Antibacterial, cytotoxic, analgesic and diuretic activities of *Rhizophora mucronata* Lam. bark


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*Rhizophora mucronata* Lam., locally known as ‘Garjon or Jhanna’ in Bangladesh is a mangrove plant of Rhizophoraceae family. Different parts of this plant have got applications in folk medicine from the ancient time. In the present study, attempts have been made for a detailed phytochemical study of the bark of the plant for antibacterial, cytotoxic, analgesic and diuretic activities. Disc diffusion method was used for the screening of antibacterial activity and cytotoxicity study was performed by brine shrimp lethality bioassay. Acetic acid induced writhing method was employed to assess the analgesic potentiality of the extract. Diuretic property of the bark extract was studied by Lipschitz method. The ethanolic extract of the barks of *R. mucronata* Lam. showed moderate antibacterial activity against both Gram-positive and Gram-negative strains. Marked inhibitory effects were found with *Escherichia coli* and *Staphylococcus epidermidis* at a concentration of 500 µg/disc. The extract showed very high level of general toxicity in the brine shrimp lethality bioassay having an LC50 value of 0.5 µg/mL. The extract of *R. mucronata* Lam. bark at concentrations of 250 mg/kg and 500 mg/kg exhibited significant (P<0.01) inhibition of writhing reflex by 36.96% and 50%, respectively while the standard diclofenac inhibition was found to be 65.21% at a dose of 50 mg/kg body weight. The extract also showed very high response to diuresis in a dose-dependent manner.

**Keywords:** *Rhizophora mucronata*, Antibacterial, Cytotoxicity, Analgesic, Diuretic, Mangrove, Rhizophoraceae.

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**Introduction**

Plants remain the primary source of many important drugs in orthodox medicine today. For instance, there are over 50 commercially available anticancer drugs approved by USFDA which are exclusively derived from natural sources1. Medicinal plants and herbs have been in use for eradication of diseases and suffering from the very beginning of human civilization. Some of the wonder drugs of modern medicine have their roots in their early knowledge of medicinal plants. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants2. The presence of diverse bioactive metabolites like alkaloid, glycosides, flavonoids, tannins, etc. in plants has formed the therapeutic basis of herbal medication.

*R. mucronata* occurs on the coasts of the Indian Ocean and the West-Pacific. It is an evergreen, mangrove tree, 25 to 30 m high, 70 cm in diam. with numerous branching arching stilt roots. Bark is brown or blackish, smooth, with horizontal fissures. For long year it is reported to be astringent and a folk remedy for angina, diabetes, diarrhoea, dysentery, haematuria and haemorrhage. Recently, anti-diarrhoeal activity of this plant has been reported3. Since no report has been found on the antibacterial, cytotoxic, analgesic and diuretic activity of the bark of this plant, present study was conducted to evaluate these properties.

**Materials and Methods**

**Plant material**

The bark of *R. mucronata* Lam. was collected from the Sathkhira range of the Sundarbans Bangladesh, the largest continuous mangrove forest in the world4 in January 2009. The fresh barks were collected from the healthy stem of the plant. A little sample was mounted on paper and the sample was taxonomically...
identified by the experts in Bangladesh National Herbarium, Mirpur, Dhaka (Accession Number DACB-34179).

Test organisms

Five Gram negative and two Gram positive bacteria have been used in this study (Table 1). The organisms were collected from the Microbiology Lab. of Pharmacy Discipline, Khulna University, Bangladesh.

Preparation of extract

The collected bark samples were separated from undesirable materials or plant parts. The samples were dried by shade drying for twenty days to avoid any possible photochemical degradation of the active constituents. Then sun dried for one day in an open sunny place afterwards grounded into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 600 g of powder was taken in a clean flat-bottomed glass container and soaked into 1400 mL of 80% ethanol. The container with its contents was sealed and kept for a period of two weeks with occasional shaking and stirring. The mixture was then filtered through white cotton followed by a finer filtration by Whatman filter paper. The filtrate (ethanol extract) thus obtained was evaporated in a rotary evaporator until became a gummy mass of (about 200 mg) reddish-black color. This was designated as crude ethanolic extract of R. mucronata Lam. which was used as sample for the subsequent studies.

Determination of antibacterial activity

Disc diffusion assay

The antibacterial activity of the crude extract was determined against the test organisms (Table 1) by the disc diffusion method. Solutions of known concentration (µg/mL) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diam. were then impregnated with known amounts of the test substances using micropipettes and the residual solvents were completely evaporated. Discs containing the test materials were placed onto nutrient agar medium uniformly seeded with the test microorganisms. Standards discs of Kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were kept at 4°C for 24 h to allow maximum diffusion of the test sample material as well as standard Kanamycin. The plates were then incubated at 37°C for 24 h to allow maximum growth of the organisms. The antibacterial activity of the test materials was determined by measuring the diameter (mm) of zone of inhibition. The experiments were carried out in triplicate and the mean values were taken.

Brine shrimp lethality bioassay

For the screening of cytotoxic activity of the bark extractives, brine shrimp lethality bioassay technique was applied which is considered to be a rapid general bioassay method for the natural products. This method has got reputation for studying cytotoxicity as well as a wide range of pharmacological activities eg. anticancer, antiviral and pesticidal properties. Dimethylsulfoxide (DMSO) solutions were made with the extract of R. mucronata and applied against the artificially hatched brine shrimp nauplii, Artemia salina in a single-day in vivo assay. For this experiment, 4 mg of ethanolic crude extract was dissolved in DMSO and the solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/mL) were obtained by serial dilution. Vincristine sulphate was used as positive control. The experiment was carried out in triplicate and the mean values were taken.

Study of analgesic activity

Analgesic activity of ethanolic extract of R. mucronata Lam. was tested using the model of acetic acid induced writhing in mice. The acetic acid induced writhing method is an analgesic assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Diam. of zone of inhibition (mm)</th>
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<tbody>
<tr>
<td>Staphylococcus epidermidis (ATCC 12228)</td>
<td>27.0</td>
</tr>
<tr>
<td>S. aureus (ATCC 6538)</td>
<td>22.5</td>
</tr>
<tr>
<td>Escherichia coli (ATCC 35218)</td>
<td>10.0</td>
</tr>
<tr>
<td>Salmonella paratyphi (ATCC 9150)</td>
<td>26.0</td>
</tr>
<tr>
<td>Shigella flexneri (ATCC 12022)</td>
<td>24.5</td>
</tr>
<tr>
<td>S. boydii (ATCC 9361)</td>
<td>21.0</td>
</tr>
<tr>
<td>S. dysenteriae (ATCC 13313)</td>
<td>22.4</td>
</tr>
</tbody>
</table>
0.6% acetic acid solution intra-peritonially and then observing the animal for specific contraction of body referred as ‘writhing’. A comparison of writhing is made between positive control (diclofenac), control and test sample given intra-peritonially 30 minutes prior to acetic acid injection. Young Swiss-albino mice aged 4-5 weeks, average weight 22-25 g were used for the experiment. The mice were purchased from the animal lab of Jahangir Nagar University, Bangladesh.

To prepare suspension of the test samples at the doses of 250 and 500 mg/kg body weight, 250 mg and 500 mg of samples were measured, respectively. The final volume of the suspension was made 20 mL for both the concentrations. Diclofenac sodium was used at a dose of 50 mg/kg body weight. Test samples, control and diclofenac were given intra-peritoneal by means of a 3 ml disposable syringe. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.6%, 10 mL/kg) was administered intra-peritoneal to each of the animals. After an interval of five minutes, the number of writhing was counted for 5 minutes after 30 minutes of giving the sample and drugs. Each mouse of all groups was observed carefully to count the number of writhing that they had made in 5 minutes.

Study of diuretic activity

Twenty adult albino mice, divided into four groups were used in this study. The animals were housed in standard metal cages and were allowed to starve 6 h before the study commences. The method of Lipschitz\textsuperscript{12} was employed for the assessment of diuretic activity. Out of four groups the first group served as control and was fed normal saline IP 25 mL/kg. Second group was fed frusemide at dose 20 mg/kg. The second and third groups were given a dose of 250 and 500 mg/kg extract (test sample), respectively. Each of these preparations was given in such a manner so that the fluid intake was the same in all cases. Immediately after the dosing the mice were placed in metabolic cages and kept at room temperature of 25 ± 0.5°C for six hours. During this period, no food was made available to them. After first, second, third, fourth, fifth, sixth hours the cumulative amount of urine excreted by each group was noted.

Results and Discussion

Results in terms of zone of inhibition (mm) obtained in the disc diffusion method have been displayed in Table 1. From the results it can be observed that 80% ethanolic extract of the bark of \textit{R. mucronata} showed activity against all the test organisms. Of the bacterial strains tested \textit{Shigella dysenteriae} (6.5 mm), \textit{Shigella boydii} (7.0 mm), \textit{Salmonella paratyphi} (8.0 mm), \textit{Shigella flexneri} (8.5 mm), \textit{Escherichia coli} (9.5 mm) showed mild sensitivity whereas \textit{Staphylococcus epidermidis} (12.0 mm) and \textit{Staphylococcus aureus} (12.5 mm) exhibited moderate sensitivity against \textit{R. mucronata}.

The lethality of crude extract to brine shrimp nauplii was determined after 24 h of exposure. Results are shown in Fig. 1. The median lethal concentration (LC\textsubscript{50}) was found to be 0.5 µg/mL for crude extract and 0.32 µg/mL for the positive control (Vincristine sulphate). The magnitude of cytotoxicity exhibited by the alcoholic crude extract was found to be significant. From this study, it can be concluded that the extracts of the bark of \textit{R. mucronata} showed significant biological activity.

The extract of \textit{R. mucronata} Lam. bark showed marked analgesic activity. At concentrations of 250 mg/kg and 500 mg/kg body weight, the extract exhibited significant (\(P<0.01\)) inhibition of writhing reflex by 36.96% and 50%, respectively while...
the standard diclofenac inhibition was found to be 65.21% at a dose of 50 mg/kg body weight (Fig. 2).

The diuretic potency of the extract of this plant is highly significant in comparison with control animals. The extract, at doses of 250 mg/kg and 500 mg/kg body weight showed a dose dependent increase in volume of urine (Table 2).

### Conclusion

The ethanolic extract of *Rhizophora mucronata* Lam. bark possesses considerable antibacterial, cytotoxic, analgesic and diuretic properties. It is obvious that the extract of the bark contains potent medicinal agent(s) which need to be isolated by further investigation.

### References