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ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA TORA* LEAVES AGAINST *CANDIDA ALBICANS*

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ABSTRACT

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents. Treatment of these infections is a real challenge to health professionals. The available antifungal drugs produce many adverse effects, show recurrence or lead to development of resistance. To overcome these problems, the development of newer antifungal compound against new targets is the matter of urgency. The present investigation was aimed at evaluation of antifungal activity of methanolic extract of *Cassia tora* leaves. The extract was prepared by hot continuous percolation method in Soxhlet apparatus using 90% methanol as an extracting solvent. The cup plate method was used to investigate the antifungal activity. The methanolic extract of *Cassia tora* leaves demonstrated significant antifungal activity against *Candida albicans*.The minimum inhibitory concentration was found to be 2mg/ml.Amphotericine-B was used as standard to compare the activity.

KEY WORDS: *Candida albican,* Antifungal activity, *Cassia tora*, Leguminoseae, Skin diseases, Candidiasis

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INTRODUCTION

Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents[1]. The incidence of fungal infection is increasing at an alarming rate, presenting an enormous challenge to health care professionals[2].

Candida albican, the agent of candidiasis is an increasingly important disease that has a world wide distribution because of the fact that it is a frequent opportunistic pathogen in AIDS patients[3]. Due to the increase of the number of immunocompromised individuals, fungal infections have increased in last two decades[4]. Among them, opportunistic systemic mycoses are associated with high mortality rates[5]. Since *Candida albicans* is found in the mouth and intestinal tract of a high percentage of humans, the fungus may spread from such location to cause skin and nail infections. Balanoposthitis has been observed in 100s of women with monilial vaginitis and cutaneous moniliasis (candidiasis) above the nipple of nursing mothers has been caused by infants with oral thrush[6].

There are many modern medicines available for the treatment of fungal diseases however they possesses a series of limitations such as undesirable side effects, development of resistance and low sensitivity against these fungal infections[7,8]. Since strains of *Candida albicans* with multiple antibiotic resistance is increasing worldwide, it is of great importance to find effective treatments for these pathogens. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drug against microbial infections[9]. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries. Traditional healers usually are cheaper and sometimes more effective than modern medicines[10].

Cassia tora Linn. (Family-Leguminoseae) is widely distributed in tropical Asian countries. It is also commonly known as 'Sickle Pod'. Various parts of the

plant are reputed for their medicinal value[11]. The plant has been well esteemed for skin diseases particularly for ring worm and an anti-inflammatory agent[12]. The leaves of *Cassia tora* contains several anthraquinone glycosides which are well known for their therapeutic value[13]. *Cassia tora* leaves have been reported to possess antireumatic activity in folklore practices[14].

The present study was designed to investigate antifungal activity of methanolic extract of *Cassia tora* leaves against *Candida albicans* and also to see if there is any rationale behind the local uses of this plant especially in the treatment of skin diseases.

MATERIALS AND METHOD

Collection of plant material

Cassia tora leaves were collected from Chalisgaon, Dist. Jalgaon (Maharashtra) in the month of October. The taxonomical identity of this plant was confirmed by Dr. J. M. Pathak, Research Director-Pharmacognosy, Zandu foundation, Mumbai. A herbarium sample (Specimen No. 2004/001) of this plant is preserved at the Department of Pharmacognosy, Principal K. M. Kundnani college of Pharmacy, Mumbai. The leaves were dried under shade, coarsely powdered and stored in airtight container. All the chemicals and reagents used were of LR grade.

Extraction of plant material

Coarsely powdered *Cassia tora* leaves were extracted with hydroalcoholic solvent (90% methanol) by hot continuous percolation method in Soxhlet apparatus. The extract was evaporated and dried under reduced pressure. One gram of dried extract was dissolved in 100 ml of 90 % methanol giving final concentration of 10 mg/ml. The extract was incorporated in the medium to give a final concentration in the range of 0.5-10 mg/ml.

Collection and Preservation of Culture

The culture of *Candida albicans* (ATCC No. 10231) was obtained from the Microbiology Department of FDA, Mumbai. Stock cultures were preserved in Sabouraud's Agar media. The composition of medium and culture requirements is mentioned in table-1.

Standardization of culture

Culture was standardized by spectrophotometric method using McFarland turbidity standard[15]. The test organism used was grown on the plates of Sabourauds Dextrose agar for 48-72 hrs. The inoculum suspension was prepared by picking 5 colonies of at least 1 mm diameter and suspending the material in 5 ml sterile 0.85% NaCl. The turbidity of the cell suspension measured at 530 nm was adjusted with saline solution to match that of 0.5 McFarland turbidity standard. This produced a cell suspension containing $1x10^6$ to $5x10^6$ cells per ml, which was then diluted 1:100 with the desired test medium to provide a starting inoculum of $1x10^4$ to $5x10^4$ cells per ml[16].

Preparation of Amphotericin - B

0.1g of Amphotericin – B (Standard) was dissolved in 100ml DMSO (Dimethyl sulfoxide) to get final concentration of 10 mg/ml.

Evaluation of Antifungal activity

The evaluation of antifungal activity of *Cassia tora* leaves extract (90% methanol) was carried out using cup plate method.

Petri plates were sterilized by dry heat in hot air oven at 160 ^{0}C for 1.5 hr. Sabourauds dextrose agar was prepared in distilled water. Molten agar (20 ml) was poured in the test tube and plugged with non-adsorbent cotton. All test tubes were sterilized by autoclaving. The test tubes were cooled up to 50 ºC. The molten agar was poured in the sterile petri-plates aseptically. The plates were kept at room temperature for 30 minutes. Then the second layer (i.e. seed layer) was prepared by pouring 5 ml molten agar medium inoculated with 0.05 ml of Standard Culture of *Candida albicans* over a solidified uninoculated base layer. Using No.4 cork borer hole was bored on each agar plate.

Plant extract was diluted at a concentration of 30, 20 and 10 mg/ml in 90% methanol and 0.1ml of each extract was applied separately in the hole made on different agar plates. The plates were then kept in refrigerator for pre-incubation diffusion for 1 hour. During one hour, the plates were allowed to come on room temperature. Plates were then incubated in an incubator at 30-32 ºC for 3 days. At the end of 3 days period, the petri-plates were observed and the diameters of zones of inhibition were recorded.

Methanol (90%) solution was used as negative control. Amphotericin B was used as a standard (Positive control). The experiments were replicated three times and the mean diameter of the zones of inhibition was measured.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) for antifungal activity of *Cassia tora* leaves extract was determined by broth dilution technique.

Sabourauds dextrose broth was prepared and poured 10ml each in the set of six test tubes. The tubes were then sterilized by autoclave. The different volume of this extract was delivered accurately to each test tube using sterile pipettes and thoroughly mixed to obtain the concentrations as mentioned in the table- 2.

The tubes were then inoculated with 0.05 ml of the standardized culture. The inoculated tubes were incubated at temperature 30 ^{0}C – 32 ^{0}C and observed for growth by monitoring the turbidity produced. The test

procedure was repeated to check the reproducibility of the results. The lowest concentration that can inhibit the growth is Minimum Inhibitory Concentration (MIC). Amphotericin - B was used as a positive control (standard).

RESULT AND DISCUSSION

The zones of inhibition (mm) obtained at various concentration of *Cassia tora* leaf extract (Figure-1, 2 and 3) and standard at concentration 10mg/ml (Figure-4) are depicted in table-3. The production of distinct zones of inhibition against *Candida albicans* suggest the presence of antifungal principle in crude extract of C*assia tora* leaves.

The least concentration of a test material responsible for inhibiting the growth of microorganism was termed as minimum inhibitory concentration (MIC).The average readings of three determinations for the MIC values of *Cassia tora* leaves extract are depicted in table - 4.

The results of the above study clearly indicated that the methanolic extract of *Cassia tora* leaves exhibits significant antifungal activity against *Candida albicans*.This also stands as a scientific support for the usage of this plant for treating skin diseases in traditional medicines.

CONCLUSION

In the present investigation, crude methanolic extract of *Cassia tora* leaves was used to evaluate the antifungal activity against *Candida albicans*.This may be the reason for getting higher MIC vaule (i.e. 2mg/ml).Probably a more refined preparation would have antifungal activity at a lower concentration. Further experimental and clinical trials need to be carried out on the individual isolate or fraction of the methanolic extract of *Cassia tora* leaves. This would also help to correlate the antifungal activity with known constituents of *Cassia tora* leaves.

Herbal formulation can be developed using active fraction of the methanolic extract to treat the candidasis.

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Table-1: Composition of Sabourauds Agar medium & culture requirements

Composition of the medium (Final pH of the medium:5.6-5.8		
Sr.No.	Ingredient	Quantity
1	Dextrose	40g
2	Peptone	10g
3	Agar	20g
4	Distilled water to make	1 liter
Culture requirements		
1	Growth condition	Aerobic
2	Temperature	28-32 ^{0}C
3	Incubation time	7 Days
4	Subculture period	30days

Table 2: Different concentrations of test and standard for determination of MIC

Test material	**Concentration (mg/ml)**					
	I	**II**	**III**	**IV**	**V**	**VI**
A) *Cassia tora* leaves extract	0.5	1.0	1.5	2.0	2.5	3.0
B) Methanol (Negative control for extract)	0.5	1.0	1.5	2.0	2.5	3.0
C) Amphotericin-B (Positive control)	0.1	0.2	0.4	0.6	0.8	1.0
D) DMSO (Negative control for standard)	0.1	0.2	0.4	0.6	0.8	1.0

Table 3: Zone of inhibition by cup plate method

Sample (mg/ml)	Zone of inhibition (mm)*
1. *Cassia tora* leaf extract	
• 10	14
• 20	20
• 30	24
2. Amphotericin -B	
• 10	32

*Diameter of cork borer is 12 mm.

Table- 4: MIC values of *Cassia tora* leaves extract and Amphotericin -B

Tested materials	MIC (mg/ml)
1. *Cassia tora* leaf extract	2.0
2. Amphotericin-B	0.6

Fig.1: Zone of inhibition – C*assia tora* leaves extract (Concentration- 10 mg/ml)

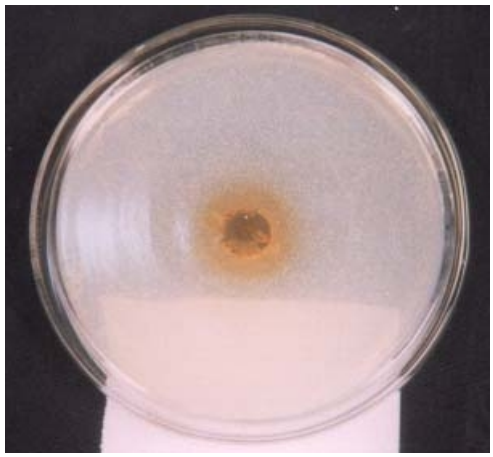

Fig.2: Zone of inhibition – *Cassia tora* leaves extract (Concentration- 20 mg/ml).

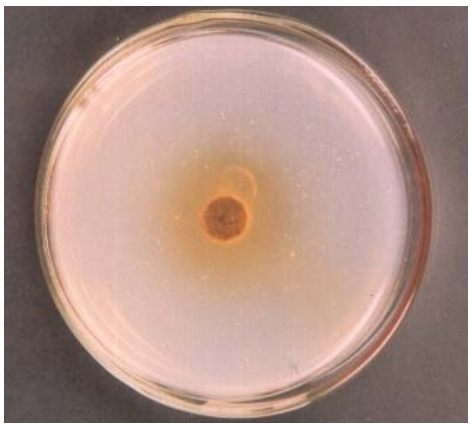

Fig.3: Zone of inhibition – *Cassia tora* leaves extract (Concentration- 30 mg/ml).

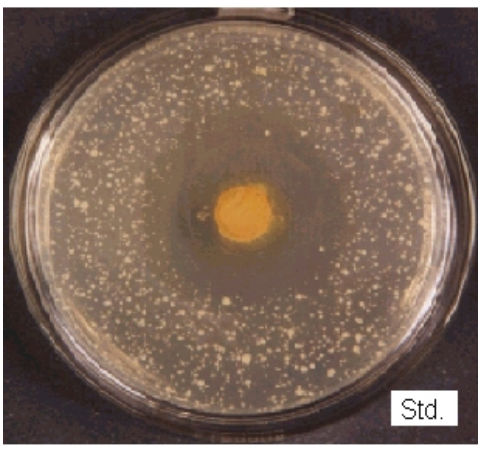

Fig. 4: Zone of inhibition - Amphotericine B (Concentration-10 mg/ml).

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