ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antinociceptive, Antibacterial and Diuretic Activities of *Cerbera odollam* Gaertn Roots

MD Ashikur Rahman[1]*, Prasanata Paul[1], Ahmed Ayedur Rahman[1]

[1]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

ABSTRACT

The crude methanolic extract of the roots of *Cerbera odollam* Gaertn. was evaluated for its possible antinociceptive, antibacterial and diuretic activities in animal models. At the dose of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive effect in acetic acid induced writhing in mice comparable to that produced by aspirin, used as standard drug (P<0.001).The extract of *C. odollam* exhibited significant in vitro antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera, Streptococcus epidermidis, Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm. Diuretic activity was proved by the electrolyte loss ratio (Na+/K+ excretion ratio was 1.38 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the standard diuretic furosemide (1.37) The obtained results tend to suggest the probable antinociceptive, antibacterial and diuretic activities of the crude extract.

Keywords: *Cerbera odollam*; Antinociceptive activity; antibacterial activity; diuretic activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cerbera odollam Gaertn. (Family: Apocynaceae) locally known as 'Dabur', 'Dhakur', is a small tree or large shrub distributed widely throughout Bangladesh, India, Malaysia, China, Australia and Philippine mostly on the sea coast. The root and leaves of the plant are traditionally used as emetic and cathartic; kernels are used as emetic; fruit is used as a cure for hydrophobia [1]. Its root and fruits are purgative and used for the treatment of rheumatism [2]. A number of research works have been performed to evaluate its biological activities as cytotoxic activity [3] effect on central nervous system [4], purgative and antirheumatic activity [5], cardiac stimulant activity [6], neurological manifestations [7], cardiotoxic activity [8], etc. Recenly two new cardenolide glycosides compound 2C(30)H(46)O(8).CH(3)OH.H(2)O, (I), and C(32)H(48)O(9), (II), respectively, are which were isolated from the seeds of Cerbera odollam [9]. The main objective of this study was to evaluate the, antibacterial and diuretic activities of the methanolic extract of roots of *Cerbera odollam* (*C. odollam*).

MATERIALS AND METHODS

Plant material

The plants were collected in February 2008 from Sundarbans Mangrove forest of Bangladesh and were identified in the National Herbarium of Bangladesh (Accession no.: 40788). The roots of *C. odollam* were pulverized into fine powder. About 600 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 1.8 liters of 90% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatmann filter paper. The filtrate thus obtained was concentrated by using a rotary evaporator.

.

Animals

Animal studies were performed in accordance with the declaration of Helsinki and the European Community guidelines for the ethical handling and the use of laboratory animals and through the clearance of Institutional Animal Ethics Committee (IAEC). Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. All the experiments were conducted on an isolated and noiseless condition.

ISSN: 0975-8585

Drugs

Aspirin (Square Pharmaceuticals Ltd, Bangladesh), Gentamycin (Square Pharmaceuticals Ltd., Bangladesh), Furosemide (Square Pharmaceuticals Ltd, Bangladesh).

Experimental

Chemical group test

The crude methanolic extract of roots of *C. odollam* was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins [10, 22]. In each test 10% (w/v) solution of the extract in methanol was taken unless otherwise mentioned in individual test.

Antinociceptive activity

The antinociceptive activity of the crude methanolic extract of *C. odollam* was studied using acetic acid induced writhing model in mice [11,12]. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with aspirin (standard drug) at the dose of 25 mg/kg of body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10 ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 minutes, the mice were observed for specific contraction of body referred to as 'writhing' for 5 min.

Antibacterial activity

The antibacterial activity of *C. odollam* extract was studied against *Staphylococcussaprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera, Streptococcus epidermidis,Shigella flexneri* and *Staphylococcus aureus* clinical isolates. All bacterial strains were kindly provided by IMTECH, Chandigarh (India). Cultures of these bacteria were grown in a nutrient broth at 37 °C and maintained on nutrient agar (Himedia, India) slants at 40 °C. The antibacterial property was studied by the disc diffusion method [13] using extract 200 mg/disc. Control disks contained solvents only (50% aqueous ethanol). Gentamycin was used as positive controls. Minimum inhibitory concentration (MIC) was evaluated by the micro dilution method using 5 mL of liquid broth with different concentrations of extract [14, 15].

Diuretic activity

Diuretic activity of the extract was investigated using the method as described by Lipschitz et al.[16] The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to

metabolic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group-2 was provided with urea solution at a dose of 500 mg/kg. Group-3 was provided with standard diuretic drug furosemide at a dose of 0.5 mg/kg.Group-4 and group-5, the test groups were treated with the methanol extract of MP at the doses of 200 and 400 mg/kg respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar. [17]

Statistical analysis:

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS

Chemical group test

Results of different chemical tests on the methanolic extract of *C. odollam* showed the presence of steroids, flavonoids, reducing sugars, gums, saponins and tannins (Table 1).

Table 1. Phytochemical properties of *C. odollam* crude extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	-ve	-ve	+ve	+ve	+ ve	+ ve	+ve	+ve

Key: +ve = Presence -ve = Absence

Antinociceptive activity

Table 2. Effect of methanolic extract of *C. odollam* on acetic acid induced writhing in mice (n=5)

Animal Group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 solution 10 ml/kg, p.o.	18 ± 1.000 (100)	—
Positive control Aspirin 25 mg/kg, p.o.	5.8 ± 0.862* (32.22)	67.78
Test group-1 Me. extract 250mg/kg, p.o.	9.2 ± 0.754* (51.11)	48.89
Test group-2 Me. extract 500 mg/kg, p.o.	5.6 ± 0.509* (31.11)	68.89

Values are expressed as mean ± SEM; Me.: Methanolic * indicates *P* <0.001 vs. control; n: Number of mice; p.o.: per oral.

Table 2 showed the effect of the methanolic extract of *C. odollam* on acetic acid induced writhing in mice. At the dose of 250 and 500 mg/kg of body weight, the extract produced 48.89% and 68.89% writhing inhibition in test animals, respectively. The results were statistically significant (*P*<0.001) and were comparable to the standard drug aspirin, which showed 67.78% writhing inhibition at the dose of 25 mg/kg.

ISSN: 0975-8585

In vitro antibacterial activity

Table 3: *In vitro* antibacterial activity of the methanolic extract of *C. odollam* crude roots extract

Bacterial stains	*Aegiceras corniculatum* (mm)	Control (mm)
Staphylococcus saprophyticus	12.22	30.00
Shigella sonnie	16.34	44.88
Salmonella typhi	14.65	36.88
Vibrio cholera	14.42	32.44
Streptococcus epidermidis	12.45	34.28
Shigella flexneri	10.76	28.90
Staphylococcus aureus	10.80	30.50

Key: Control (50% aqueous ethanol); Diameter of inhibition zones (mm)

Table 3 showed the extract of *C. odollam* exhibited significant *in vitro* antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera,Streptococcus epidermidis, Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm.

Diuretic activity

Table 4. Effect of methanolic extract of *C. odollam* on urine excretion parameters in mice

Treatment	**Dose (mg/kg; P.O)**	**Volume of urine (ml)**	**Concentration of ions (m.eq.l^{-1})**			
			Na^+	**K^+**	**Cl^-**	**Na /K^+**
Group-1 (Control)	-	2.43 ± 0.07	76.67 ± 1.24	48.75 ± 1.18*	76.55 ± 1.24	1.42
Group-2 (Urea)	500	3.74 ± 0.08	113.66±1.35**	76.56±1.27**	85.46±1.67**	1.38
Group-3 (Furosemide)	0.5	4.15±0.14	122.87±1.74*	85.46±1.67**	92.36±1.49*	1.37
Group-4 (HI)	200	4.24±0.08	118.51±1.19**	79.35±1.86**	91.74±1.68*	1.38
Group-5 (HI)	400	4.86±0.04	132.74±1.62**	92.24±1.69**	97.60±1.86*	1.45

ME: Methanolic extract of HI- *C. odollam*.; Values are expressed as mean ± SEM (Number of animals, n = 10); *indicates P<0.01, **indicates P<0.001 vs. control; [b]Collected for 5 hours after treatment

The effect of the methanolic extract of *C. odollam* on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea. Electrolyte loss showed similar ratio (Na+/K+ excretion ratio was 1.38 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.37) (Table 4).

ISSN: 0975-8585

DISCUSSION

The *odollam* tree is responsible for about 50% of plant poisoning cases and 10% of all poisoning cases in Kerala. Thus, it was called "suicide plant" [18]. But the same species of Bangladesh is not too much poisonous, and even the local people use the fleshy portion of the fruit as food. Its root is not poisonous (LD_{50}: 750 mg/kg body weight of the albino mice).

Since *C. odollam* belongs to the coastal forests, part of the plant constituents may be polar in nature. Methanol was used which has a wide range of solubility in both polar and non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness.

Antinociceptive activity of the methanolic extract of *C. odollam* root was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [19]. Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid [20]. On the basis of the result of acetic acid induced writhing test, it can be concluded that the methanolic extract of *C. odollam* might possess an antinociceptive activity.

In this experiment, methanolic extract of *C. odollam* showed moderate sensitivity to the five of the test organisms both gram positive and gram negative type of bacteria. The highest zone on inhibition (16.34 mm) was recorded against *Shigella sonnie.* Moreover, the experiment was only conducted with five species of bacteria as test samples. Therefore further research is essential to evaluate the sensitivity of the plant extract against other species of bacteria, fungi, virus of other microorganisms.

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting Na+/ K+/Cl^- co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na+ and Cl^- from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na+ and Cl^- load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

ISSN: 0975-8585

CONCLUSION

In conclusion, it could be suggested that the crude methanolic extract of antinociceptive, antibacterial and diuretic activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential confirm its pharmacological properties.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; IZ Shahid, Assistant professor, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

REFERENCES

[1] Kirtikar KR, Basu BD. Indian Medicinal Plants, 1987; vol. III, 2nd ed, pp. 1552-1553.
[2] Rollet B. Bibliography on mangroves research. 1981; 1600-1975 p. 479.
[3] Laphookhieo S, Karakai CX, Chantrapromma K. Phytochemistry 2004; 65(4): 507-10.
[4] Hien TT, Navarro-Delmasure C, Vy T. J Ethnopharmacol 1991; 34(2-3): 201-6.
[5] Yamauchi T, Abe F. Chem Pharm Bull 1987; 35: 4813-4818.
[6] Chen JS, Zheng S. Science press, Beijing. 1987; 75
[7] Iyer GV, Narendranath M. Indian J Med Res 1975; 63(2): 312-4.
[8] Kini PM, Pai KN. Indian Heart J 1965; 17(3):263-70.
[9] Chantrapromma S, Usman A, Fun HK, Laphookhieo S, Karalai C, Rat-a-pa Y, Chantrapromma K. Acta Crystallogr C. 2003; 59(Pt 2): 68-70.
[10] Evans WC. Trease and Evan's Text book of Pharmacognosy, 1989; 13th ed., p. 546.
[11] Whittle BA. Br J Pharmacol Chemother 1964; 22: 246.
[12] Ahmed F, Selim MST, Das AK, Choudhuri MSK. Pharmazie 2004; 59: 329-33.
[13] Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Mandal SC, Bhattacharya SK. J Ethnopharmacol 2002; 82: 229-237.
[14] Ahmad I, Beg AJ. J Ethnopharmacol 2007; 74: 113-23
[15] Bayoud B, Djilani SE, Legseir B, Ouahrani MR, Djilani A. J life Sci 2007; 1(1): 78-81
[16] Lipschitz WL, Hadidian Z, Kerpesar. J Pharmacol Exp Therap 1943; 79: 97-110.
[17] Godkar PB. Text Book of medical laboratory technology. Bhalani Publishing House, Mumbai. 1994; pp: 77-79.
[18] Gaillard Y, Krishnamoorthy A, Bevalot F. J Ethanopharmacol 2004; 95(2-3):123-6.
[19] Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. J Ethnopharmacol 2003; 84: 31-35.
[20] Derardt R, Jougney S, Delevalcee F, Falhout M. Eur J Pharmacol 1980; 51: 17–24.
[21] Gupta BD, Dandiya PC, Gupta M. Jap J Pharmacol 1971; 21: 293.

[22] Ghani, A. “Medicinal Plants of Bangladesh.” First Edition, The Asiatic Society of Bangladesh, Dhaka, Bangladesh, 1998; pp.110-187.