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Short Communication

Antimicrobial, cytotoxic and antioxidant activities of *Barringtonia acutangula* (L)

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ABSTRACT: *Barringtonia acutangula* (Indian Oak) is an important medicinal plant of Bangladesh and India for its antiasthmatic, anticold, antimalerial, antidiarrehal, antiprotozoal, antidysentery stimulating properties. The coarsely powdered stem bark of *B. acutangula* was extracted with petroleum ether, ethyl acetate and methanol for 8 days. The concentrated extract was then named as petroleum ether, ethyl acetate and methanol extracts respectively. The soluble portions were further fractionated with gel permeating chromatography using different solvent system. The extracts and compounds isolated from ethyl acetate were evaluated for their antibacterial, antifungal, cytotoxic and antioxidant activities. The antibacterial and antifungal activities were evaluated using the disc diffusion method using varied period of incubation while the screening of cytotoxic activity was done using brine shrimp lethality bioassay. In antibacterial testing, it was found that most microorganisms showed sensitivity to petroleum ether and ethyl acetate extracts while a large quantity of organism showed sensitivity to isolated compound from the extract. Besides, the ethyl acetate extract and its fraction 1, 2 has shown promising zone of inhibition against the fungi with few exception. In cytotoxicity test, the mortality rate of brine shrimp was found to be increased with the increase of the concentration for each sample. The LC_{50} values of plant ethyl acetate extract was found to be 12.58 µg/ml which clearly indicate that the extract was toxic to the brine shrimp. It was found that the methanol and ethyl acetate extracts showed higher free radicals scavenging activity with IC_{50} 3.84 µg/ml and 9.83 µg/ml respectively. From this investigation it has been found that this plant showed a number of promising biological activities including antibacterial, antifungal cytotoxic and antioxidant. This is first report of the biological activity of the stem bark of *B. acutangula*. Therefore, further investigation is required to establish this plant as a good source of natural medicine.

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INTRODUCTION

Plants have formed the basis for traditional systems of medicine, which have been used for thousands of years in many countries and extensively documented. The number of higher plant species (Angiosperms & Gymnosperms) on these planet estimated at 250,000 [1] of which only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically [2].

Barringtonia acutangula (Lecythidaceae) is a shrub or small bushy, multi-stemmed,evergreen tree, with spreading crown, which occur in the fresh water swamp forests of Sylhet ("Haor"). Also occur along the cannels and roadside ditches throughout the country.

There are various medicinal uses of *B. acutangula*. Juice of the leaves is given in diarrhoea and dysentery. Roots are cooling, aperient and expectorant, stimulating and emetic; supposed to be similar to Chincona in its properties. The fruit is astringent to the bowels, lactagogue, vulnerary, anthelmintic; useful in biliousness, bronchitis, sore eyes, gleet, lumber pain, nasal catarrh,coughs, cold, asthma and hallucinations. Seeds are carminative and emetic; with juice of ginger used in catarrh of the nose and respiratory passages; applied to chest to relieve pain and cold; to the abdomen to relieve colic and flatulence. The bark is given as astringent in diarrhoea and blenorrhagia, and as a febrifuge in malaria. Rubbed with water on the chest to relieve pains and colds, to the abdomen to relieve colic and flatulence. Fruit used as anthelmintic and as astringent in gingivitis. Decoction of bark used as mouthwash in gum problems. EtOH(50%) extract of root is hypoglycaemic and that of stem bark is antiprotozoal [3].

Crude extracts showed good activity against all test organisms: Gram negative and positive bacteria and two fungi. It was especially effective against *Bacillus subtilis* and *Aspergillus niger*, comparable to kanamycin and fluconazole [4]. Extract of *B. acutangula* was non-toxic, and possesses antimalarial activity justifying indigenous medicinal use in Sri Lanka [5]. *B. acutangula* may act as a chemopreventive agent, providing antioxidant properties and protection from free radicals [6]. *Barringtonia acutangula* leaves exhibit potential antibacterial and antifungal activity[7].

An ethyl alcohol extract of coarsely powdered leaves evaluated by various assays showed CNS depressant behavior with maximum inhibition of neuronal activity [8]. Study evaluated a methanol extract of seeds and leaves for antinociceptive, antidiarrheal and neuropharmacological effects in mice. Both extracts showed dose-dependent antinociceptive effect, significant inhibition of defecation in diarrheal models and decreased motor activity in both

hold cross and open field test [9]. The aqueous extract of fruit showed significant hypoglycemic potential comparable to standard drug glibenclamide [10].

Since this plant has a number of biological activities, so present study was undertaken to investigate its antibacterial, antifungal , cytotoxic and antioxidant activities simultaneously for the first time in Bangladesh.

METHODS AND MATERIALS

The plant materials (stem bark) were collected from shariatpur in the month of March 2013. After drying in sunlight and grinding. Plant material is extracted by petroleum ether, ethyl acetate and methanol each time for 7 days. It was then filtered and concentrated using a buchi rota vapour 6g petroleum ether, 10g ethyl acetate, 8g methanol extracts were collected. The antibacterial assay was performed in vitro disc diffusion method [11].

Fifteen pathogenic bacteria were selected for the test and pure culture of the bacteria were collected from the Department of Microbiology and Institute of Nutrition and Food Science of the University of Dhaka and later the culturing were mentioned in the Department of Biochemistry and Molecular Biology. For experimental purpose sterilized filter paper disc (6mm) were taken by this foreceps in the plants. Petroleum ether, Ethyl acetate and Methanol extracts and pure compounds, BA-3 and BA-4 (50 µg/disc) were applied in the disc with the help of micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of the solvent. Cyprofloxacin (30 µg/disc) was used as standard disc for comparison purpose and control disc also placed. Finally the plates were incubated at 37^0 C for 24 hours in an incubator. The discs were then placed in such a way that each one was not close within 15mm to the edge of the plates and enough apart to prevent overlapping of the zone of inhibition. After incubation the antibacterial activity of the test samples was determined by measuring the diameter zone of inhibition in terms of mm with a transparent scale.

The antifungal activity of the plant extracts was also performed by disc diffusion method using the same procedure as antibacterial activity test but incubation period of the plates containing different fungus was 48 hours at room temperature. Potato Dextrose agar (PDA) was used to gain the maximum growth of fungus at optimum condition (P^H 5.6 , room temperature) and grisofulvin was used as a standard.

Cytotoxic activity was performed by Brine Shrimp Lethality test [12]. The lethality test involves the culture of brine shrimp, Artimia salina nauplii, the nauplii should be grown in the sea water . Sea water contains 3.8% NaCl. Accordingly 3.8% NaCl solution was made by desolving 38g NaCl in 1000ml distilled water and filtered off. The P^H of the brine water thus maintained between 8 to 9 using $NaHCO_3$. Constant temperature (37^0 C) and sufficient light were ensured to give sufficient aeration. After 48 hours , matured shrimp as nauplii (lervae) was collected and 30 nauplii were used for each concentration (µg/ml) of experiment. For the sample extract 4mg were initially dissolve in 100 ml of pure dimethyl sulfoxide (DMSO) to make hydrophilic before adding 1.9 mL of water to get a concentration of 400 µg/ml which was used as a stock solution A. Then a series of dilution was made to produce 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.563 µg/ml, 0.781 µg/ml. For each concentration, one test tube containing the same volume of DMSO diluted upto 10 ml with sea water and 30 shrimp nauplii was used as a negative control group. After 24 hours, the test tubes were observed that the number of survived naulpii in each test tube was counted by probit analysis to determine LC50 . Vincristine sulfate was used as the positive control and DMSO as the negative control for the brine shrimp nauplii.

Free radical scavenging activity of the plant extractions against stable DPPH (2,2 diphenyl -2-picryl hydrazyl) were determined spectrophotometrically [13]. The antioxidant activity of petroleum ether extract, ethyl acetate extract and methanol extract were evaluated by comparing with the standard (ascorbic acid) on the basis of scavenging activity of the stable DPPH free radical. 175 ml solution of DPPH (20 µg/ml) was prepared by dissolving 3.5 mg of DPPH in spectral grade methanol in a 250 ml volumetric flask. Ascorbic acid with the same concentration (20 µg/ml) was used as a standard. Different concentrated solutions of extracts were made by dissolving dried extracts ranging from 1 µg/ml to 500 µg/ml in spectral grade methanol solvent. DPPH solution (freshly prepared) was added to each solution and kept in dark for 30 minutes to complete the reaction. After 30 minutes, absorbance of each test tube was taken by a spectrophotometer at 517nm and inhibition of free radical DPPH oxidant was calculated in percentage

(1%) as follows: $(I\%=1-A_{sample}/ A_{blank}) \times 100$

The IC50 was determined from % inhibition Vs concentrated graph (Table 3).

RESULT AND DISCUSSION

From the above experiment it was found that the gram positive bacterial strains exhibited promising activity against petroleum ether extracts except *Bacillus polymyx*. Among the gram negative bacteria all strains also showed promising activity except *Shigella boydii* and *Klebsiella sp.* Among gram positive bacteria, all strains displayed good activity against petroleum ether except *Bacillus cereus* and *Bacillus polymyx*. The gram negative bacteria also showed promosing activity except *Shigella boydii, Shigella flexnari type-1, Shigella dysenteriae, Klebsiella sp and Salmonella paratyphi*. The methanol extract did not show any activity

Shigella boydii is not sensitive to BA-3, *Escherchia coli* and *Bacillus polymyx* were also not sensitive to BA-3. All the other gram positive bacteria were sensitive to BA-3.In case of compounds BA-4 all gram positive bacteria are sensitive except *Bacillus polymyx* and *Staphylococcus aureus* but some gram negative bacteria such as *Shigella boydii* and *Klebsiella* species were not sensitive to the compounds.

The crude petroleum ether, ethyl acetate and methanol extracts and compounds were investigated against fungi. This crude extract was used in concentration 3µg /disc and the activity observed is list bellow in table 2. The petroleum ether and ethyl acetate extracts and compounds have shown promising zone of inhibition against the fungi except *Aspergillus fumigate, Rhizopus oryzae* and *Candida krusi*. The grisofulvin showed antifungal activity.

Table1: Antibacterial and antifungal activity of the extracts and compounds of *Barringtonia acutangula*

Organism (gram positive bacteria)	Zone of inhibition (mm) by petroleum ether extract	Zone of inhibition (mm) by ethyl acetate extract	Zone of inhibition (mm) by methanol extract	Compounds BA-3 (zone of inhibition in mm)	Compounds BA-4 (zone of inhibition in mm)	Zone of inhibition shown by standard (ciprofloxacine)
Bacillus cereus	8	-	-	8	9	18
Bacillus polymyx	-	-	-	-	-	15
Bacillus subtilis	10	8	-	7	8	18
Bacillus megaterium	9	7	-	9	7	16
Sarcina lutea	11	8	-	6	8	20
Staphylococcus aureus	12	9	-	-	-	19
Organism (gram negative bacteria)						
Vibrio minicus	11	10	-	9	7	19
Vibrio cholera	12	9	-	7	8	21
Salmonela typhi	10	9	-	8	-	20
Shigella boydii	-	-	-	-	-	22
Shigella flexnari type-1	9	-	-	6	7	19
Shigella dysenteriae	8	-	-	8	9	18
Pseudomonus sp.	13	10	-	7	9	17
Klebsiella sp.	-	-	-	-	-	15
Escherchia coli	14	11	-	-	8	18
Salmonella paratyphi	10	-	-	9	8	16

Table 2: Antifungal activity of the extracts and compounds of *Barringtonia acutangula*

Name of fungus	Zone of inhibition (mm) by petroleum ether extract	Zone of inhibition (mm) by ethyl acetate extract	Zone of inhibition (mm) by methanol extract	Compounds BA-3 (zone of inhibition in mm)	Compounds BA-4 (zone of inhibition in mm)	Zone of inhibition shown by standard (Grisofulvin)
Candida arrizae	10	8	-	10	9	12
Aspergillus fumigate	-	-	-	-	-	-
Aspergillus niger	9	7	-	7	10	12
Rhizopus oryzae	-	-	-	-	-	8
Candela albicans	8	8	-	10	7	11
Saccharromyces cerevisae	7	-	-	-	6	-
Candida krusii	-	-	-	-	-	7

Two isolated compounds BA-3 and BA-4 were tested against fungi concentration of 100 micro gram per disc for each. The antifungal activity was determined after 48h of incubation at room temperature and the result is listed bellow in table. As the control discs did not show any zone of inhibition their data was not mentioned in the table.

Bioactive compounds are almost always toxic at higher dose. *In vivo* lethality in a simple zoological organism can be used as a marker for screening and fractionation in the discovery of new bioactive natural products as an antitumor agent. In this bioassay, the ethyl acetate extract of *Barringtonia acutangula* showed a significant cytotoxic activity in the brine shrimp lethality bioassay indicates that the compounds are biologically active.

Test sample showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase of the concentration for each sample. The percent mortality of the brine shrimp nauplii was calculated for every concentration for each sample. A plot of log of concentration of the sample versus percent mortality showed an approximate linear correlation between them. From the graph the LC_{50} values of plant extract is found to be 12.58 µg/ml. It is evident from the result that the extract was toxic to the brine shrimp.

In DPPH the IC_{50} of methanol extract, petroleum ether, ethyl acetate extracts of *B.acutangula* were evaluated 3.84 µg/ml, 10.50 µg/ml, 9.53 µg/ml respectively.

Table 3: Determination of LC50 of brine shrimp lethality bioassay for plant extract of *Barringtonia acutangula*

Sample No.	Conc of plant extract (µg/ml)	Log C	% of mortality of brine shrimp			Mean	LC_{50}
			Expt 1	Expt 2	Expt 3		
1	400.00	2.602	100	100	100	100	16.6184 µg/ml
2	200.00	2.301	90	100	90	93.33	
3	100.00	2.000	90	90	90	90	
4	50.00	1.699	80	90	80	83.33	
5	25.00	1.398	70	70	70	70	
6	12.50	1.097	60	50	50	53.33	
7	6.25	0.796	40	40	40	40	
8	3.125	0.495	30	20	30	23.33	
9	1.56	0.194	20	20	20	20	
10	0.00	-----	00	00	00	00	

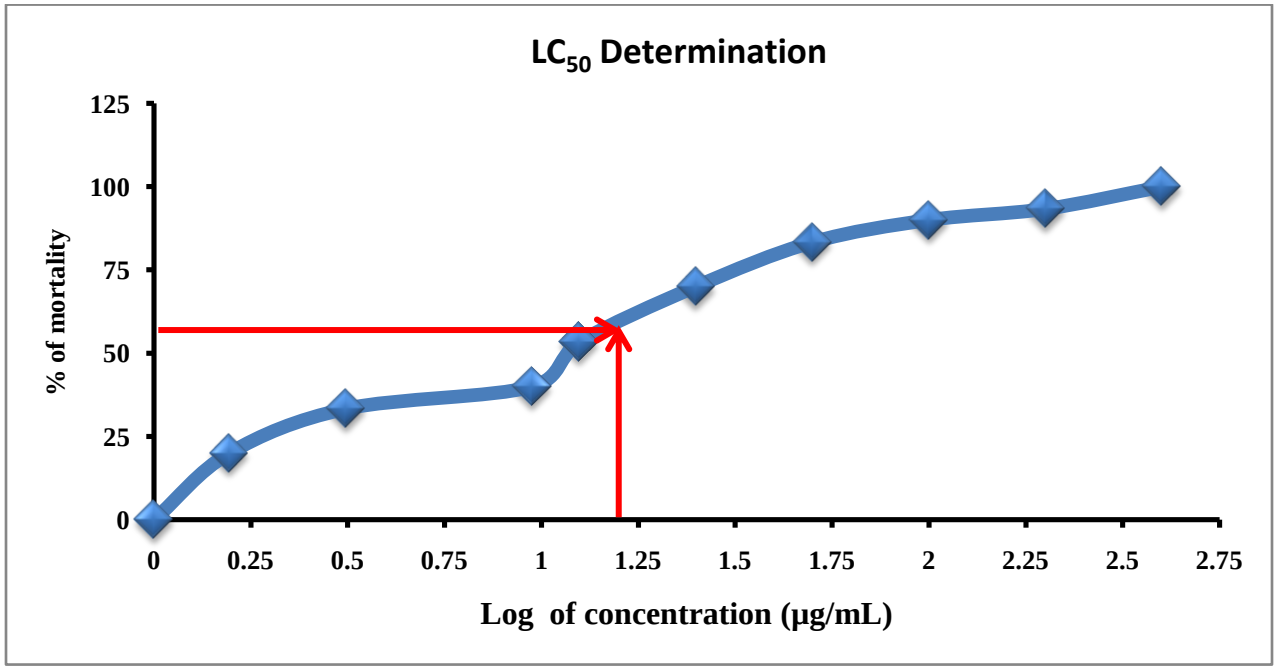

Figure 1: Graphical presentation for LC_{50} of crude extract

Table 4: Antioxidant scavenging of *B.acutangula* extraction.

Test sample	IC_{50} (µg/ml)	Regression line	R2	Activity
Ascorbic acid	10.92	Y=25.74+31.07	0.950	Standard
Petroleum ether extract	10.50	Y=28.64+20.25	0.988	Moderate
Ethyl acetate extract	9.53	Y=27.99+22.59	0.996	Higher
Methanol extract	3.83	Y=21.63+37.36	0.985	Highest

The ascorbic acid used as standard show IC50 of 10.92 µg/ml. by comparing the results with ascorbic acid it was found that the methanol and ethyl acetate extraction showed highest free radical scavenging activity with IC50 3.83 µg/ml and 9.53 µg/ml respectively. From this investigation it can be concluded that the plant has potential role in scavenging free radicals due to antioxidant activity.

From this investigation it has been found that this plant showed a number of promising biological activities including antibacterial, antifungal and cytotoxic and antioxidant. Therefore it would be a good source of natural medicine. This is the first report of the biological activity of the stem bark of this plant. To achieve this further investigation on this plant is required.

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