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# 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 $IC_{50}$ 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. acutangul*, *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 neoclerodane 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.

## Materials and Methods

Plant material

The bark of *B. racemosa*, was collected from the Sundarbans, Karamjol region, Bangladesh. It was collected in November' 2011 at the day time. During collection, any type of adulteration was strictly avoided.

The sample was identified and authenticated by the experts at Forestry and Wood Technology Discipline (FWT), Khulna University, Khulna, Bangladesh, where a voucher specimen (Accession number- 39631) has been submitted for further reference.

### 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 plat 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 (ICCDR, 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 assessment.

### 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:

$$\text{Percent inhibition} = [(\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}] \times 100.$$

$IC_{50}$ 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 mg/kg, p.o.) in suspension form. Control group was treated with 1% tween-80 in distilled water (10 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.

### Antibacterial Activity

### Disc Diffusion Assay

Antibacterial activity of the ethanol bark extract of *B. racemosa* was assessed by disc diffusion assay (18). The extract was prepared using DMSO at desired concentration. Sterile blank discs (BBL, Cocksville, USA) were impregnated with the test extract at the concentrations of 250 and 500 μg/disc using micropipette. Then discs were dried. Dried sample discs, standard antibiotic discs (Ciprofloxacin 5 μg/disc, Oxoid Ltd., UK) and control discs (contain DMSO) were placed on nutrient agar medium seeded with bacteria in Petri dishes using sterile forceps. Then Petri dishes were incubated at 37 °C for 16 h. After incubation period, zone of inhibition was measured using digital slide calipers.

### Statistical Analysis

Results were expressed as mean ± SEM. Significance of the results were analyzed by Student's *t*-test. The results were considered as statistically significant when $P < 0.001$ in comparison to control.

## Results

Results of Phytochemical Screening

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

| Phytochemical groups | Results |
|---|---|
| Reducing sugars | + |
| Alkaloids | + |
| Glycosides | + |
| Steroids | - |
| Gums | + |
| Saponins | - |
| Flavonoids | + |
| Tannins | - |
| Terpenoids | + |

+ = Presence - = Absence

Table 1: Phytochemical screening of *B. 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. 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 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 defection 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 ($PGE_2$ and $PGE_{2\alpha}$) 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 no 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.

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## References


1. Grosvenor PW, Gothard PK, McWilliam NC, Supriono A, Gray DO. Medicinal plants from Riau province, Sumatra, Indonesia. Part 1: Uses. J Ethnopharmacol 1995; 45:75-95.
2. Khan MR, Omoloso AD. Antibacterial, antifungal activities of Barringtonia asiatica. Fitoterapia 2002; 73:255-260.
3. Bourdy G, Walter A. Maternity and medicinal plants in Vanuatu. I. The cycle of reproduction. J Ethnopharmacol 1992; 37:179-196.
4. Khan S, Jabbar A, Hasan CM, Rashid MA. Antibacterial activity of Barringtonia racemosa. Fitoterapia 2001; 72:162-164.
5. Thomas TJ, Panikkar B, Subramoniam A, Nair MK, Panikkar KR. Antitumour property and toxicity of Barringtonia racemosa Roxb seed extract in mice. J Ethnopharmacol 2002; 82:223-227.
6. Deraniyagala SA, Ratnasooriya WD, Goonasekara CL. Antinociceptive effect and toxicological study of the aqueous bark extract of Barringtonia racemosa on rats. J Ethnopharmacol 2003; 86:21-26.
7. Behbahani M, Ali AM, Muse R, Mohd NB. Anti-oxidant and anti-inflammatory activities of Barringtonia racemosa. J Med Plant Res 2007; 1:95-102.
8. Hussin NM, Muse R, Ahmad S, Ramli J, Mahmood M, Sulaiman MR, Shukor MYA, Rahman MFA, Aziz KNK. Antifungal activity of extracts and phenolic compounds from Barringtonia racemosa L. (Lecythidaceae). Afr J Biotechnol 2009; 8:2835-2842.
9. Sun HY, Long LJ, Wu J. Chemical constituents of mangrove plant Barringtonia racemosa. Zhong Yao Cai 2006; 29:671-672.
10. Gowri PM, Radhakrishnan SVS, Basha SJ, Sarma AVS, Rao LM. Oleanane-type isomeric triterpenoids from Barringtonia racemosa. J Nat Prod 2009; 72:791-795.
11. Hasan CM, Khan S, Jabbar A, Rashid MA. Nasimaluns A and B: neo-clerodane diterpenoids from Barringtonia racemosa. J Nat Prod 2000; 63:410-411.
12. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109-110.
13. Kokate CK. Practical Pharmacognosy. 4th ed. Delhi: Vallaph Prakashan, 1994; p. 107-111.
14. Khandelwal KR. Practical Pharmacognosy. 11th ed. Pune: Nirali Prakashan, 2004; p. 149-153 & 157-159.
15. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B. Free radical scavenging ability of methanolic extract of Hyoscyamus squarrosus leaves. Pharmacologyonline 2009; 2:796-802.
16. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesics screening. Fed Proc 1959; 18:412-417.
17. Talukder C, Saha S, Adhikari S, Mondal HK, Islam MK, Anisuzzman M. Evaluation of antioxidant, analgesic and antidiarrhoeal activity of Flacourtia jangomas (Lour.) Raeusch. leaves. Pharmacologyonline 2012; Arch.3:20-28.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45:493-496.
19. Beecher GR. Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 2003; 133:3248S-3254S.
20. Ortega R. Importance of functional foods in the Mediterranean diet. Public Health Nutr 2006; 9:1136-1140.
21. Gorzalczany S, Marrassini C, Miño J, Acevedo C, Ferraro G. Antinociceptive activity of ethanolic extract and isolated compounds of Urtica circularis. J Ethnopharmacol 2011; 134:733-738.
22. Shang XF, Wang JH, Li MX , Miao XL, Pan H, Yang YG, et al. Antinociceptive and anti-inflammatory activities of Phlomis umbrosa Turcz extract. Fitoterapia 2011; 82:716-721.
23. Chakraborty A, Devi RKB, Rita S, Sharatchandra K, Singh TI. Preliminary studies on anti-inflammatory and analgesic activity of Spilanthes acmella in experimental animal models. Indian J Pharmacol 2004; 36:148-150.
24. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, Saeedi F, Kamalinejad M, et al. Antinociceptive and anti-inflammatory effects of Elaeagnus angustifolia fruit extract. J Ethnopharmacol 2000; 72:287-292.
25. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavanoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol 2001; 33:2-16.
26. Choi J, Jung HJ, Lee KT, Park HJ. Antinociceptive and antiinflammatory effects of saponin and sapogenin obtained from the stem of Akebia quinata. J Med Food 2005; 8:78-85.
27. Saha S, Islam MK, Anisuzzman M, Hasan MM, hossain F, Talukder C. Evaluation of antioxidant, analgesic and antidiarrhoeal activity of Phoenix paludosa Roxb leaves. International Journal of Medical Sciences and Pharmacy 2012; 2:46-52.
28. Gaginella TS, Stewart JJ, Olsen WA, Bass P. Actions of recinoleic acid and structurally related fatty acids on the gastrointestinal tract. II. Effect on water and electrolyte absorption in vitro. J Pharmacol Exp Ther 1975; 195:355-361.
29. Nell G, Rummel W. Action mechanism of secretagogue drugs. In: Csaky TZ (Ed.). Pharmacology of Intestinal Permeation (Vol. 2). Berlin: Springer-Verlag, 1984; p. 464-474.
30. Galvez A, Zarzuelo ME, Crespo MD, Lorente M, Ocete A, Jimenez J. Antidiarrhoeic activity of Euphorbia hirta extract and isolation of active flavonoid constituents. Planta Med 1993; 59:333-336.
31. Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. Br J Pharmacol 1994; 113:1127-1130.
32. Mascolo N, Izzo AA, Gaginella TS, Capasso F. Relationship between nitric oxide and platelet activating factor in castor oil induced mucosal injury in the rat duodenum. Naunyn Schmiedebergs Arch Pharmacol 1996; 353:680-684.
33. Saha S, Anisuzzman M, Islam MK, Mondal H, Talukder C. Antibacterial and cytotoxic potential of Dalbergia spinosa Roxb. leaves. Int J Pharm Sci Res 2013; 4:512-515.

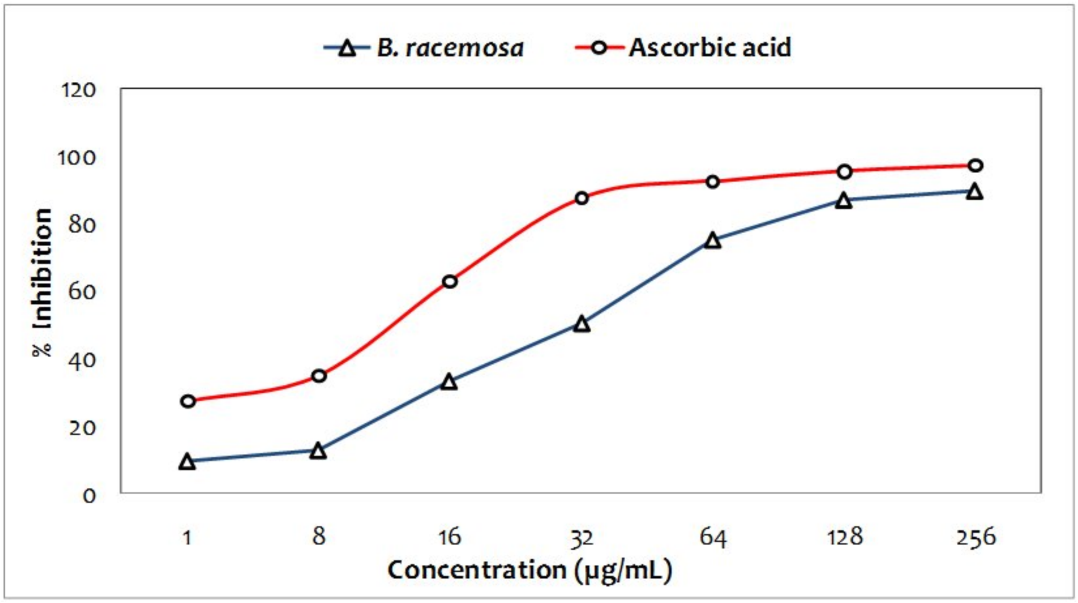


Figure 1: DPPH scavenging activity of *B. racemosa* bark

| Treatment *n* = 5 | Dose (mg/kg) | No. of writhes | % Inhibition |
|---|---|---|---|
| Control | --- | 34.80±0.60 | --- |
| Diclofenac sodium | 25 | 7.60±0.90* | 78.16 |
| Extract | 250 | 22.20±1.40* | 36.20 |
| | 500 | 12.60±1.05* | 63.80 |

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

| Treatment *n*=5 | Dose (mg/kg) | Onset of diarrhoea (min) | No. of stools after 4 h | % Inhibition of defecation |
|---|---|---|---|---|
| Control | --- | 47.80±2.55 | 22.20±1.15 | --- |
| Loperamide | 3 | 180.40±2.42* | 3.40±0.50* | 84.68 |
| Extract | 250 | 83.60±1.88* | 12.00±0.86* | 45.94 |
| | 500 | 120.40±1.43* | 7.00±0.70* | 68.46 |

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.

| Bacterial strains | Type of bacteria | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Blank | Extract (250 µg/disc) | Extract (500 µg/disc) | Ciprofloxacin (5 µg/disc) |
| *Staphylococcus aureus* | Gram (+) | - | 12.14 | 13.46 | 32.00 |
| *Staphylococcus epidermidis* | Gram (+) | - | 12.32 | 15.04 | 21.80 |
| *Escherichia coli* | Gram (-) | - | 13.07 | 14.75 | 22.45 |
| *Shigella dysenteriae* | Gram (-) | - | 12.22 | 14.74 | 21.52 |
| *Vibrio cholerae* | Gram (-) | - | 14.12 | 14.62 | 22.18 |
| *Proteus sp.* | Gram (-) | - | 6.96 | 10.00 | 16.52 |

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


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