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Antifungal activity of *Cassia fistula* Linn. against some pathogenic fungi

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Abstract

Antifungal activity of number of phytochemicals has been reported. In the present study, aqueous and methanolic leaf extracts of *Cassia fistula* has been investigated for antifungal activity against some pathogenic fungal strains viz. *Candida albicans, Microsporum gypseum* and *Aspergillus niger* using the standard disc diffusion method. Methanolic extract had shown the maximum activity (13mm) against *A. niger*. Extracts showed a concentration dependent antifungal activity, with higher concentrations of 100 and 200 mg/ml showing greater zones of inhibition than with lower concentrations. The Minimum Inhibitory Concentration (MIC) ranged from 0.78 to 3.12 mg/ml. The in vitro findings justify the use of *Cassia fistula* in traditional medicine practice for the treatment of some fungal infections. However, study on the toxicity of the crude extracts and the compounds isolated from this plant should be assessed to ensure their eligibility to be used as sources of modern medicines.

Keywords: *Cassia fistula*, Disc diffusion, Minimum inhibitory concentration, Phytochemicals

Introduction

India is one of the most popular countries in the world, where traditional medicine system is practiced in primary health care. Medicinal plants are used in the treatment of much life threatening diseases [1]. Thus, today quality assurance is thrust area for the evaluation of traditionally used medicinal plants and herbal formulation. According to World Health Organization (WHO) report, about 80% of the world's population is taking interest in indigenous medicinal plant remedies [2]. Indian medicinal plants are considered as a vast source of several pharmacologically principles and compounds that are commonly used as home remedies against multiple ailments [3]. The use of medicinal plants to treat human diseases has its roots in pre-historical time. These plants and derived products play an important role in the primary health care of people. At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominates in homeopathic or ayurvedic medicines [4]. Screening of various bioactive compounds from plants has lead to the discovery of new medicinal drugs which have effective protection and treatment roles against various diseases [5-7]. Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulteration and side effects [8]. Therefore, the need of hour is to search alternative source to treat diseases.

Cassia fistula known as the golden shower tree is one of the important medicinal plant of Fabaceae family. It is native to Southern Asia, from Southern Pakistan, East through India to Myanmar and South to Sri Lanka. It is the national tree of Thailand and its flower is Thailand's national flower. It is also state flower of Kerala in India. It is a popular herbal medicine and is cultivated as an ornamental plant throughout the India [9]. *C. fistula* is widely used ethno medicinally against skin diseases, liver troubles, tuberculosis, rheumatism, leucoderma and diabetes [10, 11]. The leaves are laxative and used externally as emollient, a poultice is used for chilblains, in insect bites, swellings and facial paralysis [12]. The leaves and bark, mixed with oil are applied to pustules, insect bites [13]. Considering the medicinal potential of *Cassia fistula,* the present work has been designed to evaluate the antifungal activity of the plant against some pathogens in an attempt to use it as an alternative source for the treatment of fungal diseases.

Materials and Methods

Plant Material

Fresh leaves of *Cassia fistula* were collected from the botanical garden of RBS College, Agra during 2011 – 2012. The collected leaves were washed and dried under shade at room temperature and then powdered with a grinder and then stored in air tight container.

Extraction

Aqueous Extract

For aqueous extract leaves were separately homogenised with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whattman No. 1 filter paper [14]. The extraction was carried out at room temperature.

Organic Extract

Organic extract was prepared by Soxhlet extraction method following [15]. A thimble was prepared by using a 0.5mm whattman filter paper. A weighed amount of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with solvent (methanol) for the period of about 48 hours or 22 cycles or till the solvent in the siphon tube of the extractor became colourless. After that extract was filtered and solvent evaporated from extract in rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove trace of solvent The crude extract was kept in refrigerator at 4 C to evaluate antifungal activity.

Test Organisms

The pure culture of test fungal strains used in the study included: *Aspergillus niger* (MTCC 282), *Candida albicans* (MTCC 227) and *Microsporum gypseum* (MTCC 2819). The test organisms were obtained from microbial type culture collection (MTTC), institute of microbial technology, Chandigarh. The fungal cultures were maintained on sabouraud's dextrose agar slants and stored at 4ºC prior to use.

Antifungal Assay

In vitro antifungal activity of selected plant extracts was determined by disc diffusion method [16]. Crude extract was dissolved in suitable solvent (dimethyl sulfo oxide). Different concentration (200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml) was prepared by serial dilution. Empty sterile disc having a diameter of 6 mm were impregnated with 25µl plant extract and incubated for 15 minutes for proper diffusion of extract. On the other hand, some colonies from the pure culture were mixed (emulsified) in nutrient broth (7µl/ml broth). This broth was inoculated on entire surface of sabouraud's agar plate with the culture moistened cotton swab. With the help of sterile forceps, discs loaded with herbal extract were placed on inoculated surface of agar plate. These plates were incubated for 24 - 72 hours at 25-30 C and the zone of inhibition was taken as measure of the antifungal activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

Minimum Inhibitory Concentration

For MIC the crude plant extract was made into suspension by using a suitable solvent (DMSO). Different concentrations from stock (200 mg/ml) were prepared by serial dilution. Few drops of over night broth culture of test organism were inoculated into each dilution and incubated at 25 to 30ºC for 24 – 72 hrs. The minimum concentration of the extract which inhibited the growth of test organism was recorded as MIC.

Activity Index (AI)

The activity index as a measure of antifungal activity in comparison to standard drug was calculated by the following formula:

$$AI = \frac{\text{Zone of Inhibition shown by extracts}}{\text{Zone of Inhibition shown by drug}}$$

Results and Discussion

Antifungal activity

Crude methanolic and aqueous extract of leaves of *Cassia fistula* were assessed for antifungal activity against *C. albicans, M. gypeseum* and *A. niger*. Both the extracts inhibited the growth of test fungi (Figure. 1 & 2) as indicated by the zones of inhibition. The maximum zones of inhibition (13.00 ± 2.12, 12.50 ± 2.35 and 12.00 ± 1.88mm) were found against *A. niger, C. albicans* and *M. gypseum ,* respectively at 200mg/ml concentration (Table 1). The results were comparable to standard drug (18.00 ± 2.82, 20 ± 2.82 and 13.33 ± 0.93 mm against *A. niger, C. albicans* and *M. gypseum,* respectively). The investigation showed that the extracts demonstrated a concentration dependent antifungal activity with higher concentrations of 200 and 100 mg/ml showing greater zones of inhibition than with lower concentrations of 6.25 and 3.12 mg/ml. Our results are in agreement with [17, 18] who observed gradual decrease in biological activity with the decrease in concentration in *Celtis australis* and *Holoptelea integrifolia*, respectively. The better antifungal activity of methanolic leaf extract might be due to the presence of various secondary metabolites.

In the present study aqueous extract was found to be comparatively less promising as compared to methanolic extract, with maximum zone of inhibition (11.66 ± 0.94 mm) against *A. niger* (Table 2).Similar results were also reported by [19-21] in *Cassia alata*, *Cassia fistula* and *Flacoartia indica*. Thus in addition to the microorganism tested, the extractive solvent was a determinant factor for the extraction of antifungal agents in this study. These results also agree favorably with the suggestions of [22] that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvent used. The aqueous extract showed less antifungal activity as compared to the organic extract possibly because of polarity of antimicrobial compounds that make them more readily extractable in the organic solvent and insufficient quantities of the active compound in the crude aqueous extract [23].

Table 1: Antifungal activity of *Cassia fistula* methanolic leaf extract.

Solvent	Test Organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Activity Index (AI)
Methanol	*Aspergillus niger*	200	13.00 ± 2.12	0.72
		100	12.16 ± 1.65	0.67
		50	12.00 ± 1.41	0.66
		25	10.16 ± 1.17	0.56
		12.5	9.16 ± 1.17	0.50
		6.25	9.00 ± 0.70	0.50
		3.125	9.00 ± 0.70	0.50
		Drug	18.00 ± 2.82	
	Candida albicans	200	12.50 ± 2.35	0.61
		100	11.50 ± 2.12	0.57
		50	10.83 ± 1.64	0.54
		25	9.16 ± 1.17	0.45
		12.5	9.50 ± 0.70	0.42
		6.25	7.33 ± 0.94	0.36
		3.125	7.33 ± 1.17	0.36
		Drug	20 ± 2.82	
	Microsporum gypseum	200	12.00 ± 1.88	0.90
		100	11.16 ± 1.17	0.83
		50	10.50 ± 0.93	0.78
		25	9.83 ± 0.94	0.73
		12.5	8.50 ± 0.70	0.63
		6.25	7.83 ± 0.47	0.58
		3.125	7.50 ± 0.70	0.52
		Drug	13.33 ± 0.93	

Data represents an average value of three replicates; value represents mean ± standard deviation
Itraconazole was used as drug against *M. gypseum* and Fluconazole against *A. niger* and *C. ablicans.*

Table 2: Antifungal activity of *Cassia fistula* aqueous leaf extract.

Solvent	Test Organism	Concentration (mg/ml)	Zone of Inhibition (mm)	Activity Index (AI)
Aqueous	*Aspergillus niger*	200	11.66 ± 0.94	0.57
		100	10.00 ± 0.93	0.52
		50	9.33 ± 0.93	0.49
		25	8.50 ± 0.70	0.42
		12.5	7.83 ± 0.47	0.38
		6.25	6.16 ± 0.46	0.32
		3.125		
		Drug	19.00± 2.8	
	Candida albicans	200	10.66 ± 1.88	0.62
		100	9.16 ± 1.65	0.53
		50	9.00 ± 1.41	0.52
		25	9.66 ± 0.94	0.56
		12.5	8.70 ± 0.70	0.51
		6.25	8.60 ± 0.70	0.50
		3.125	8.30 ± 0.46	0.48
		Drug	17.00 ± 2.36	
	Microsporum gypseum	200	10.00 ± 1.41	0.65
		100	9.00 ± 1.17	0.59
		50	9.00 ± 1.41	0.59
		25	9.66 ± 1.17	0.63
		12.5	8.83 ± 0.94	0.58
		6.25	7.50 ± 0.70	0.49
		3.125	7.00 ± 0.47	0.46
		Drug	15.16 ± 1.65	

Data represents an average value of three replicates; value represents mean ± standard deviation;
Itraconazole was used as drug against *M. gypseum* and Fluconazole against *A. niger* and *C. ablicans.*

Table 3: Minimum inhibitory concentration of leaf extract of *Cassia fistula*.

Extract	Test Organisms	MIC
Methanolic leaf	*spergillus niger*	1.56
	andida albicans	1.56
	icrosporum gypseum	0.78
Aqueous leaf	*spergillus niger*	3.12
	andida albicans	1.56
	icrosporum gypseum	1.56

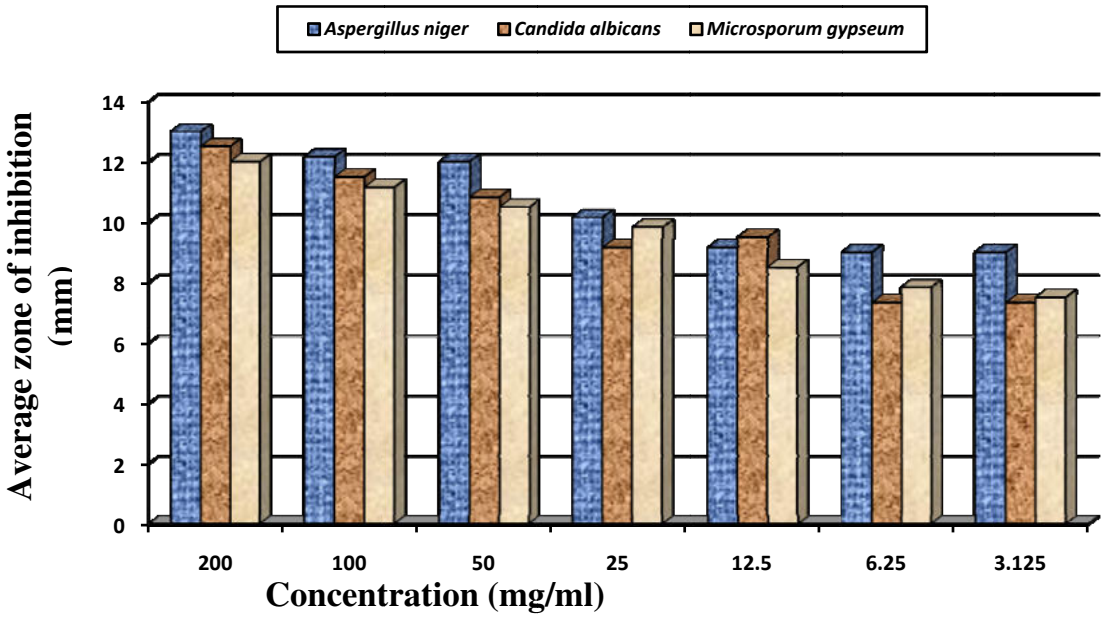

Figure. 1 – Graphical representation of antifungal activity of methanolic leaf extract of *Cassia fistula* against different fungi.

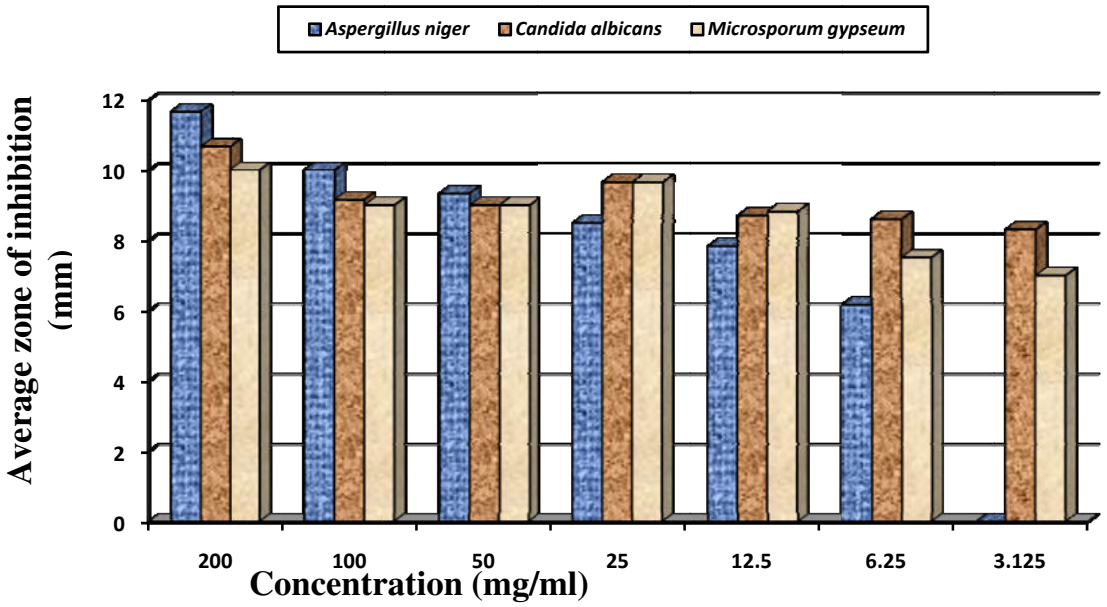

Figure. 2 – Graphical representation of antifungal activity of aqueous leaf extract of *Cassia fistula* against different fungi.

Minimum Inhibitory Concentration

Activity index and minimum inhibitory concentration of both the test extracts for *C. albicans, M. gypseum* and *A. niger* are given in Table 3. The MIC ranged from 0.78 to 3.12 mg/ml. High MIC values are indication of low activity while low MIC value are indication of high activity.

Conclusion

The findings of the present study reveal that methanol was comparatively better extractive solvent than distilled water for the antifungal activity of the test plant. Plant extracts worked in dose dependent manner i.e. the antifungal activity decreased gradually with the decrease in concentration of extract. The study provides an insight into the usage of the plant species in traditional medicine

for the treatment of common fungal infections. However, further work on clinical trials, pharmaceutical dosage formulation and development should be carried out for its safety and efficacy.

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