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ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST CLINICAL PATHOGENS

Tinki Kanoje and *Kavita Sharma

Department of Botany, Govt., D. B. Girl's PG Autonomous College, Raipur, Chhattisgarh, India

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Corresponding author

ABSTRACT

The present investigation determines the antifungal activity of the plant extracts of viz. *Discorea hispida* (tubers), *Sterculia urens* (Leaves), *Dillenia indica* (leaves), *Celastrus paniculatus* (leaves), *Desmodium gangeticum* (leaves) against the pathogens viz. *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* (locally isolated). polar extracts (aqueous and methanolic) had strong antifungal activity in comparison to the non polar extracts (petroleum ether and hexane) against the pathogens studied. The results showed that, aqueous and methanolic extract of tubers of *Discorea hispida* had most significant antifungal activity against *Aspergillus niger* followed by aqueous and methanolic extract of *Dillenia indica*, *Celastrus paniculatus* and *Desmodium gangeticum*.

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INTRODUCTION

Nature has been a source of medicinal agents from thousands of years. Leaves, flowers, stems, roots, seeds, fruit and bark of the plants are the constituents of medicinal properties (Ababa *et al.,* 2007). A very rich botanical wealth and large number of diverse types of plants grow in different parts of the country and plant material used in traditional medicine are readily available in rural areas at relatively cheaper costs than modern medicine. Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of some known drugs. Components with medicinal properties from plants play an important role in conventional Western medicine. In 1984, at least 25% of the Western medicine issued in the US and Canada were derived from or modeled after plant natural products and 119 secondary metabolites were used globally as drugs (Farnsworth, 1994). It has been estimated that 14-28% of higher plant species are used medically. Only 15% of all angiosperms have been investigated chemically and 74% of pharmaceutically active plant derived components were discovered after following up on ethno medical use of the plant (Farnsworth, 1991). Ethno medicinal plants have been used for preparation of an alternative treatment in several cases of infectious diseases and represent a rich source of antimicrobial agents (Prince and Prabhakaran, 2011; Upadhyay *et al.,* 2010). Different pharmacological activities of medicinal plants were investigated (Mathur *et al*., 2011a, b). The present study is about the determination of antifungal activity of different solvent extracts of the plants viz. *Discorea hispida* (Tuber), *Sterculia urens* (Leaves), *Dillenia indica* (leaves), *Celastrus paniculatus* (leaves), *Desmodium gangeticum* (leaves) against clinical fungal pathogens.

MATERIALS AND METHODS

The chemicals and reagents used in the present study were of analytical grade procured from Ranchem, Mumbai, India and media was procured from Hi-Media, Mumbai.

Collection of plant materials

The plants parts viz. *Discorea hispida* (Tuber), *Sterculia urens* (Leaves), *Dillenia indica* (leaves), *Celastrus paniculatus*

(leaves), *Desmodium gangeticum* (leaves) were taxonomically identified and specimen were stored for future reference in Department of Botany, Govt. D. B. Girl's PG Autonomous College, Raipur, Chattisgarh, India

Preparation of plant extracts

Plant parts were separated, washed with distilled water, dried under shade and pulverized. The method of Alade and Irobi (1993) was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of each of the powdered plant material were soaked separately in different solvents i.e. hexane, petroleum ether, methanol and distilled water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C.

Saccharomyces cerevisiae (locally isolated) were inoculated into separately Sabouraud's dextrose broth and incubated at 28 ^{0}C for 72 h. The suspension was checked to provide approximately, 10^8 CFU/ml. Determination of diameter of zone of inhibition by well diffusion method. The agar well diffusion method (Perez *et al*., 1993) was modified. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts (500 µg/ml) and DMSO in which the extracts were dissolved. DMSO was used as negative controls. Flucanazole (25 mg/ml) was used as standard for determination of antifungal activity. The plates were kept at 28^{0}C for 48 h for determination of antifungal activity of any of the extracts and solvent blanks. The procedure for assaying antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

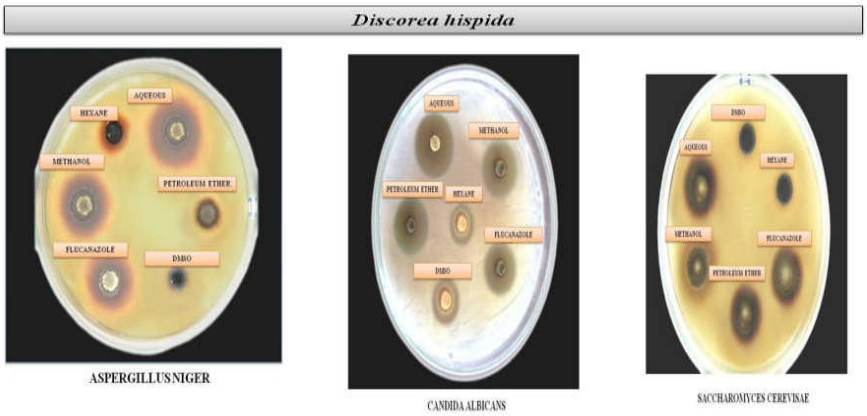

Figure 1(a): Antifungal activity of *Discorea hispida* against *Aspergillus niger*; *Candida albicans* and *Saccharomyces cerevisiae*

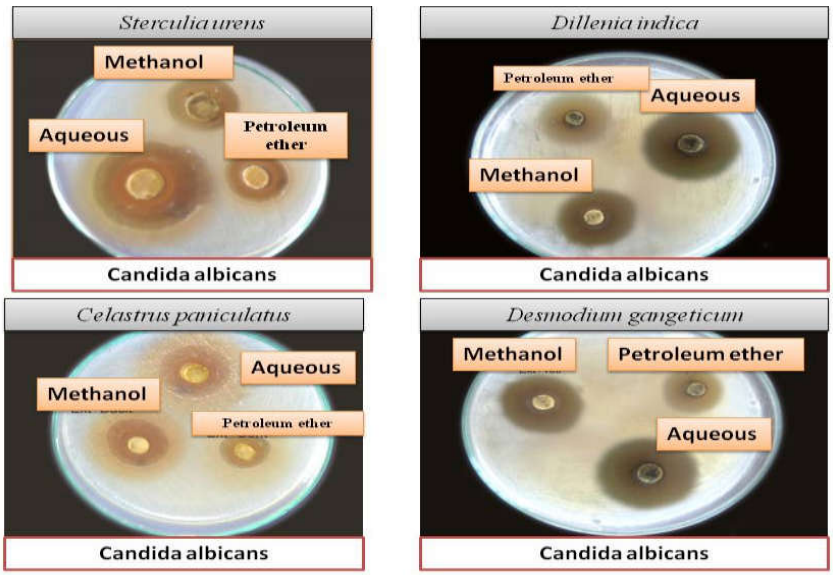

Figure 1(b): Antifungal activity of *Sterculia urens* and *Dillenia indica* against *Candida albicans*

Culture Media

For antifungal activity, Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India was used for antifungal test.

Inoculum of the microbes

The fungal cultures used for the study viz. *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231 and

Determination of Minimum Inhibitory Concentration (MIC)

MIC value of potent plant extracts was determined by the method adopted by Vollekova *et al*., 2001 and Usman *et al.,* 2007, with some modifications. Plant extract was prepared in highest concentration (500 µg/ml) in sterile distilled water and is serially diluted with N-saline (0.85 % NaCl) and similar quantity of fungal suspension was added to different test tubes

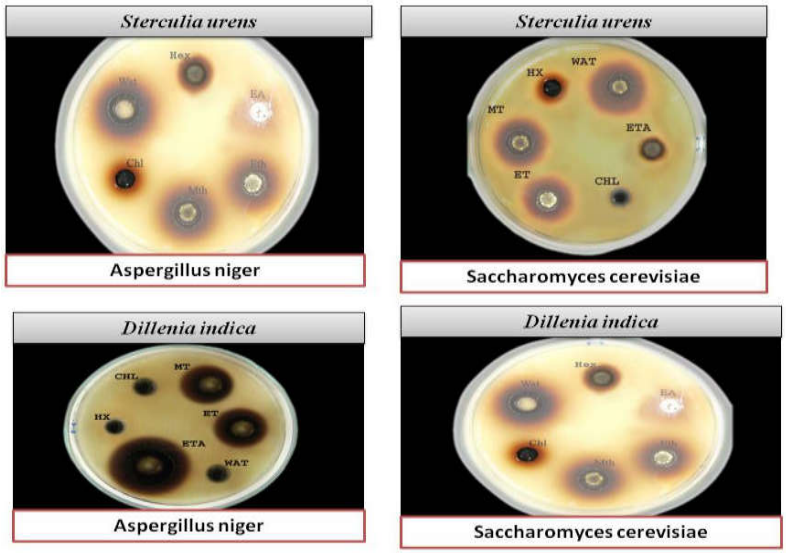

Figure 1(c): Antifungal activity of *Sterculia urens* and *Dillenia indica* against *Aspergillus niger* and *Saccharomyces cerevisiae*

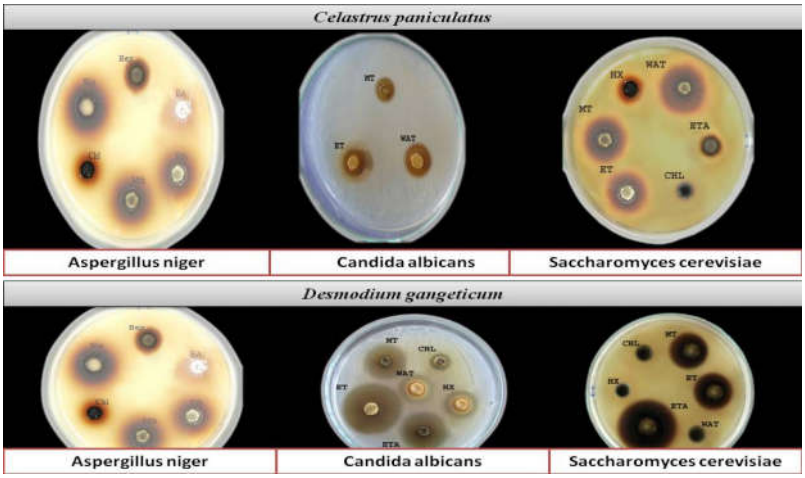

Figure 1(d): Antifungal activity of *Celastrus paniculatus* and *Desmodium gangeticum* against fungal pathogens viz. *A. niger*; *C. albicans* and *S. cerevisiae*

Table 1. Antifungal activity of the plants extracts

Plants/parts	Solvent extracts	Diameter of zone of inhibition (mm)		
	@ 500 µg/ml	*Aspergillus niger*	*Candida albicans*	*Saccharomyces cerevisiae*
Discorea hispida (Tubers)	Distilled water (Aqueous)	57.0	45.0	43.0
	Methanol	53.0	35.0	28.0
	Petroleum ether	20.0	28.0	32.0
	Hexane	15.0	12.0	NA
Sterculia urens (Leaves)	Distilled water (Aqueous)	28.0	35.0	28.0
	Methanol	23.0	28.0	24.0
	Petroleum ether	18.0	15.0	18.0
	Hexane	12.0	NA	11.0
Dillenia indica (Leaves)	Distilled water (Aqueous)	36.0	27.0	30.0
	Methanol	30.0	25.0	26.0
	Petroleum ether	25.0	18.0	17.0
	Hexane	22.0	NA	10.0
Celastrus paniculatus (Leaves)	Distilled water (Aqueous)	35.0	26.0	24.0
	Methanol	32.0	25.0	25.0
	Petroleum ether	12..0	22.0	15.0
	Hexane	NA	NA	11.0
Desmodium gangeticum (Leaves)	Distilled water (Aqueous)	35.0	28.0	27.0
	Methanol	30.0	25.0	23.0
	Petroleum ether	18.0	22.0	14.0
	Hexane	NA	NA	NA
Flucanazole (25 mg/ml)	-	25.0	22.0	32.0
DMSO	-	NA	NA	NA

*NA, No activity

Table 1. Antifungal activity of the potent polar plants extracts

Plants/parts	Solvent extracts	MIC & MFC (µg/ml)					
		Aspergillus niger		*Candida albicans*		*Saccharomyces cerevisiae*	
		MIC	MFC	MIC	MFC	MIC	MFC
Discorea hispida (Tubers)	Distilled water (Aqueous)	50	150	70	170	120	200
	Methanol	60	190	125	200	180	225
Sterculia urens (Leaves)	Distilled water (Aqueous)	150	256	175	267	175	300
	Methanol	200	250	178	278	185	215
Dillenia indica (Leaves)	Distilled water (Aqueous)	150	265	175	267	150	256
	Methanol	150	256	178	225	156	216
Celastrus paniculatus (Leaves)	Distilled water (Aqueous)	100	150	120	167	156	215
	Methanol	125	156	128	226	125	167
Desmodium gangeticum (Leaves)	Distilled water (Aqueous)	100	135	150	220	175	250
	Methanol	120	150	170	250	156	250
Flucanazole (25 mg/ml)	-	170	230	200	270	120	170
DMSO	-	NA	NA	NA	NA	NA	NA

*NA, No activity

and incubated for 48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of fungus gets killed is known as minimum lethal concentration (MLC) or minimum fungicidal concentration (MFC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of fungus is known as Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

The present results suggests that, all the plants used for the study viz. *Discorea hispida* (tubers), *Sterculia urens* (Leaves), *Dillenia indica* (leaves), *Celastrus paniculatus* (leaves), *Desmodium gangeticum* (leaves) had potent antifungal activity against the pathogens viz. *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* (locally isolated). The results showed that, polar extracts (aqueous and methanolic) had strong antifungal activity in comparison to the non polar extracts (petroleum ether and hexane) against the pathogens studied. The results showed that, aqueous and methanolic extract of tubers of *Discorea hispida* had most significant antifungal activity against *Aspergillus niger* followed by aqueous and methanolic extract of *Dillenia indica*, *Celastrus paniculatus* and *Desmodium gangeticum*. The results showed that, *Candida albicans* was the most sensitive pathogen against aqueous and methanolic extract of Discorea hispida followed by *Sterculia urens*, *Desmodium gangeticum*, *Celastrus paniculatus* and *Dillenia indica*. It was found that, Saccharomyces cerevisiae was the most sensitive organism against *Discorea hispida* followed by *Dillenia indica*, *Desmodium gangeticum* and *Celastrus paniculatus*. The results are shown in Table 1 and Figure 1 (a-d). The potent polar (aqueous and methanol) extracts were screened for MIC and MFC/MLC values. The MFC/MLC values lies in the range from 300-150 µg/ml while MIC values were found to be in the range from 200-50 µg/ml. The results are shown in Table 2.

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