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BIOACTIVITY ASSESSMENT OF A WIDELY DISTRIBUTED MANGROVE PLANT: *BARRINGTONIA ACUTANGULA*

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Abstract

One of the widely distributed mangrove plant *Barringtonia acutangula* (L) (Family: Lecythidaceae) has been used in folk medicine in the treatment of tuberculosis, skin disease, inflammation, hemorrhoids and diarrhea.The aim of the investigation was to evaluate the phytochemical, antibacterial, antioxidant ,cytotoxic and anthelmintic activity of bark extract of *Barringtonia acutangula* (L) . In order to investigate the antioxidant activity 2,2-diphenyl-1-picryl-hydrazil (DPPH) free radical scavenging was used. Antibacterial activity was evaluated by disc diffusion method. Cytotoxic activity was evaluated by brine shrimp lethality bioassay. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate. Phytochemical analysis of the extract indicated the presence of gums, glycosides, alkaloids, and flavonoids. Extract showed DPPH scavenging activity (53.80 µg/mL) that was comparable to standard ascorbic acid (20.83 µg/mL). In disc diffusion assay experimental extract showed potent antibacterial activity. In the brine shrimp lethality bioassay the extract showed LC_{50} at 5.82 µg/ml and LC_{90} at 80 µg/ml. Extracts showed moderate anthelmintic activity in a dose-dependent manner.

Keywords: *Barringtonia acutangula, Antioxidant activity, Antibacterial activity, cytotoxic activity, anthelmentic activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH).*

Introduction

Mangrove plants are specialized plants that grow in the tidal coasts of tropic and subtropic regions of the world. In Bangladesh, Mangrove forest- the Sundarbans is consisting of a versatile ecosystem where natural extracts from plants show several pharmacological actions and provide contention for their traditional uses in local folk [1].

Barringtonia acutangula(L.)Gaertn (Lecythidaceae) is a 5–8 m tall, evergreen tree with obovate leaves, rough fissured grey bark, red flowers on pendulous racemes, and about 20 cm long four-sided fruits. The plant is also called Indian Oak and is locally known as Hijal in Bangladesh. It grows in the tropical areas of Southeast Asia, Australia, and Africa, frequently occurring on the bank of rivers, ponds, lakes, and low-lying areas [2][3]. The plant is traditionally used in diarrhea, flatulence, hemorrhoids, inflammation, skin diseases, leprosy, arthralgia, dysmenorrhea, and as anthelmintic [4][5].The leaf juice is used in diarrhea and seeds are used in chest pain, cold, abdominal colic, and gonorrhea [3]. Acutangulic acid, saponins, acutagenol A, acutagenol B, barringtogenols B, C, and D, stigmasterol, β-sitosterol, and β-amyrin have been isolated from leaf [6]. The leaf also contains a good amount of phenolic compounds [7]. Seeds are reported to contain a triterpenoid glucoside [8]. Pharmacological properties such as antibacterial activity of stem bark and twigs [9]. antioxidant and hepatoprotective activities of leaves [10][11]. hypolipidemic and antioxidant activities of root , [12][13] and anti-scorpion venom activity have been reported [14]. However, there are few experimental studies on bark of *Barringtonia acutangula.* So, the present study has been designed to investigate the methanol extracts of bark of *Barringtonia acutangula* (family: Lecythidaceae) for their phytochemical group determination and selected pharmacological activities (antibacterial, antioxidant, cytotoxic and anthelmintic activity).

Methods

Chemicals and reagents

2, 2-Diphenyl-1-picryldydrazyl (DPPH.), Ascorbic acid and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol were obtained from Merck (Darmstadt, Germany).

Microorganisms

Five species of both Gram positive and Gram negative bacteria were used for antibacterial assay. The bacterial strains used for the investigation were *Salmonella typhi, Bacillius megaterium, Vibrio parahaemolyticus, Bacillus cereus, Vibro mimicus.* The bacterial strains were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B).

Plant materials and extraction

The bark was collected in June, 2019 from Kolaroa, Satkhira, Bangladesh and identified by experts at Bangladesh National Herbarium, Bangladesh. A voucher specimen (DACB 64293) has been submitted there for future reference.

Preparation of crude extract

The collected plant bark were separated from undesirable materials and shade-dried and finally ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). About 400 gm of bark were macerated in 1 liters methanol (98%), for a period of 14 days accompanying routine shaking and stirring. After filtration and evaporation, using rotary vacuum evaporator (Bibby RE200, Sterilin Ltd., UK), the weight of the obtained crude extract was 8.6 gm. Then the crude extract was stored in a refrigerator at 4 °C.

Phytochemical screening

Different phytochemical groups such as carbohydrates, alkaloids, glycosides, flavonoids, tannins, phenols, gums and saponins were identified by characteristic color change using standard chemical tests [15]. Lead acetate, alkaline reagent, ferric chloride and ammonia tests were used for detection of flavonoids. Alkaloids were detected using the Dragendroff's, Mayer's, Hager's and Wagner's tests. For identification of tannin potassium dichromate test, ferric chloride, potassium hydroxide and lead acetate tests were followed. Legal's test, Keller-Kiliani and Borntrager's tests were performed to identify glycosides. Salkowski test and froth test were used to detect

the presence of saponins . Molisch test was performed for detecting the existence of gums in the samples. Carbohydrates are detected by Molisch test, Benedict's test and Fehling's test.

Antioxidant activity

The antioxidant potential of the extracts was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical [16] [17]. At first different concentrations (400-1.57 µg/ml) of crude and fractionated extracts were prepared. In 2mL of each of the different concentration 6mL of freshly prepared DPPH solution (0.004%) was added. They were kept for 30 minutes in dark at room temperature to complete the reaction. Then absorbance was measured at 517 nm against suitable blank. In this assay well known free radical scavenger, ascorbic acid was used as standard. IC50 value, the concentration of sample required to scavenge 50% of the DPPH free radicals was determined by using the following formula:

% inhibition = {(Blank absorbance - Sample absorbance) / Blank absorbance} × 100

Antibacterial activity by Disc diffusion method

Antibacterial activity of was tested by using the disc diffusion method [19]. In this method-measured amount of the test samples were dissolved in definite volumes of solvent to prepare solutions of desired concentration (µg/ml). The sterile Matricel (BBL, Cocksville, USA) filter paper discs were impregnated with known amount of test substances using micropipette and dried. Kanamycin (5µg/disc) was used as standard. Disk of sample, standard and control were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop. The plates were then kept at 400C for facilitating maximum diffusion and then in an incubator for 12-18 hour to allow the growth of the microorganisms. After the incubation period the diameter of the zone of inhibition was measured in term of millimeter. The experiments are carried out duplicate manner.

Cytotoxic activity

Cytotoxic activity of extract was carried out according to the Meyer method [20]. Artemia salina leach (brine shrimp eggs) was used as the test organism. It was hatched in simulated sea water. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. Different concentration of extract (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/mL) was prepared using dimethyl sulfoxide (DMSO) in sea water. A set of seven test tubes were used where 10 shrimps were taken and a solution of different concentration was applied on it. At last, the final volume was adjusted with saline water and kept for 24 h. Vincristine sulfatewas used as standard. The lethal concentration LC50 of the test samples after 24 h was obtained by a plot of percentage of the shrimps kill ed against the sample concentration.

Anthelmintic activity:

The anthelmintic assay was carried as per the method of Ajayieoba *et al* [21].with minor modifications. Earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil, the average size of earthworm being 6-8 cm. They were washed with tap water for the removal of the adhering dirt. The assay was performed on adult earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug. Briefly, 20 ml formulations containing three different concentrations, each of crude methanol bark (5, 10 and 15 mg/ml in double distilled water) were prepared and six earthworms (same size) were placed in it. Both the test solution and standard drug solution were freshly prepared and 'time for paralysis' was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The 'time for death' of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°. A maximum time period of 120 min was ascertained for the paralyzing as well as death time of *Pheretima posthuma* . Piperazine citrate (10 mg/ml) was used as reference standard with distilled water as the vehicle control. From the observations made, a dose dependent

paralytic effect much earlier and the time of death was observed (Table 4).

Statistical analysis

One-way ANOVA followed by Dunnett's test were performed and the results were considered statistically significant when $p < 0.05$. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

Results

Phytochemical screening

Phytochemical studies showed that alkaloids, glycosides, flavonoids and gums were present, while steroids, tannins and saponins were absent in the extract (Table 1).

Antioxidant activity

Extract was subjected to free radical scavenging activity. In this investigation, the leaves extract showed free radical scavenging activity (IC50=53.80 µg/ml) while ascorbic acid was 20.83 µg/ml (Table 2).

Antibacterial activity

The extract showed good activity against *Bacillius megaterium* and *Vibrio parahaemolyticus* but reduced activity against *Bacillus cereus, Vibro mimicus* and *Salmonella typhi* (Table 3).

Cytotoxic activity

The cytotoxic activity of the extracts was assayed by the brine shrimp lethality bioassay test. The effect of the extract was dose dependent. In this assay the bark extracts showed LC50 value of 5.82 µg/mL whereas standard vincristine sulphate showed LC50 value of 0.003 µg/mL. No mortality was found in control group.

Anthelmintic activity

Evaluation of anthelmintic activity was compared with reference standard piperazine citrate. Extracts showed moderate anthelmintic activity in a dose-dependent manner. The methanol extract of bark of *B. acutangula* caused paralysis at 22.33 min. and time of death at 45.00 min. for *Pheretima posthuma* respectively. The reference drug piperazine citrate showed the time of paralysis and time of death as 25.00 and 64.00 min, respectively (Table 4).

Discussion

In the present study, phytochemical tests revealed the existence of alkaloid, flavonoid, gums and glycosides. These phytochemical compounds presence can be correlated to the biological activities of *B. Acutangula.* Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo [22] [23]. In the quantitative DPPH radical scavenging assay, *B. Acutangula* bark extract explored potent free radical scavenging activity (IC50 53.80µg/mL).

The antibacterial activity of *Barringtonia acutangula* is shown in table 3. In the present study,bark of *the Barringtonia acutangula* were tested against Gram-negative and Gram-positive organisms. From the results *Barringtonia acutangula* could inhibit different typical pathogenic bacteria. Thus, *Barringtonia acutangula* could be considered as excellent broad-spectrum antibacterial agents. Phytocompounds extracted from the plant source can serveas a prototype to develop less lethal and efficient drug incontrolling the development of micro-organism. The presence of bioactive compounds in the plant which can be responsible for the observed antibacterial property.

The brine shrimp lethality bioassay has been used extensively in the primary screening of the crude extracts to evaluate the toxicity towards brine shrimps, which could also provide an indication of possible cytotoxic properties of the test materials [20]. It has been established that the cytotoxic compounds generally exhibit significant activity in the brine shrimp lethality bioassay, and this assay can be recommended as a guide for the detection of antitumour and pesticidal compounds because of its simplicity and low cost. Earlier reports in several plant extracts showed a good correlation of this bioassay with the cytotoxic activity. Present study showed that the experimental plant extracts possess moderate amount of cytotoxic activity. From the experiment it can be interpreted that the extracts may contain minor amount of cytotoxic compounds.

Considering the methanol extract of bark showed comparable activity in a dose dependent mannar for anthelmintic activity, it would be important to identify the key phytoconstituents.

However the results of this study affirm the traditional uses of the plant in the treatment of painful conditions such as arthralgia, chest pain, dysmenorrhea, inflammation, hemorrhoids, in diarrhea and psychological disorders.

Conclusion

The present study highlights the possible use of bark extracts of *B. acutangula* as source of antioxidant, antibacterial, anthelmintic agents. Chemical and pharmacological studies are required to isolate the bioactive compounds and elucidate the precise mechanisms responsible for the pharmacological activities of the plant. It seems quite possible that bark of *B. acutangula* contains chemical constituents with antibacterial, antthelmintic, and antioxidant properties which may be used as lead compound for new drug development.

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Table 1: Phytochemical screening of extract

Test Sample	Steroids	Alkaloids	Glycosides	Flavonoids	Tannins	Gums	Saponins
Extract	-	+	+	+	-	+	-

Table 2: IC_{50} values of standard and extract in antioxidant assay

Test Sample	IC $_{50}$ (µg/ml)
Ascorbic acid	**20.83**
Extract	**53.80**

Table 3. Average zone of inhibition of extract against different bacterial strains

Kanamycin (30*)	Control	Extract (250*)	Extract (500*)	Bacterial Strains
28	0	19	22	*Bacillius megaterium*
28	0	11	16	*Bacillus cereus*
26	0	8	11	*Vibro mimicus*
25	0	10	14	*Salmonella typhi*
25	0	18	21	*Vibrio parahaemolyticus*

***** = µg/Disc

Table 4:

Groups	Conc. (mg/ml)	Paralyzing time (min)	Death time (min)
Distilled water	-	-	-
Extract	5	55.16±0.47	70.09±0.39
	10	37.77±0.57	39.66±0.82
	15	22.38±0.64	45.00±0.78
Piperazine citrate	10	25.07±0.88	64.23±0.58

Anthelmintic activity of bark of *B. acutangula*

Results are exprtessed as mean ± SEM, N=3

Fig. 1 : DPPH radical scavenging activity of bark of *B. acutangula*

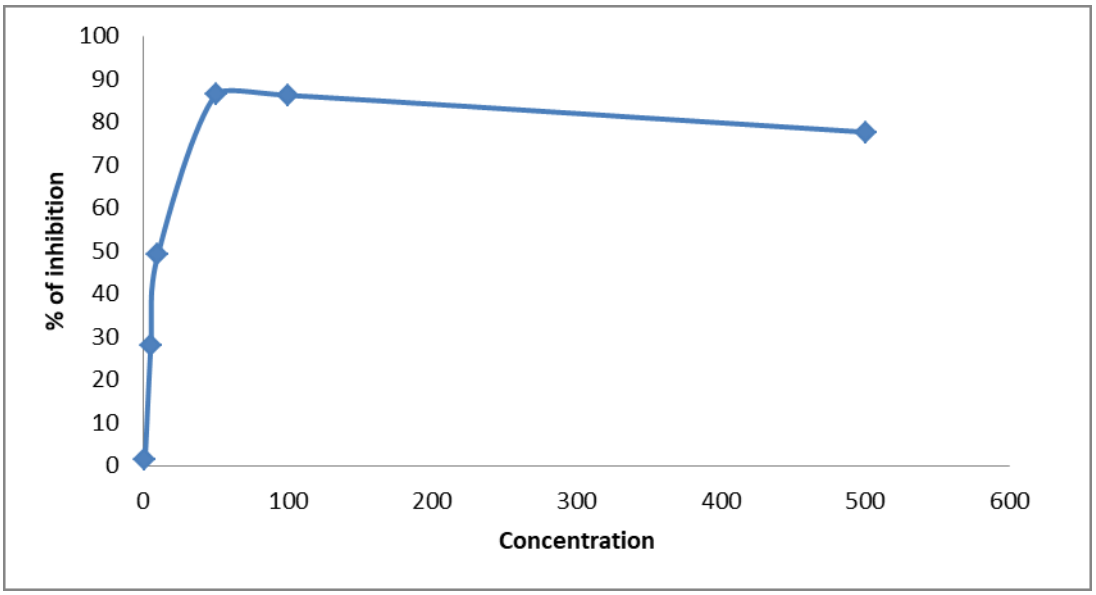

Fig. 2: LC_{50} of bark of *Baringtonia acutangula*.