol, beta-sitosterol-beta-D glucoside, ferulic, palmitic, stearic, succinic and d-tartaric acids, uridine, quercitrin and isoquercitrin are isolated from leaves^{4,5}. Antibacterial⁵, anti-platelet aggregation, hepatoprotective, cAMP-phosphodiesterase inhibitory activities, antifungal, antiyeast, anti-inflammatory, estrogenic and antiestrogenic, hypolipidemic, antimutagenic, antioxidant activities have been evaluated³.

The dermatophytes are a group of closely related filamentous fungi that infect only superficial keratinized tissues- the skin, hair and nails. They cause a variety of clinical conditions, collectively known as dermatophytes and popularly called tinea or ringworm¹. A large number of fungi, including yeasts, dermatophytes and other moulds, grow on human skin, hair and nails. The term 'keratinolytic' is used for fungi exhibiting the enzymatic ability to attack and utilize keratin⁶. Therefore the objective of this work was to explore the antifungal properties of *Cassia tora* leaves.

EXPERIMENTAL

Collection and extraction of plant material

The leaves of *Cassia tora* plants were collected from Kolar area of the Bhopal city, Bhopal. The leaves were washed and shade dried. The leaves were grounded into powder form using the grinder². Powder was first defatted with petroleum ether and then extracted with methanol which is further evaporated to dryness to obtain alkaloid extract. Extract were

obtained in powder form (300 mg lipid and alkaloid compound was obtained) by maceration for 5 to 7 days³.

Sampling of fungal disease

Sampling was done by swabbing process from two different patients. Swab was stored in 0.1% peptone water.

Media preparation and identification

PDA (potato dextrose agar) media was prepared. Fungal sample was inoculated in media by pour plate method, incubated at 25°C for 3-5 days. Five different colonies were obtained in two sample plates. Each colony was again inoculated in PDA and PDB (potato dextrose broth), incubated at 25°C for 3-5 days. All colonies were identified on the morphological basis by slide preparation.

Antifungal test by well diffusion method

Four different concentrations (100%, 75%, 50%, 25%) of plant extract were prepared. The petroleum ether and methanolic extracts of *C. tora* leaves extracts were screened for antifungal activity by agar well diffusion method⁷ with sterile cork borer of size 6.0 mm. Prepared five PDA plates and marked 1, 2, 3 and 4 of patient-1 (P-1) and one plate has marked patient-2 (P-2). Each plate divide into 4 parts and marked 100%, 75%, 50% and 25%. Spread the preincubated culture on plates and formed wells. 5 µL of each conc. poured in wells according to marking. Then incubates all the plates at 25°C for 3-5 days. Antifungal activities were then measured indicated by the clear zones of inhibition⁸.

Observation

Clear inhibition zones at 5 µL/100 µL of *Cassia tora* leaves extracts were observed.

Table 1: Sample of Patient-1 and Patient-2 shows zone of inhibition on different concentration of lipid compounds

Conc.	Zone of inhibition				
	P-1 (1)	P-1 (2)	P-1 (3)	P-1 (4)	P-2
100%	8 mm	11 mm	-	-	4 mm
75%	5 mm	10 mm	-	-	3 mm
50%	4 mm	5 mm	-	-	-
25%	4 mm	5 mm	-	-	-

Table 2: Sample of Patient-1 and Patient-2 shows zone of inhibition on different concentration of alkaloid compounds

Conc.	Zone of inhibition				
	P-1 (1)	P-1 (2)	P-1 (3)	P-1 (4)	P-2
100%	-	9 mm	6 mm	6 mm	-
75%	-	7 mm	5 mm	5 mm	-
50%	-	6 mm	3 mm	5 mm	-
25%	-	4 mm	3 mm	3 mm	-

Samples collected from two different patients infected from ring worm disease -



Fig. 1: Patient-1 (P-1) back portion of the body infected from ring worm

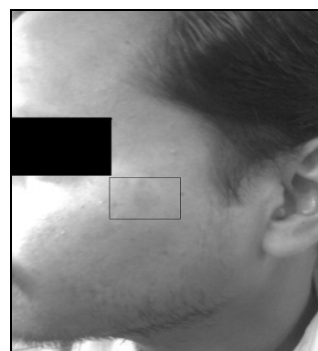


Fig. 2: Patient-2 (P-2) cheek portion nearby eye infected from ring worm



Fig. 3: Plant of *Cassia tora*



Fig. 4: Fungal colonies were obtained from P-1 (4 different colonies were identified)

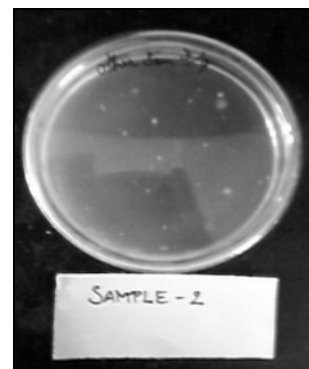


Fig. 5: Fungal colonies were obtained from P-2 (One type of colony was found)

Different concentration of lipid of *C. tora* showed zone of inhibition on different fungal samples -



Fig. 6: Different lipid conc. showed 4 different zone of inhibition on P-1 (1)

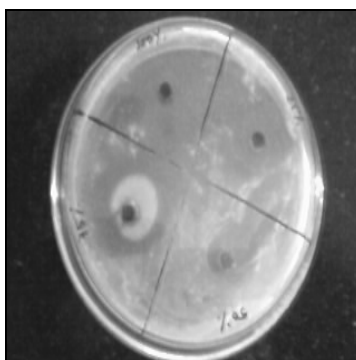


Fig. 7: Different lipid conc. showed 4 different zone of inhibition on P-1 (2)

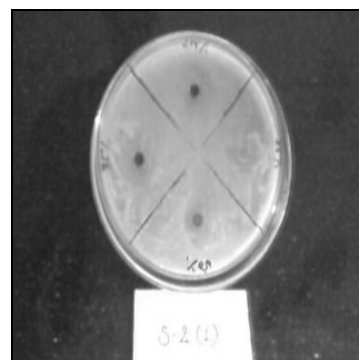


Fig. 8: Different lipid conc. showed 2 different zone of inhibition on P-2

Different concentration of alkaloid of *C. tora* showed zone of inhibition on different fungal samples -

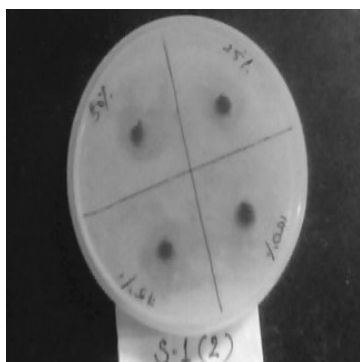


Fig. 9: Different alkaloid conc. showed 4 different zone of inhibition on P-1(2)

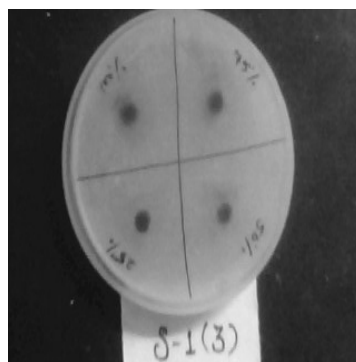


Fig. 10: Different alkaloid conc. showed 4 different zone of inhibition on P-1(3)

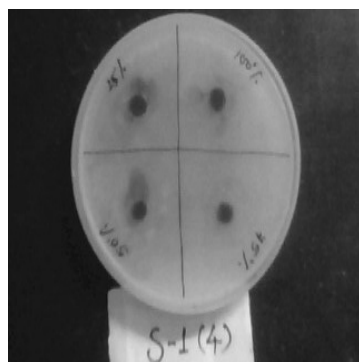
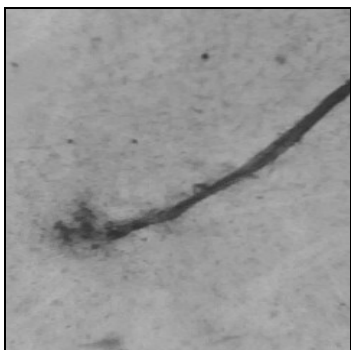
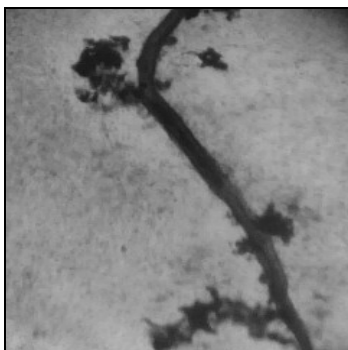


Fig. 11: Different alkaloid conc. showed 4 different zone of inhibition on P-2

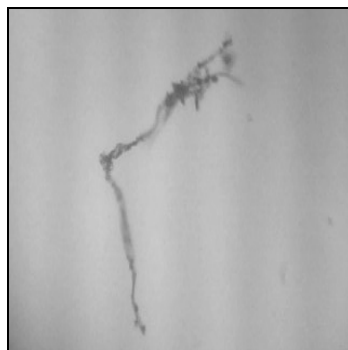
Slides of identified fungal colonies obtained from patients, they shown sensitivity against *C. tora* -



Slid-1: Hyphae of the class *Trichophyton*, sensitive agained lipid compound of *C. tora* founded in P-1(1)



Slid-2: Hyphae of the class *Epidermophyton*, sensitive agained lipid compound of *C. tora* founded in P-1(2)



Slid-3: Hyphae of the class *Trichophyton*, sensitive agained lipid comp *C. tora* founded in P-2



Slid-4: Hyphae of the class *Trichophyton*, sensitive agained alkaloid compound of *C. tora* founded in P-1(2)



Slid-5: Hyphae of the class *Trichophyton*, sensitive agained alkaloid compound of *C. tora* founded in P-1(3)



Slid-6: Hyphae of the class *Trichophyton*, sensitive agained alkaloid compound of *C. tora* founded in P-1(4)

RESULTS AND DISCUSSION

Traditionally in Malaysia, *Cassia tora* is used externally to treat fungal infections⁹. In India its promising use in case of Daad (ringworm) has given it a name Dadmari, Mari

means to kill¹⁰. The results from this current study showed that petroleum ether and methanol extraction of *C. tora* had antifungal properties on Dermatophytes (ringworm fungus, Trichophyton and Epidermatophyton).

Traditional medicines hold a great promise as source of easily available therapy for skin disease to the people, particularly in tropical developing countries, including India. It is in this context that the people use several plant derived preparations to cure skin diseases¹¹.

Many plants have been shown to have antifungal properties including *C. tora*. The growth inhibition effect of leaves extract of *C. tora* on ring worm fungus studied comparatively with lipid and alkaloid compound of *C. tora*. However, the petroleum ether extraction of the leaves of *C. tora* was able to inhibit the growth of Dermatophytes, but it is effective only on some type of Dermatophytes.

On the other hand methanol extraction of the leaves of *C. tora* showed minutely less inhibition than petroleum ether extract but it is effective on maximum type of Dermatophytes. In this study, four dilutions (100%, 75%, 50% & 25%) were used which are prepared from 100 μ l plant extract per ml of dissolution chemical.

So it is concluded that alkaloid is more effective than lipid compound of *C. tora* on Dermatophytes in *in vitro* condition.

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