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Identification of antifungal principle from leaf extracts of *Bauhinia scandens* L.

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Environment-friendly antifungal principle from diethyl ether fraction of 50% aqueous ethanolic extract of leaves of *Bauhinia scandens* L. was screened against plant pathogens like *Helminthosporium oryzae*, *Fusarium oxysporum*, *Rhizoctonia oryzae* and *Aspergillus niger* by antifungal assays using spore germination and agar-cup methods.Comparing with the control set without test solution, all the fungi showed remarkable inhibitions in their growth in the presence of the diethyl ether fraction of aqueous ethanolic extract of *B.scandens* except *Aspergillus niger*.The antifungal activity could be due to the presence of various secondary metabolites. Plants of *Bauhinia* species can be used to discover bioactive natural products serving as leads in the development of new agricultural research activities.

Keywords: Antifungal activity, *Bauhinia scandens*, Diethyl ether fraction, spore germination method, agar-cup method

INTRODUCTION

During the last decade, phytochemicals have been successfully linked with many biological parameters and much work is now-a days in progress towards a non-chemical and environment-friendly approach of management against various types of phytopathogens. The present investigation is a calculated attempt to search whether the plant *Bauhinia scandens* (Fam: Fabaceae) possesses any antifungal activity. Different parts of legumes have been found to have antifungal and antimicrobial activities against different plant pathogenic fungi (Jacob and Nair, 2022; Bhalodia and Shukla, 2011). Boonphong *et al*. (2007) demonstrated that some of the isolated compounds from *Bauhinia purpurea* showed antimalarial, antibacterial, antifungal, anti-inflammatory and cytotoxic activities.

Here, leaf extracts of *Bauhinia scandens*, a member of subfamily Caesalpinoidae, and a woody climber, exhibited antifungal effects against pathogenic fungi like *Helminthosporium oryzae*, *Fusarium oxysporum*, and *Rhizoctonia oryzae* assayed by spore germination and agar-cup methods.

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MATERIALS AND METHODS

Collection of Plant materials

Leaves of *Bauhinia scandens* L. were collected from Jorethang (1056 feet high from sea level) of Sikkim, India, during April-May.

Preparation of Plant Extract

Sundried leaves (1 kg) of *Bauhinia scandens* were ground to powder and extracted in 50% aqueous ethanol (3 l) at room temperature for seven days. The extract was filtered, and the filtrate was charcoalised. The charcoalised fraction was filtered repeatedly through Whatman No. 42 filter paper and a clear brown filtrate was obtained. The filtrate was concentrated under reduced pressure in a vacuum evaporator and a deep brown-coloured residue was obtained.

The brown-coloured residue was fractionated successively over petroleum ether (60^0-80^0 C), diethyl ether and chloroform. Each fraction was collected, separately dried over anhydrous sodium sulphate, concentrated and preserved at 2^0 to 4^0 C. Each fraction was then subjected to antifungal assays for locating the antifungal properties of the species.

Antifungal Assay

The antifungal assays were done following the standard (a) spore germination method and (b) agar-cup method.

Antifungal assay by spore germination method

Source of fungal strains: Strains of *Helminthosporium oryzae* and *Fusarium oxysporum* were collected from the stock culture, Department of Botany, University of Kalyani, India.

Each fraction of the brown-coloured residue was diluted with propylene glycol (a commonly used vehicle) in the concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml. A control set was maintained with propylene glycol. All the dilutions were sterilized by filtration using membrane filter (0.02 µ pore size).

Different dilutions of the test solutions were placed on sterilized grooved slides to which one drop of fungal spore suspension (30-40 spores/ microscopic field) each of *Helminthosporium oryzae* and *Fusarium oxysporum* of seven days old culture was added. A control set was prepared identical to these and taking propylene glycol instead of test solution. Each treatment was replicated three times. These were then incubated in humid condition for 24 hours at 20^0-25^0 C. The number of spores germinated were counted and the percentage inhibition of spore germination was calculated as follows :

$$\text{Percentage inhibition of spore germination} = \frac{\text{Total number of inhibited spores}}{\text{Total number of spores}} \times 100$$

Antifungal assay by agar-cup method

Source of fungal strains: Strains of *Rhizoctonia oryzae* and *Aspergillus niger* were obtained from the stock culture of Department of Botany, University of Kalyani, India.

Preparation of fungal medium

Potato Dextrose Agar medium was prepared by adding appropriate amounts of potato infusion, bacteriological agar and dextrose, volume made up to 1000 ml of distilled water. The medium was autoclaved for 15 min at 121^0C and pH of the medium was maintained to 5.6±0.2 at 25^0C. The total amount of melted autoclaved PDA medium was cooled to 45^0-50^0 C.

In 9 cm diameter Petri plates, PDA medium was added to fungal suspension of *Rhizoctonia oryzae* and *Aspergillus niger* in aseptic conditions. After thorough mixing, the mixture was allowed to solidify. With the help of a sterile cork borer (0.8 cm diameter), small wells were made in the middle of each plate.

In the wells, different dilutions of test solution as stated above were taken. A control plate was maintained by adding propylene glycol in the well. The Petri plates were sealed with strips of parafilm and incubated at 28^0±1^0C.

The diameters of the fungal inhibition zones were measured everyday after the growth till when the fungal growth in control set reached the size of the Petri plate. Each treatment was replicated thrice, and mean values were taken.

RESULTS AND DISCUSSION

Results (Table1) indicate 28.53% and 62.83% inhibition of spore germination of *Helminthosporium oryzae* by 100 mg/ml and 200 mg/ml of the sample administered respectively. At 25 mg/ml and 50 mg/ml of the diethyl ether fraction, no inhibition was however noticed. The rest of the fractions, i.e., petroleum ether and chloroform did not produce any inhibition of spore germination of *Helminthosporium oryzae*.

The results (Table2) indicate that the inhibition percentage against spore germination of *Fusarium oxysporum* was 17.93%, 48.68% and 76.50% with 50 mg/ml, 100 mg/ml, and 200 mg/ml of diethyl ether fraction respectively. In this case no inhibition was produced by 25 mg/ml of the applied fraction. The other two fractions, i.e., petroleum ether and chloroform, did not exhibit any inhibition as found in case of *Helminthosporium oryzae* (Table1). Results (Table 3) further indicate that there was

Table 1: Antifungal screening of different fractions obtained from leaf extracts of *Bauhinia scandens* against *Helminthosporium oryzae* (by spore germination method)

Test Fungus	Dose (mg/ml)	Total no. of spores			Total no. of inhibited spores			Inhibition (%)		
		PE	DE	Chl	PE	DE	Chl	PE	DE	Chl
Helminthosporium oryzae	Control	30	35	32	0	0	0	0	0	0
	25	37	35	30	0	0	0	0	0	0
	50	33	37	32	0	0	0	0	0	0
	100	33	38	32	0	11	0	0	28.53	0
	200	36	35	33	0	22	0	0	62.83	0
LSD at 5%									1.415	
S.E.									0.459	

PE = Petroleum ether fraction, DE = Diethyl ether fraction,Chl = Chloroform fraction
MIC of DE = 100 mg/ml Each treatment was replicated thrice and mean values were taken.

Table 2: Antifungal screening of different fractions obtained from leaf extracts of *Bauhinia scandens* against *Fusarium oxysporum* (by spore germination method)

Test Fungus	Dose (mg/ml)	Total no. of spores			Total no. of inhibited spores			Inhibition (%)		
		PE	DE	Chl	PE	DE	Chl	PE	DE	Chl
Fusarium oxysporum	Control	36	30	38	0	0	0	0	0	0
	25	36	30	35	0	0	0	0	0	0
	50	37	39	38	0	7.0	0	0	17.93	0
	100	33	39	37	0	11	0	0	48.69	0
	200	36	34	35	0	22	0	0	76.50	0
LSD at 5%						1.060			1.574	
S.E.						0.353			0.525	

PE = Petroleum ether fraction, DE = Diethyl ether fraction, Chl = Chloroform fraction
MIC of DE = 50 mg/ml
Each treatment was replicated thrice and mean values were taken.

inhibition of the diameter of zone of colony growth of *Rhizoctonia oryzae* by 50 mg/ml, 100 mg/ml and 200 mg/ml. 25 mg/ml concentration of the fractions did not produce any inhibition in the colony growth of the fungus. The minimum inhibitory concentration was 50 mg/ml of the sample. The petroleum ether and the chloroform fractions did not produce any inhibition in the growth of the fungal colony. None of the concentrations of all the fractions showed any activity against *Aspergillus niger*.

The present investigation agrees with other studies where it is shown that leaf extracts of other species of *Bauhinia* have antifungal activities (Ghosh *et. al*. 2013). The activities of bark extracts of *Bauhinia tomentosa* against pathogenic fungi like *Candida albicans* and *Candida tropicalis* have also been proved (Gopalakrishnan and Vadivel, 2011).

CONCLUSION

It was concluded from the results that the antifungal activity of leaf extracts of *Bauhinia scandens* was located in the diethyl ether fraction.This fraction can be taken up for further investigation by characterizing it chemically. The above findings strongly suggest that leaf extracts of *Bauhinia*

Table 3 : Antifungal screening of different fractions obtained from leaf extracts of *Bauhinia scandens* against *Rhizoctonia oryzae* (by agar-cup method)

Test fungus	Dose (mg/ml)	Diameter of inhibition zone (cm)			MIC (mg/ml)		
		PE	DE	Chl	PE	DE	Chl
Rhizoctonia oryzae	Control	0	0	0			
	25	0	0	0			
	50	0	1.1	0	0	50	0
	100	0	2.1	0			
	200	0	2.7	0			
LSD at 5%			0.125				
S.E.			0.042				

PE = Petroleum ether fraction, DE = Diethyl ether fraction, Chl = Chloroform fraction
Each treatment was replicated thrice and mean values were taken.

scandens can be cited as a potential non-hazardous antifungal agentagainst plant pathogens like *Helminthosporium oryzae*, *Fusarium oxysporum* and *Rhizoctonia oryzae*.

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