BIOACTIVITY STUDIES ON BARRINGTONIA RACEMOSA (LAM.) BARK

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Abstract

Barringtonia racemosa (Lam.), a mangrove tree belongs to the family, Lecythidaceae, well known in traditional medicine systems in Bangladesh for the treatment of various ailments. In the present study, several pharmacological investigations were carried out to ascertain bioactivity of the ethanol bark extract. Antioxidant activity of the extract was evaluated by free-radical-scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Acetic acid induced writhing method was utilized to evaluate analgesic activity. For the evaluation of anti-diarrhoeal activity, castor oil induced diarrheal model was applied. Antibacterial activity assessment was performed by Disc diffusion assay. The extract showed IC₅₀ value of 31.90 µg/mL in DPPH assay. The extract showed significant (p<0.001) and dose dependent analgesic activity with 36.3 and 63.8% inhibition of writhing at the doses of 250 and 500 mg/kg body-weight, respectively. The extract exhibited significant (p<0.001) reduction in the total number of faeces as well as prolongation of onset of diarrhoea at both doses of 250 and 500 mg/kg body-weight. In antibacterial test, the extract, showed activity against the bacterial strains namely S. aureus, S. epidermidis, E. coli, S. dysenteriae, V. cholera, and proteus sp. at the doses of 250 and 500 µg/disc. The results suggest that the ethanol bark extract of B. racemosa could be used as potential antioxidant, analgesic, anti-diarrheal, and antibacterial agent and demands further investigations to identify underlying mechanisms responsible for bioactivities.

Key words: Barringtonia racemosa, Lecythidaceae, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, writhing test, disc diffusion assay, castor oil
**Introduction**

A tropical plant, *Barringtonia racemosa* belongs to the family, Lecythidaceae, moderate in size, available in the West Coast of India, Sundarbans, Assam and Andaman Islands, and Malaysia. It is an evergreen tree with potential traditional medicine value in Bangladesh. In Bangladesh, local health practitioners of Sundarbans, use its fruits to treat cough, asthma, and diarrhoea meanwhile the seeds are beneficial in the treatment of colics and ophthalmic problems.

Previous studies, on some of the *Barringtonia* species namely *B. asiatica*, *B. acutangula*, *B. lanceolata*, and *B. edulis*, definitely demonstrate that *B. racemosa* might have potential medicinal properties (1,2,3). In previous report, the ethanol extract of *B. racemosa* roots, has shown potential antibacterial activity against some pathogenic bacterial strains (4). Seed extract of the plant has exhibited promising antitumor property in mice (5). An aqueous extract of bark was reported with antinociceptive activity in rats (6). Leaves extract has shown antioxidant and anti-inflammatory activity (7). Bartogenic acid, isolated from fruits, has shown anti-arthritic activity in rats (8). Previous phytochemical study has revealed the presence of 3,3'-dimethoxy ellagic acid, dihydromytecetin, gallic acid, bartogenic acid, and stigmasterol in ethyl acetate extract of stem bark (9). An oleanane-type isomeric triterpenoids from methanol extract of fruits (10), and two neo-clerodane diterpenoids namely nasimaluns A and B from ethanol extract of roots (11) were isolated and reported.

In the present study, ethanol extract of bark of *B. racemosa*, was subjected to several pharmacological investigations to ascertain its antioxidant, analgesic, anti-diarrhoeal and antibacterial activities in different in vivo and in vitro models.

**Preparation of plant extract**

Shade drying was applied for drying bark and the dried bark was grinded into coarse powder with a suitable mechanical grinder. The powder was stored in an air-tight container, and kept in a cool, dark, and dry place. The powdered plant material was extracted by cold extraction method. Powder material of 300 gm was soaked in 800 mL of ethanol in a glass container for ten days accompanying regular shaking and stirring. After ten days, the extract was separated from the plant debris by filtration with clear cotton plug. The residue was again soaked in 450 mL of ethanol for three days, and then filtration was performed to remove plant debris. The filtrate (ethanol extract) was evaporated using rotary vacuum evaporator (Bibby RE200, Sterilin Ltd., UK). Then dried extract was taken in an air-tight container, and stored in refrigerator at 4 °C to avoid any possible fungal attack. The yield was 6.33% of dried plant material.

**Experimental Animals**

Young Swiss-Albino mice, age 4-5 weeks, average weight 20-25 gm, were collected from International Centre for Diarrheal Disease Research, Bangladesh (ICCCDR, B) and kept in optimum environmental condition (temperature 25±0.5 °C, humidity 55-60%, and 10 h light: 14 h dark cycle) for one week in the animal house of Pharmacy Discipline, Khulna University for adaptation to the experimental environment. They were kept in standard plastic
polypropylene cages with proper ventilation. All animals were bestowed with standard ICCDR, B formulated rodent pellet diet and water ad libitum. Experiments were performed in accordance with animal ethics guidelines of Institutional Animal Ethics Committee (12).

Test Pathogens

The pathogenic bacteria were collected from ICCDR, B and stored in Microbiology Laboratory of Pharmacy Discipline, Khulna University. Both Gram-positive and Gram-negative bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Shigella dysenteriae*, *Vibrio cholerae*, and *Proteus* sp. were used for antibacterial assay.

Chemicals and Reagents

Ascorbic acid, acetic acid, and dimethyl sulfoxide (DMSO) were purchased from Merck, Germany. 1,1-diphenyl-2-pycrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. Ltd., (St. Louis, MO, USA). Tween-80 and castor oil were purchased from Loba Chemie Pvt. Ltd., India. Solvents and all other reagents were of analytical grade.

Standard Drugs

Diclofenac sodium and loperamide were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Phytochemical Screening

The ethanol bark extract *B. racemosa* was subjected to different preliminary phytochemical tests to identify major phytochemical groups (13,14).

In Vitro Antioxidant Activity

DPPH Scavenging Assay

Free radical scavenging activity of the ethanol bark extract was substantiated by DPPH assay (15). Sample was prepared in ethanol at different concentrations of 256, 128, 64, 32, 16, 8, and 1 µg/mL. Sample of 1 mL of each concentration was added to 3 mL of 0.004% ethanol solution of DPPH. Incubation period of 30 min was allowed at room temperature in dark place to complete any reaction that is to be occurred. Then absorbance was measured by UV spectrophotometer at 517 nm against blank. Ascorbic acid was used as standard free radical scavenger and activity of the extract was compared with it. Activity of the sample was calculated using the following formula:

\[
\text{Percent inhibition} = \left( \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \right) \times 100.
\]

\(IC_{50}\) value was determined from % inhibition versus concentration (µg/mL) graph.

Analgesic Activity

Acetic Acid Induced Writhing Test

Acetic acid induced writhing test was performed according to the method of Koster et al., 1959 (16). The experimental mice were screened randomly and divided into four groups (n = 5) to carry out the present pharmacological investigation. Test groups received the ethanol bark extract at the doses of 250 and 500 mg/kg body-weight in oral route. The positive control group was treated with diclofenac sodium (25 mg/kg, p.o.). Control group received 1% tween-80 in distilled water orally at the dose of 10 mL/kg. After 30 min, each mouse was given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid at the dose of 10 mL/kg to induce writhing. After 5 min, the number of writhing was counted for the period of 10 min for each mouse. The percent inhibition of writhing for each group was calculated and compared with the control for the assessment of analgesic activity.

In Vivo Anti-diarrhoeal Activity

Castor Oil Induced Diarrhoea

The experiment was carried out according to the method described by Talukder et al., 2012 (17).
Experimental mice were selected based on their sensitivity to castor oil-induced diarrhoea and divided into four groups \((n = 5)\). Test groups were treated with the bark extract \((250\) and \(500\) mg/kg, p.o.) and positive control group was provided with loperamide \((3 \text{ mg/kg, p.o.})\) in suspension form. Control group was treated with 1% tween-80 in distilled water \((10 \text{ mL/kg, p.o.})\). Each mouse was provided with 0.5 mL of castor oil in oral route after the interval of 60 min for inducing diarrhoea. Each mouse was housed in individual plastic transparent cage and floor was lined with clean white blotting paper which was changed in every hour throughout the observation period of 4 h. Onset of diarrhoea and the number of stool for each mouse was counted. For the assessment of anti-diarrhoeal activity, onset of diarrhoea and percent inhibition of defecation were compared with the control group.

### Results

**Results of Phytochemical Screening**

In phytochemical screening, the ethanol bark extract of \(B. \text{ racemosa}\) exhibited the presence of reducing sugars, alkaloids, glycosides, gums, flavonoids, and terpenoids (Table 1).

<table>
<thead>
<tr>
<th>Phytochemical groups</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Gums</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

\(+ = \text{Presence} - = \text{Absence}\)

Table 1: Phytochemical screening of \(B. \text{ racemosa}\) bark

**Activity in DPPH Scavenging Assay**

The DPPH radical scavenging activity of the extract was in concentration dependent manner. Activity was gradually increased with the concentration at low concentration level. But, at high concentration, the graph reached plateau state. The ethanol bark extract showed IC\(_{50}\) value of 31.90 µg/mL while standard ascorbic acid showed IC\(_{50}\) value of 12.40 µg/mL. see Figure 1.

**Activity in Acetic Acid Induced Writhing Test**

The ethanol bark extract of \(B. \text{ racemosa}\) exhibited dose dependent inhibition of writhing in comparison to the control. The extract showed 36.20 and 63.80% inhibition of writhing at the doses of 250 and 500 mg/kg, respectively while diclofenac sodium showed 78.16% inhibition of writhing in mice. see Table 2.

**Effect on Castor Oil Induced Diarrhoea**

In castor oil induced diarrhoea, the ethanol bark extract...
extract showed considerable increase in latent period of diarrhoea as well as decline in frequency of defecation in dose dependent and significant (p<0.001) manner. The extract showed 45.94 and 68.46% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively while loperamide showed 84.68% inhibition of defecation at the dose of 3 mg/kg.

see Table 3.

**Activity in Disc Diffusion Assay**
The ethanol bark extract showed antibacterial activity against all the tested bacterial strains with zone of inhibition ranging from 6.96 to 14.12 mm.  
see Table 4.

**Discussion**
DPPH scavenging assay, was carried out, for evaluating in vitro antioxidant activity of the ethanol bark extract of *B. racemosa*. After accepting electron or hydrogen radical, DPPH is converted into stable DPPH-H form. When this conversion occurs, deep violet colour of DPPH turns into light yellow colour. Unconverted DPPH is detected by UV spectrophotometer at 517 nm against blank and percent inhibition was calculated. It is most widely used in vitro assay to evaluate scavenging ability of plant extract or compounds. Phytochemical investigation revealed the presence of flavonoids in the bark extract, and it is well established that flavonoids are responsible for antioxidant properties (19,20). The extract exhibited concentration dependent DPPH radical scavenging activity which was strongly comparable to the standard antioxidant ascorbic acid.

Acetic acid induced writhing test, most common and cheap method, for evaluating in vivo analgesic activity in mice, more specifically for evaluating peripheral analgesic activity. In writhing test, peripherally acting analgesic activity of the sample is evaluated by inducing writhing through the sensitization of pain receptors by prostaglandins release (21,22). It is a sensitive conventional model in which local peritoneal receptors are responsible pain sensation (23). The phytochemicals of *B. racemosa* namely terpenoids, reducing sugars, gums, flavonoids, and alkaloids may be responsible for potential analgesic activity in mice (24-26). The most probable mechanism of peripheral analgesic activity may be the inhibition of prostaglandins (PGE2 and PGE2α) synthesis (27).

Castor oil induced diarrhoeal model is very much rational for evaluating in vivo anti-diarrhoeal activity in mice, because of the involvement of prostaglandins in causation of diarrhoea by castor oil through the release of ricinoleic acid that causes irritation of the intestinal mucosa and subsequently increases bowel movement and poor absorption, ultimately watery diarrhoeal stools possess (28). Numerous mechanisms are already reported in previous reports to clarify the causes of castor oil induced diarrhoea such as inhibition of intestinal Na+, K+-ATPase activity (29), stimulation of prostaglandins formation through irritation of the intestinal mucosa (30), activation of adenylate cyclase mediated active secretion (31) and involvement of nitric oxide (32).

In disk diffusion assay, the ethanol bark extract was profound to inhibit bacterial growth against all the tested bacterial strains with considerable zone of inhibition. But in disk diffusion assay, non polar compounds are not evaluated properly, because agar media is prepared with water, so poor diffusion of non polar compounds results (33). As a result, the antibacterial activity was mainly the attribution of polar compounds present in the bark extract. But it is must to declare that antibacterial activity was promising against all the tested bacterial strains.

**Conclusion**
The ethanol bark extract of *B. racemosa* showed potential antioxidant, analgesic, ant-diarrhoeal, and antibacterial activity. The results demand further investigations in much bigger resolution to identify underlying mechanism as well as active compounds responsible for bioactivities.

**Acknowledgments**
Special thanks and gratification to International Centre for Diarrhoeal Disease Research, Bangladesh
(ICCDR, B) for providing test animals and also to the authorities of Pharmacy Discipline, Life Science School, Khulna University for aiding instrumental and technical support.

References

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Figure 1: DPPH scavenging activity of *B. racemosa* bark

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of writhes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>34.80±0.60</td>
<td>--</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>25</td>
<td>7.60±0.90*</td>
<td>78.16</td>
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<tr>
<td>Extract</td>
<td>250</td>
<td>22.20±1.40*</td>
<td>36.20</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>12.60±1.05*</td>
<td>63.80</td>
</tr>
</tbody>
</table>

Table 2: Effect of *B. racemosa* bark on acetic acid induced writhing in mice

Results are expressed as mean ± SEM,
SEM= Standard error of mean,
*P < 0.001 versus control, Student's t-test
Table 3: Effect of *B*. *racemosa* bark on castor oil induced diarrhoea in mice

Results are expressed as mean ± SEM, SEM= Standard error of mean, *P < 0.001 versus control, Student's t-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Onset of diarrhoea (min)</th>
<th>No. of stools after 4 h</th>
<th>% Inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>47.80±2.55</td>
<td>22.20±1.15</td>
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<tr>
<td>Loperamide</td>
<td>3</td>
<td>180.40±2.42*</td>
<td>3.40±0.50*</td>
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<tr>
<td>Extract</td>
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<td>83.60±1.88*</td>
<td>12.00±0.86*</td>
<td>45.94</td>
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<tr>
<td></td>
<td>500</td>
<td>120.40±1.43*</td>
<td>7.00±0.70*</td>
<td>68.46</td>
</tr>
</tbody>
</table>

Table 4: Antibacterial activity of *B*. *racemosa* bark in disk diffusion assay

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Type of bacteria</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blank (250 µg/disc)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Gram (+)</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Gram (+)</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Gram (-)</td>
<td>-</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Gram (-)</td>
<td>-</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Gram (-)</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus sp.</em></td>
<td>Gram (-)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4: Antibacterial activity of *B*. *racemosa* bark in disk diffusion assay