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Effect of antifungal activity of seven species of *Bauhinia* against *Helminthosporium oryzae, Fusarium oxysporum* by spore germination method and *Rhizoctonia oryzae, Aspergillus niger* by agar cup method

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ABSTRACT

The antifungal activity of seven different species of *Bauhinia* namely *Bauhinia acuminata* L., *Bauhinia variegata* L., *Bauhinia purpurea* L., *Bauhinia scandens* L., *Bauhinia vahlii* W. and A., *Bauhinia racemosa* Lam, *Bauhinia malabarica* L. were tested against four plant pathogenic fungi like *Helminthosporium oryzae*, *Fusarium oxysporum* by spore germination method and *Rhizoctonia oryzae*, *Aspergillus niger* by agar cup method respectively. The dried plant leaves were extracted with three liters of 50% of aqueous ethanol at room temparature for seven days. The individual extract was filtered separately. Each extract was charcoalised and concentrated under reduced pressure and a dark brown residual solid was obtained in each case. The residue obtained in each case was diluted and subjected to antifungal assay for locating the antifungal properties of each species.

KEYWORDS

Antifungal activity;
Bauhinia scandens L.;
Helminthosporium oryzae;
Fusarium oxysporum;
Rhizoctonia oryzae;
Aspergillus niger.

INTRODUCTION

Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer (or) modern antibiotics that have been produced in the last three decades[8,14]. Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in disease treatment found more especially in the developing countries cannot be over emphasized[24]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine[4,12]. Legumes are used medicinally in different countries and are sources of many potent and powerful drugs[9]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts of legumes are used including root, stem, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in small quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[23]. Although hundreds of plant species have been tested for antimicro-

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bial properties, the vast majority of them have not been adequately evaluated[13]. Herbal remedies are often sought by patients with chronic disease especially patients with cancers to provide symptom relief[11,19,22]. Barnes et al.[19], have also reported that, natural product was one of the therapies most commonly used by adults and children in the U.S. Increase in death associated with cancer[7], the callous side effects of some of the cancer chemotherapies[15] and the recurrence of drug-resistant tumors as well as the lack of selectivity of anticancer drugs[18], have triggered the search for more natural cancer fighting agents, particularly those derived from plants. The need for a continuous search for novel natural products that can act as cancer chemopreventive and/or chemotherapeutic agents, have triggered an increase in the interest on plant-derived secondary metabolites, with potential anticancer activity[10].

Bauhinia (a member of subfamily Caesalpinoidae or Caesalpiniaceae), a genus of 250 species of trees, shrubs and climbers, distributed throughout the tropical region with nearly 40 species occurring in India, has gained a position for medicinal and other economic values. *B. purpurea* is native to Southern China and India, and have been used to treat stomach tumors, ulcers, wounds, glandular swellings, diarrhea and fever[25]. There was little report on the anti-inflammatory activities of the aqueous extract of *B. purpurea* leaves and phytochemistry study has also revealed the presence of flavonoids, triterpenes, tannins and steroids[25]. The plant *Bauhinia* contain kaempferol, quercetin and isorhamnetin[4] havepacharin and bauhiniastatins 1-4[20] and dihydrodibenzoxepins (1-8), a dihydrobenzofuran, a novel spirochromane-2,1'-hexenedione and a new bibenzyl[21]. Furthermore, Boonphong et al.[21] demonstrated that, some of the isolated compounds exerted anti-mycobacterial, antimalarial, antifungal, anti-inflammatory activities.

There are some previous reports on anti-tumour activity of 1-O-alkyl glycerol isolated from the leaves of *Bauhinia scandans* L.[3] and anti-microbial activity[1] from this laboratory. In this study we have studied the antifungal effect of seven different species of *Bauhinia* against four plant pathogenic fungi like *Helminthosporium oryzae, Fusarium oxysporum, Rhizoctonia oryzae, Aspergillus niger* by spore germination and agar cup method.

EXPERIMENTALS

Plant collection

Healthy, disease free, mature leaves of *Bauhinia acuminata* L., *Bauhinia variegata* L., *Bauhinia purpurea* L., *Bauhinia scandens* L., *Bauhinia vahlii* W. and A., *Bauhinia racemosa* Lam, *Bauhinia malabarica* L. were collected from the Sevak gram of West Bengal (500 feet higher from sea level) during the month of September.

Preparation of plant extracts

About 2g of fresh, healthy and sun dried leaves of *Bauhinia acuminata* L., *Bauhinia variegata* L., *Bauhinia purpurea* L., *Bauhinia scandens* L., *Bauhinia vahlii* W. and A., *Bauhinia racemosa* Lam, *Bauhinia malabarica* L. were ground to a fine powder and then extracted separately for three times with three liters of 50% of aqueous ethanol at room temparature for seven days. The individual extract was filtered separately. Each extract was charcoalised and concentrated under reduced pressure and a dark brown residual solid was obtained in each case. The residue obtained in each case was then subjected to antifungal assay for locating the antifungal properties of each species.

Preparation of sample solutions

The dark brown solid residual mass obtained after concentrating the crude extract separately from each species was subjected to antifungal bioassay. The test solution was prepared by dissolving the dark brown residual mass in a few drops of propylene glycol and then diluting with sterile water[16] in the concentrations of 50mg/ml, 100 mg/ml, 200 mg/ml for each species. A few drops of propylene glycol diluted with sterile water were used as control. All the dilutions were sterilised by filtration using membrane filter (0.02μ pore size).

Fungal strains

Pure cultures of *Helminthosporium oryzae*, *Fusarium oxysporum*, *Rhizoctonia oryzae* and *Aspergillus niger* were procured from the Depatmental stock culture, Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal. All the fungi were

grown on PDA medium (pH- 6.3) and incubated at 28°C.

Media preparation

The potato tubers were peeled off and weighed for about 250 g tubers were chopped into small pieces into the sterile conical flask. After boiling the supernatant were collected and dextrose (20g) with agar (20g-Microbiology Grade) to dissolve the ingredients. The pH of the medium was adjusted to 6.3. The total volume of the medium was adjusted toone liter. Finally the medium was sterilized in autoclave at 121^0C for 17 minutes.

Screening for antifungal assay

(a) Spore germination method

Different dilutions of test solutions obtained from each species of *Bauhinia* was separately placed on sterilized grooved slides to which one drop of fungal spore suspension (30-40 spores/ microscopic field, at 400 X magnification) each of *Helminthosporium oryzae* and *Fusarium oxysporum* of seven days old culture was added. One control was prepared identical to these and taking propylene glycol instead of test solution. These were then incubated in humid condition for 24 hours at 23^0C -28^0C. All the above observations were taken in triplicate on each fungus/ extracts/ concentration combinations. The numbers of spores germinated were counted and the percentage of inhibition of spore germination was calculated as follows-

$$\text{Percent inhibition of spore germination} = \frac{\text{Total number of inhibited spores}}{\text{Total number of spores}} \text{X100}$$

(b) Agar cup method

Antifungal activity was screened by agar cup method[5,6,17,]. Diffenent concentrations (50 mg/ml, 100 mg/ml, 200 mg/ml) of sample solution obtained from each species were tested against two plant pathogenic fungi like *Rhizoctonia oryzae* and *Aspergillus niger* to access their antifungal nature. The PDA medium was poured in to the sterile petri plates and allowed to solidify under the sterile environment of the laminar air flow cabinet. The test fungal cultures were evenly spread over the media by sterile cotton swabs. Then wells of 9 millimeter were made in the medium using sterile cork borer. 100 µl volume of each extract obtained from seven different species of *Bauhinia* having different concentrations (50 mg/ml, 100 mg/ml, 200 mg/ml) were transferred into the separate wells which was made within the PDA medium. Plates containing the pure cultures of *Rhizoctonia oryzae* and *Aspergillus niger* were allowed to incubated at 29^0C for 24-48 hours. After the incubation period was over the plates were observed for formation of clear inhibition zone around the well indicated the presence of their antifungal nature. The zone of inhibion was recorded in millimeter scale. The final measurement was taken when the control reached the full size within the petridish. If a culture grew in an irregular shape, two or more measurements were made and an average was recorded. From the growth of the diameter of the fungal colony, the effective concentration for colony growth inhibition was calculated. One control set was prepared identical to these and taking propylene glycol instead of test solution. All the above observations were taken in triplicate on each fungus/ extracts/ concentration combinations.

RESULTS AND DISCUSSION

Antifungal efficacy of crude extracts of seven different species of *Bauhinia* against *Helminthosporium oryzae* and *Fusarium oxysporum* by spore germination method

TABLE 1 indicates that the crude extracts were administered in the antifungal test (by spore germination method) against *Helminthosporium oryzae* and *Fusarium oxysporum* in three different doses viz., 50 mg/ml, 100 mg/ml and 200 mg/ml. *Bauhinia scandens* L. produced 60%, 82.35%, 96.87% inhibition of spore germination of *Helminthosporium oryzae* on administration of 50 mg/ml, 100 mg/ml and 200 mg/ml of the crude extract respectively. *Bauhinia variegata* L. showed 52.94% inhibition at 50 mg/ml, 72.85% inhibition at 100 mg/ml, 81.22% inhibition of spore germination at 200 mg/ml. *Bauhinia malabarica* L. exhibited 38.88% inhibition at 50 mg/ml, 41.66% inhibition at 100 mg/ml, 54.28% inhibition of spore germination at 200 mg/ml. The remaining species of *Bauhinia* showed negligible inhibition of spore germination of *Helminthosporium oryzae*.

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TABLE 1 : Antifungal efficacy of crude extracts of seven different species of *Bauhinia* against *Helminthosporium oryzae* and *Fusarium oxysporum* by spore germination method.

Species screened	Dose (mg/ml)	Total number of spores of *Helminthosporium oryzae*	Total number of spores of *Fusarium oxysporum*	Total number Of inhibited spores of *Helminthosporium oryzae*	Total number of inhibited spores of *Fusarium oxysporum*	Inhibition percentage of *Helminthosporium oryzae*	Inhibition percentage of *Fusarium oxysporum*
control	0	35±0.35	30±0.35	0	0	0	0
Bauhinia acuminata L.	50	37±0.22	33±0.67	0	0	0	0
Bauhinia acuminata L.	100	38±0.33	33±0.45	3±0	0	7.89	0
Bauhinia acuminata L.	200	35±0.22	37±0.55	4±0.07	3±0.3	11.42	8.1
Bauhinia variegata L.	50	34±0.32	39±0.56	18±0.45	0	52.94	0
Bauhinia variegata L.	100	35±0.35	38±0.07	29±0.67	2±0.2	72.85	5.26
Bauhinia variegata L.	200	36±0.18	38±0.04	35±0.7	5±0.02	81.22*	13.15
Bauhinia purpurea L.	50	37±0.33	37±0.45	0	0	0	0
Bauhinia purpurea L.	100	34±0.22	34±0.11	0	3±.9	0	8.82
Bauhinia purpurea L.	200	32±0.56	35±0.45	1±0	4±0.07	3.12	11.42
Bauhinia vahlii W. and A.	50	35±0.22	32±0.07	0	0	0	0
Bauhinia vahlii W. and A.	100	38±0.35	33±0.11	2±0	1±0.6	5.26	3.03
Bauhinia vahlii W. and A.	200	34±0.45	30±0.22	5±0.5	3±0.3	14.7	10
Bauhinia malabarica L.	50	36±0.55	36±0.11	14±0.33	0	38.88	0
Bauhinia malabarica L.	100	36±0.56	35±0.33	15±0.07	1±0.0	41.66	3.12
Bauhinia malabarica L.	200	35±0.07	36±0.45	19±0.35	3±0.11	54.28	8.33
Bauhinia racemosa Lam.	50	33±0.33	37±0.67	0	0	0	0
Bauhinia racemosa Lam.	100	32±0.35	36±0.07	0	0	0	0
Bauhinia racemosa Lam.	200	34±0.18	38±0.71	2±0.11	0	5.26	0
Bauhinia scandens L.	50	35±0.33	36±0.71	21±0.35	22±0.22	60	61.1
Bauhinia scandens L.	100	34±0.56	35±0.45	28±0.56	28±0.07	82.35	80**
Bauhinia scandens L.	200	32±0.45	35±0.45	31±0.45	34±0.35	96.87*	97.14**

The observed Values were expressed as mean ± standard deviation. Calculation was done with the help of spread sheet software Mocrosoft Excel 2010; *Indicates significance at (P<0.01); **Indicates significance at (P<0.05).

When the effect of crude extracts of seven species of *Bauhinia* were examined against spore germination of *Fusarium oxysporum*, it was found that *Bauhinia scandens* L. exhibited 61.10% inhibition at 50 mg/ml, 80% inhibition at 100 mg/ml, 97.14% inhibition of spore germination at 200 mg/ml. The rest of the species showed least activity against *Fusarium oxysporum*.

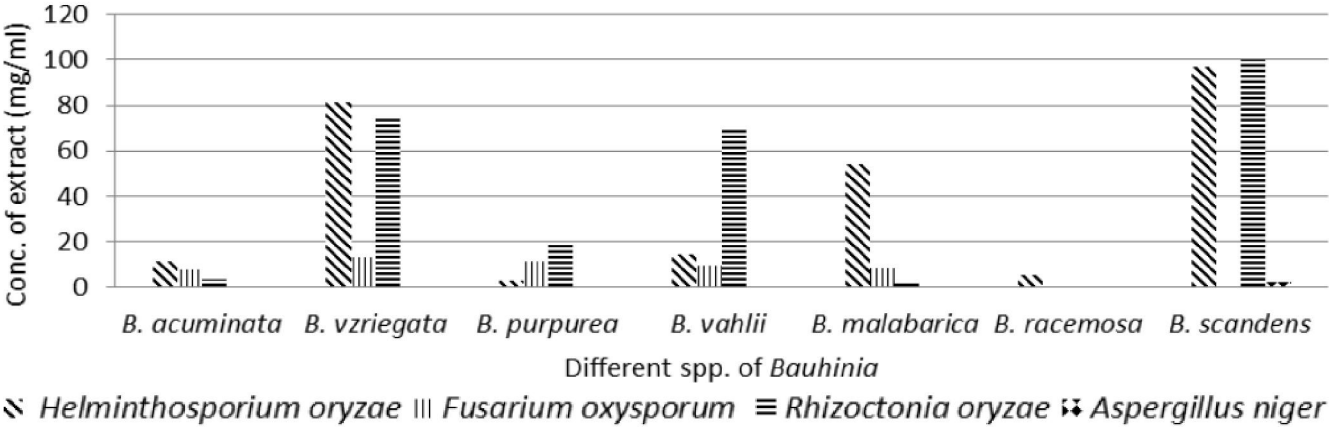

Chart 1 : Comparative account of antifungal activities of crude extract (200mg/ml) of the different spp. Of *Bauhinia* against four pathogenic fungi.

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TABLE 2 : Antifungal efficacy of crude extracts of seven different species of *Bauhinia* against *Rhizoctonia oryzae* and *Aspergillus niger* by agar cup method.

Species screened	Dose (mg/ml)	Diameter of inhibitory zone (in cm) of *Rhizoctonia oryzae*	Diameter of inhibitory zone (in cm) of *Aspergillus niger*	Inhibition percentage of *Rhizoctonia oryzae*	Inhibition percentage of *Aspergillus niger*
Control	1ml	0	0	0	0
Bauhinia acuminata L.	50	0	0	0	0
Bauhinia acuminata L.	100	0	0	0	0
Bauhinia acuminata L.	200	0.32±0.2	0	3.66	0
Bauhinia variegata L.	50	6.32±0.1	0	70.3	0
Bauhinia variegata L.	100	6.56±0.7	0	72.92	0
Bauhinia variegata L.	200	6.79±1.2	0	75.53**	0
Bauhinia purpurea L.	50	0.85±1.6	0	9.46	0
Bauhinia purpurea L.	100	1.62±1.4	0	18.1	0
Bauhinia purpurea L.	200	1.8±1.8	0	20.06*	0
Bauhinia vahlii W. and A.	50	5.32±0.7	0	59.21	0
Bauhinia vahlii W. and A.	100	5.76±2.2	0	64.11	0
Bauhinia vahlii W. and A.	200	6.35±1.5	0	70.63**	0
Bauhinia malabarica L.	50	0	0	0	0
Bauhinia malabarica L.	100	0	0	0	0
Bauhinia malabarica L.	200	0.18±0.5	0	2.08	0
Bauhinia racemosa Lam.	50	0	0	0	0
Bauhinia racemosa Lam.	100	0	0	0	0
Bauhinia racemosa Lam.	200	0	0	0	0
Bauhinia scandens L.	50	9±0	0	100**	0
Bauhinia scandens L.	100	9±0	0	100	0
Bauhinia scandens L.	200	9±0	0.21±0.2	100	2.4

The observed Values were expressed as mean ± standard deviation. Calculation was done with the help of spread sheet software Mocrosoft Excel 2010; *Indicates significance at (P<0.01); **Indicates significance at (P<0.05).

Antifungal efficacy of crude extracts of seven different species of *Bauhinia* against *Rhizoctonia oryzae* and *Aspergillus niger* by agar cup method

TABLE 2 indicates the details of antifungal assay by agar cup method against *Rhizoctonia oryzae* and *Aspergillus niger*. Here, again 100% inhibition of colony growth of *Rhizoctonia oryzae* was shown by *Bauhinia scandens* L. at 50 mg/ml, 100 mg/ml, 200 mg/ml. *Bauhinia variegata* L. showed 70.30%, 72.92%, 75.53% and *Bauhinia vahlii* W. and A. showed 59.21%, 64.11%, 70.63% inhibition at 50 mg/ml, 100 mg/ml, 200 mg/ml of the crude extract respectively. *Bauhinia malabarica* L., *Bauhinia racemosa* Lam. and *Bauhinia acuminata* L. showed negligible or no activity against *Rhizoctonia oryzae.*

None of the experimental species of *Bauhinia* showed any activity against *Aspergillus niger*.

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