Research Article

www.ijrap.net

EVALUATION OF NEUROPHARMACOLOGICAL, ANTIBACTERIAL AND ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF THE BARK OF *CYNOMETRA RAMIFLORA* LINN.

(LEGUMINOSAE)

Siraj Md. Afjalus^{1*}, Salahuddin Malik¹, Hossain Emrul¹, Safi Sanjana², Yasmin Farjana³

¹Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

²Department of Clinical Pharmacy & Pharmacology, Faculty of Pharmacy, Dhaka University, Dhaka-1000, Bangladesh

³Pharmacy Discipline, Stamford University Bangladesh, Dhaka-1217, Bangladesh

Received on: 02/12/12 Revised on: 20/01/13 Accepted on: 10/02/13

*Corresponding author

E-mail: saeed_2567@yahoo.com

DOI: 10.7897/2277-4343.04220

Published by Moksha Publishing House. Website www.mokshaph.com

All rights reserved.

ABSTRACT

The crude methanolic extract of the bark of *Cynometra ramiflora* Linn. (Leguminosae) growing in southeast part of Bangladesh has been evaluated for its possible neuropharmacological, antibacterial and antinociceptive properties. The extract of *Cynometra ramiflora* bark potentiated the pentobarbital induced sleeping time in mice, decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test. Acute toxicity test showed that the plant might be safe for pharmacological uses. Antibacterial activity of *Cynometra ramiflora* bark was tested by disk diffusion method. The extract exhibited significant antibacterial activity against *Vibrio cholerae*, *Salmonella typhi*, *Staphylococcus aureus* and exhibited moderate antibacterial activity against *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Enterococci*, *Shigella boydii*, *Shigella flexneri*, *Staphylococcus epidermis*. The methanolic extract exhibited statistically significant writhing inhibition in acetic acid-induced writhing model in white albino mice. The crude extract produced 48.62% inhibition of writhing at the dose of 250 mg/kg body weight and 63.89% inhibition of writhing at the dose of 500 mg/kg body weight, while the standard drug Diclofenac-Na inhibition was found to produce 69.45% at a dose of 25 mg/kg body weight.

Key words: *Cynometra ramiflora*, neuropharmacological, antibacterial, acetic acid induced writhing model, antinociceptive

INTRODUCTION

Cynometra ramiflora Linn. (Leguminosae) also referred to as Balitbitan, belangan, gal mendora, gulos, gulus etc. It is an erect, small tree up to 10 meter tall. Leaves are imparipinnate, 30-38 centimeters long. Leaflets 2-4 pairs and an odd one, opposite or sub opposite, 7.5-18 centimeters by 3-6.3 centimeters, oblong-elliptic, sub falcate, sub acute, glabrous on both surfaces, base rounded very inequilateral except that of the terminal leaflet. Male flowers about 4 millimeters long, in axillary lax branched sparingly lepidote panicles is equaling the leaves; peduncles long. Fruit depressed, globular, 5-12.5 centimeters diameter, dehiscing by 3 valves. Seeds rounded, trigonous, with an orange-colored aril.¹

Cynometra ramiflora is found in undisturbed and secondary forest at low and medium altitudes. Also found in limestone areas. It is distributed from India, Sri Lanka and southern China to New Guinea, West Pacific and Australia. *Cynometra ramiflora* is cultivated for its beautiful, pendant inflorescences covered with large, conspicuous, attractive, silvery bracts. According to Guerrero the leaves are used as an antihyperpetic, like those of *Cassia alata*. Burkill quotes Rheede, who says that the roots purge; that the leaves are used in Malabar to make a lotion for skin diseases; and that oil is drawn from the seeds for the same purpose. Crevost and Petelot quote de Lanessan, who states that the roots are purgative. It is used as timber for interior and light constructions, novelties, plywood and decorative veneers; fuel wood and charcoal.¹

Methanolic extract of the *Cynometra ramiflora* was found to lower the postprandial blood glucose level of the

sucrose loaded rats significantly. To our knowledge this is the first report describing the antihyperglycaemic potential of *Cynometra ramiflora*. The extracts of *Cynometra ramiflora* will be further fractionated in order to search for the antidiabetic ingredients and also comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antihyperglycaemic effect of *Cynometra ramiflora*.²

MATERIALS AND METHODS

Plant material collection and extraction

For this present investigation, *Cynometra ramiflora* was collected from Karamjal, Sundarban, Bangladesh and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No: 31149). The collected plant parts (bark) were separated from undesirable materials or plants or plant parts. They were sun-dried for one week. The plant parts were grind 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 400 gm of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 80% 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 whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (Methanol extract) obtained was evaporated under ceiling fan and in a water-bath until dried. It rendered a gummy concentrate of

reddish black color. The gummy concentrate was designated as crude extract of Methanol.

Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example saponins were identified by shaking with distilled water, flavonoids were detected by adding a few drops of concentrated Hydrochloric acid (HCl), reducing sugar was identified by 5 ml Fehling's solution, tannins with 1 ml of 10% Lead acetate solution, gums with Molish reagent and Sulfuric acid.

Drugs

Diazepam (Square Pharmaceuticals Ltd., Bangladesh) Diclofenac Sodium (Orion Pharma Ltd., Bangladesh).

Animals

Young Swiss-albino mice of either sex, weighing 20-25 gm, 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 $25.0 \pm 2.0^{\circ}\text{C}$ and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experiment met the national guidelines on the proper care and use of animals.

Pharmacological Studies

Neuropharmacological activity

Pentobarbital-induced hypnosis

Neuropharmacological activity of *Cynometra ramiflora* extract was tested by pentobarbital induced hypnosis method. The test animals were divided into four groups consisting of seven mice in each group. Group I was the control group, Group II was positive control, Groups III and IV were the experimental groups. The experimental groups were administered with the methanolic extract of *Cynometra ramiflora* at dose of 250 and 500 mg/kg body weight orally, while positive control was treated with Diazepam (1 mg/kg p.o.) and control with vehicle (1% v/v Tween 80 in water). Each mouse was placed in an observation box (a rectangular open box composed of hardboard floor (36 cm²) with a surrounding wall 30 cm height. 30 minutes later, Pentobarbital (40 mg/kg, i.p.) was administered to each mouse to induce sleep. The total sleeping time were recorded for both control as well as for treated groups. The animals were observed for the latent period (time between Pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

Exploratory behaviour

This experiment was performed by: (1) Open field test (2) Hole cross test and (3) Hole board test. The test animals were divided into four groups consisting of seven mice in each group. Group I was the control group, Group II was the positive control and Groups III and IV were the experimental groups. The experimental groups were

administered with the methanolic extract of fruits of *Cynometra ramiflora* at dose of 250 and 500 mg/kg of body weight per orally (p.o.), while the animals of Group I (control) were supplied with 0.1% (v/v) Tween-80 (p.o.) at the dose of 10 ml/kg of body weight and Group II was treated as 'positive control' by giving diazepam intraperitoneally 1 mg/kg (i.p.). The observations were made on 0 minute before injection and 30, 60, 90, 120, 180 and 240 minutes after injections of the test samples and control.

Acute toxicity test

The acute toxicity of *Cynometra ramiflora* extract was determined in animal model (Rat) with slight modifications. Rats fasted for 16 hours and were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800 and 1600 mg/kg p.o.) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 48 hours for signs of acute toxicity. The number of deaths within this period was recorded.

Antibacterial activity

Antibacterial activity of the methanolic extract of bark of *Cynometra ramiflora* was tested by using the disc diffusion method^{3,4}. Antibiotic disc (Kanamycin 30 µg/disc) was used as a standard. In this method, measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile Matricel (BBL, Cocksville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried were used as positive and negative control, respectively. These discs were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening. The plates were then kept at 40°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates were then kept in an incubator for 12-18 hour to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in term of millimeter. The experiments were carried out three times and the mean of the reading were recorded. Both gram positive and gram-negative bacterial strains were taken for the test. The bacterial strains used for the investigation were Gram negative bacteria include *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi*, *Enterococci*, *Vibrio cholerae*, *Shigella boydii*, *Shigella flexneri* and Gram negative bacteria include *Staphylococcus aureus*, *Staphylococcus epidermis*. These organisms were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B)

Table 1: Effect of methanolic extract of *Cynometra ramiflora* on pentobarbital induced hypnosis in mice

Animal group	Treatment	Time of onset of sleep (minute)	Total sleeping time (minute)
I (Control)	1% Tween 80 solution (10 ml/kg, p.o.) + Pentobarbital, i.p. (40 mg/kg)	15.69 ± 1.63	38.87 ± 3.48
II (Positive Control)	Diazepam (1 mg/kg, p.o.) + Pentobarbital, i.p. (40 mg/kg)	4.34 ± 0.16**	93.06 ± 1.20**
III (Test Group - I)	Met. <i>C. ramiflora</i> (250 mg/kg, p.o.) + Pentobarbital, i.p. (40 mg/kg)	7.36 ± 1.30**	60.80 ± 2.87**
IV (Test Group - II)	Met. <i>C. ramiflora</i> (500 mg/kg, p.o.) + Pentobarbital, i.p. (40 mg/kg)	6.25 ± 0.84**	75.31 ± 3.51**

Values are expressed as mean ± SEM; Met., Methanolic; **indicates $P < 0.001$; p.o., per oral; i.p.- Intraperitoneal.

Table 2: Effect of *Cynometra ramiflora* on animal model in Hole cross test; a= $P < 0.001$; p.o. = per oral

Animal Group / Treatment	0 minute	30 minutes	60 minutes	120 minutes	240 minutes
Control	8.42±	9.85±	10±	9.28±	8.42±
1% Tween- 80 solution in water 10 mg/kg ; p.o.	0.629	0.459	0.487	0.68	6.49
Test Group – I	7.42±	6.07±	4.89±	3.07±	2.90±
Methanolic Extract 250 mg/kg ; p.o.	0.571	0.845 ^a	0.337 ^a	0.501 ^a	0.218 ^a
Test Group – II	8.28±	6.16±	4.27±	3.33±	1.66±
Methanol Extract 500 mg/kg ; p.o.	0.386	0.731 ^a	0.617 ^a	0.183 ^a	0.127 ^a

Table 3: Effect of *Cynometra ramiflora* on animal model in Hole board test; a= $P < 0.001$; p.o. = per oral

Animal Group / Treatment	0 minute	30 minutes	60 minutes	120 minutes	240 minutes
Control	114.15±	112±	123.36±	106.36±	110.16±
1% Tween- 80 solution in water 10 mg/kg ; p.o.	2.36	1.94	1.24	1.62	1.38
Test Group – I	101.75±	89.25±	83.17±	17.54±	85.15±
Methanolic Extract 250 mg/kg ; p.o.	1.37	1.54 ^a	1.96 ^a	1.47 ^a	2.85 ^a
Test Group – II	117.33±	82.45±	79.83±	74. ±	80±
Methanol Extract 500 mg/kg ; p.o.	2.13	2.34 ^a	2.52 ^a	1.63 ^a	2.74 ^a

Table 4: Effect of *Cynometra ramiflora* on animal model in Open field test; a= $P < 0.001$; p.o. = per oral

Animal Group / Treatment	0 minute	30 minutes	60 minutes	120 minutes	240 minutes
Control	15.66±	17.58±	13.89±	16.50±	18.26±
1% Tween- 80 solution in water 10 mg/kg ; p.o.	2.32	1.64	1.68	2.43	2.12
Test Group – I	19.86±	16.62±	19.29±	15.31±	15.40±
Methanolic Extract 250 mg/kg ; p.o.	2.74	2.35	1.30 ^a	1.67 ^a	2.10 ^a
Test Group – II	17.59±	14.75±	11.36±	9.53±	10.43±
Methanol Extract 500 mg/kg ; p.o.	1.55	2.26 ^a	1.21 ^a	1.43 ^a	2.37 ^a

Table 5: Zone of inhibition of *Cynometra ramiflora* barks extracts against bacterial strains (Gram Negative and Gram Positive) with respect to Kanamycin

Bacterial strains	Standard Drug, kanamycin (30µg/disc)	Diameter of zone of inhibition (mm)	
		Methanolic extract of <i>Cynometra ramiflora</i> (250 µg/disc)	Methanolic extract of <i>Cynometra ramiflora</i> (500 µg/disc)
Gram Negative			
<i>Salmonella typhi</i>	28	8	15
<i>Enterococci</i>	25	7	14
<i>Salmonella sonnei</i>	24	8	13
<i>Escherichia coli</i>	31	9	12
<i>Vibrio cholerae</i>	25	9	16
<i>Shigella boydii</i>	29	8	14
<i>Shigella flexneri</i>	28	7	13
<i>Shigella dysenteriae</i>	23	8	14
Gram Positive			
<i>Staphylococcus aureus</i>	25	10	15
<i>Staphylococcus epidermis</i>	31	10	12

Table 6: Result of the antinociceptive effect of methanolic extract of *Cynometra ramiflora*

Animal group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control	14.4±2.37 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	4.4±0.50* (30.55)	69.45
Test group - 1 Methanolic extract 250 mg/kg, p.o.	7.4±0.93** (51.38)	48.62
Test group - 2 Methanolic extract 500 mg/kg, p.o	5.2±0.97*** (36.11)	63.89

Values are expressed as Mean S.E.M (n=5), * $P < 0.1$, ** $P < 0.025$, *** $P < 0.005$, % = Percentage, p.o. = per oral.

Antinociceptive activity

Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice^{5,6}. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug Diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 250 mg/kg and 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 minutes prior to acetic acid (0.7%) injection. Then after an interval of 15 minutes, the number of writhes (squirms) was counted for 5 minutes.

RESULTS

Neuropharmacological activity

Pentobarbital-induced hypnosis test

Statistical analysis of the data obtained in this test show that (Table 1) both 250 and 500 mg/kg dose of methanolic extract of barks of *Cynometra ramiflora* prolong the duration of the pentobarbitone-induced sleeping time. The total sleeping time was about 75 and 61 minutes at dose of 500 and 250 mg/kg of body weight, respectively whereas in positive control group it was about 93 minutes.

Exploratory behavior test

The Hole crossed by the mice of the control group increased gradually along with time from 0 minute to 60 minutes. In case of *Cynometra ramiflora* (250 mg/kg) the number of hole crossed was gradually decreased along with time from 0 minute to 120 minutes. This lowered number of movement was significant ($p < 0.001$) from 30 minutes to 240 minutes. In case of *Cynometra ramiflora* (500mg/kg) dose level the movement is also gradually decreased along with time. Also this decreased was significant at the statistical level (Table 2). Above all from this hole cross test, it can be observed that the extract may have an increasing effect with increasing the dose. From the Table 3 we can observe that the overall number of movements of the control group was similar from 0 minute to 240 minutes. In the group of *Cynometra ramiflora* (250 mg/kg) the number of movements was decreased from its initial value of 0 minute to 120 minutes. The values are significant ($p < 0.001$) within the 30 minutes to 240 minutes. In the group of *Cynometra ramiflora* (500 mg/kg) the number of movements is also decreased from its initial value of 0 minute to 240 minutes. The values are more significant ($p < 0.001$) within the 30 minutes to 120 minutes. From this experiment we found that, *Cynometra ramiflora* may have depressant effect on the central nervous system and the effect is increased with increase in dose. From the Table 4 we can observe that the number of hole dipped by the mice of the control group overall was increased from 30 minutes to 240 minutes. In the group of *Cynometra ramiflora* (250mg/kg and 500 mg/kg) the number of hole dipped by the mice was decreased from its initial value of 0 minute to 120 minutes. But the values of *Cynometra ramiflora* (500 mg/kg) were decreased more in comparison to *Cynometra ramiflora* (250 mg/kg). Above all from this

head dip test, we can observe that the extract may have a depressant effect on the central nervous system (CNS) and the effect is increased with increase in dose.

Acute toxicity test

In acute toxicity study, oral administration of graded doses (200, 400, 800 and 1600 mg/kg p.o.) of the methanolic extract of *Cynometra ramiflora* to rats did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group after 48 hours of administering the extract to the animals. *Cynometra ramiflora* was safe up to a dose level of 1600 mg/kg of body weight.

Antibacterial activity

The methanolic extract of the barks of *Cynometra ramiflora* was tested for anti-bacterial activity against a number of both gram positive and gram-negative bacteria. Standard antibiotic discs of Kanamycin (30 µg/disc) were used for comparison purpose. The Table 5 showed that the methanolic extract of the barks of *Cynometra ramiflora* (250 µg/disc and 500 µg/disc) showed moderate anti-bacterial activity against *Escherichia coli* zone of inhibition was 9 mm and 12 mm respectively. Zone of inhibition of other strains includes, *Staphylococcus epidermis* (10 mm and 12 mm), *Shigella dysenteriae* (8 mm and 14 mm), *Enterococci* (7 mm and 13 mm), *Shigella sonnei* (8 mm and 14 mm), *Staphylococcus aureus* (10 mm and 15 mm), *Salmonella typhi* (8 mm and 15 mm), *Shigella flexneri* (7 mm and 13 mm), *Shigella boydii* (8 mm and 14 mm) and *Vibrio cholerae* (9 mm and 16 mm) respectively.

Antinociceptive activity

The antinociceptive effect of the methanolic extract of the bark of *Cynometra ramiflora* was tested on acetic acid-induced writhing in mice. At dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced about 48.62% & 63.89% writhing inhibition in test animals (Table 6). The results were statistically significant ($P < 0.100$ & $P < 0.025$) and were comparable to the standard drug Diclofenac sodium, which showed about 69.45% writhing inhibition at the dose of 25 mg/kg ($P < 0.005$).

DISCUSSION

Plants are employed as important source of medication in many traditional medications^{7, 8, 9}. The most important step in evaluating drug action on the CNS is to observe the behaviour of the test animals. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. Both the doses of the crude extract produced a significant increase in the hypnotic effect induced by the Pentobarbitone, in a dose-dependent manner, thus, suggesting a profound sedative activity. Like many other centrally acting drugs, barbiturates acts on the cerebral cortex to produce their actions¹⁰. Pentobarbital, a barbiturates class hypnotic drug by an allosteric modification of gamma-amino-butyric acid type A (GABA_A) receptor increases the chloride conductance and potentiates GABA_A mediated postsynaptic inhibitors¹¹. The extract potentiates

Pentobarbitone induced sleeping time that may be attributed to an action on the central mechanism involved in the regulation of sleep^{12,13}. The prolongation of Pentobarbital-induced sleeping time may be attributed to an inhibition of Pentobarbital metabolism¹⁴. The extract prolongation of Pentobarbital sleep suggests that barbitol-induced hypnosis is a good index of CNS depressant activity¹⁵. Again, the extract significantly decreased the locomotor activity as shown by the results of the open field, hole cross and hole board tests. The locomotor activity is a measure of the level of excitability of the CNS¹⁶ and any decrease of this activity may be closely related to sedation resulting from depression of the CNS¹⁷. Extracts of *Cynometra ramiflora* decreased locomotor activity which indicates its CNS depressant activity. GABA_A is the major inhibitory neurotransmitter in the CNS. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs elicit their action through GABA_A, therefore it is possible that extracts of *Cynometra ramiflora* may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts¹⁸.

Antibacterial activity was tested by using the disc diffusion method. Disc diffusion method is widely acceptable for the preliminary screening of antibacterial activity. It is essentially a qualitative or semi qualitative test indicating the sensitivity or resistance of microorganisms to the test materials. The extract was found active against both gram positive and gram negative bacteria and the inhibitory effects on tested species was concentration dependent. It is well known that plant containing various phytochemical constituents such as flavonoids, saponins and steroids have antimicrobial activity¹⁹. Plant containing Quercetagenin-7-arabinosyl-galactoside, a flavonoid has been used extensively to treat infectious disease²⁰. The flavone baicalein is reported to be largely responsible for antimicrobial effects²¹. Flavonoid rich plant extracts from species of *Hypericum*²², *Capsella*²³ and *Chromolaena*²⁴ have been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity²⁵⁻³³. It has been reported that saponins have potent antimicrobial activity. The antibacterial activity of *Cynometra ramiflora* probably due to the presence of flavonoids and saponins that revealed in phytochemical studies. The zone of inhibition varies within the ranges of 7-10 mm and 12-16 mm at the dose of 250 and 500 µg/disc respectively. The highest zone of inhibition was found against *Vibrio cholerae* (16 mm) at 500 µg/disc. The results support the traditional use of this plant as a remedy of infectious skin infections and gonorrhea. Present study reveals that the bark extract of *Cynometra ramiflora* shows significant dose-dependent antinociceptive effect. Oral administration of *Cynometra ramiflora* extracts produced peripheral antinociceptive effect in acetic acid-induced writhing test. Pain sensation in acetic acid-induced writhing method is elicited by triggering localized inflammatory response resulting in release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin (PG)

biosynthesis³⁴. In other words, the acetic acid-induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase product³⁵. The increase in PG levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability³⁶. The acetic acid-induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of PG synthesis, a peripheral mechanism of pain inhibition³⁷. The effect of the extracts against the noxious stimulus may be an indication that it significantly stimulated the production of irritants and hereby reduction in number of writhes in the animals was not well enough. Phytochemical screening of the methanol extract of *Cynometra ramiflora* reveals the presence of tannin, flavonoids, reducing sugars, gums and saponins. Tannins inhibit PG synthesis by modifying the production of COX-1 and COX-2 and lipoxygenase (LOX) involved in the PG synthesis³⁸. The plant is also reported to contain saponins. There is growing interest in natural saponins due to both improved extraction and structural analysis techniques of these compounds, and also by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic³⁹. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins. In conclusion, it can be suggested that the crude methanolic extract of *Cynometra ramiflora* may possess neuropharmacological, antibacterial and antinociceptive activity, which correlates well with the traditional uses of the plant. Therefore further researches are essential to find out the active principle responsible for these activities.

ACKNOWLEDGEMENTS

The authors are thankful to Professor Dr. Samir Kumar Sadhu and Dr. Jamil Ahmed Shilpi, Associate Professor, Pharmacy Discipline, Life Science School, Khulna University, Bangladesh for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation. The authors are also grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental mice and bacterial strains.

REFERENCES

1. *Cynometra ramiflora*.htm [homepage on the Internet]. Available from: <http://www.asianplant.net/>
2. Priti Tiwari, Neha Rahuja, Rajesh Kumar. Search for antihyperglycemic activity in few marine flora and fauna. Indian Journal of Science and Technology, 2008; 1(5)
3. McLaughlin JL, Anderson JE, Chang CJ. Bioactive components of *Allamanda schottii*. Journal of Natural Products 1988; 51(2): 307-308. <http://dx.doi.org/10.1021/np50056a018>
4. Roland R. Antibiotics - An introduction. Hoffman La-Roche & Co, Switzerland, 1982; 70-71.
5. Khan MA, Kust GS, Barth H-J & Boer B. Sabkha Ecosystems. 2006; Vol: II: p 129-153.
6. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 1989 13th ed, Cambridge University Press, London, p 546.
7. Grover JK, S Yadav, V Vats. Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol. 2002; 81(1): 81-100. [http://dx.doi.org/10.1016/S0378-8741\(02\)00059-4](http://dx.doi.org/10.1016/S0378-8741(02)00059-4)
8. Keung WM, BL Vallee. Kudzu root: An ancient chinese source of modern antidipsotropic agents. Phytochemistry. 1998; 47 (4): 499-506. [http://dx.doi.org/10.1016/S0031-9422\(97\)00723-1](http://dx.doi.org/10.1016/S0031-9422(97)00723-1)
9. Neves JM, C Matos, C Moutinho, G Queiroz, LR Gomes. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J. Ethnopharmacol. 2009;

- 124(2):270-283. <http://dx.doi.org/10.1016/j.jep.2009.04.041>
PMid:19409473
10. Bowman WC, Rand MJ (1980). Text book of pharmacology. Blackwell Scientific publication, New York. p 267.
11. Katzung BG (2004). Basic and clinical pharmacology. McGraw Hill, New York. p 356.
12. N'Gouemo P, Ngumby-bina C, Baldt-Moulinier M. Some Neuropharmacological effects of an ethanolic extract of *Maprounea africana* in rodents. *J. Ethnopharmacol.* 1994; 43(3):161-166. [http://dx.doi.org/10.1016/0378-8741\(94\)90037-X](http://dx.doi.org/10.1016/0378-8741(94)90037-X)
13. Chindo BA, Amos S, Odutola AA, Vongtau HO, Abbah J, Wambebe C, Gamaniel KS. Central nervous system activity of the methanolic extract of *Ficus platyphylla* stem bark. *J. Ethnopharmacol.* 2003; 85:131-137. [http://dx.doi.org/10.1016/S0378-8741\(02\)00376-8](http://dx.doi.org/10.1016/S0378-8741(02)00376-8)
14. Kaul PN, Kulkarni SK. New drug metabolism inhibitor of marine origin. *J. Pharm. Sci.* 1978; 67:1293-1296. <http://dx.doi.org/10.1002/jps.2600670927> PMid:690837
15. Fujimori H, Cobb D. Central nervous system depressant activity of MA 1337, 3 [3,4-m-chlorophenyl-1-piperazyl propyl]- 2- 4 (1H, 3H) quinoxalinedone hydrochloride. *J. Pharmacol. Exp. Ther.* 1965; 148:151-157. PMid:14302151
16. Mansur RM, Martz W, Carlini EA. Effects of acute and chronic administration of *Cannabis sativa* and (-) 9-trans tetrahydrocannabinol on the behaviour of rats in open field arena. *Psychopharmacology* 1980; 2:5-7.
17. Ozturk Y, Aydinli S, Beis R, Baser KHC, Berberoglu H. Effect of *Hypericum pericum* L. and *Hypericum calycinum* L. extracts on the central nervous system in mice. *Phytomedicine* 1996; 3(2):139-146. [http://dx.doi.org/10.1016/S0944-7113\(96\)80027-4](http://dx.doi.org/10.1016/S0944-7113(96)80027-4)
18. Kaul PN, Kulkarni SK. New drug metabolism inhibitor of marine origin. *J. Pharm. Sci.* 1978; 67:1293-1296. <http://dx.doi.org/10.1002/jps.2600670927> PMid:690837
19. T.P. Tim Cushman, Andrew J. Lamb Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 2005; 26: 343–356. <http://dx.doi.org/10.1016/j.ijantimicag.2005.09.002>
20. Tereschuk ML, Riera MV, Castro GR, Abdala LR. Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. *J Ethnopharmacol* 1997; 56:227–232. [http://dx.doi.org/10.1016/S0378-8741\(97\)00038-X](http://dx.doi.org/10.1016/S0378-8741(97)00038-X)
21. Tsao TF, Newman MG, Kwok YY, Horikoshi AK. Effect of Chinese and western antimicrobial agents on selected oral bacteria. *J Dent Res* 1982; 61:1103–1106. <http://dx.doi.org/10.1177/00220345820610091501> PMid:6963285
22. Dall'Agnol R, Ferraz A, Bernardi AP. Antimicrobial activity of some *Hypericum* species. *Phytomedicine* 2003; 10:511–516. <http://dx.doi.org/10.1078/094471103322331476> PMid:13678236
23. El-Abyad MS, Morsi NM, Zaki DA, Shaaban MT. Preliminary screening of some Egyptian weeds for antimicrobial activity. *Microbios* 1990; 62:47–57. PMid:2336037
24. Aladesanmi AJ, Sofowora A, Leary JD. Preliminary biological and phytochemical investigation of two Nigerian medicinal plants. *Int J Crude Drug Res* 1986; 24:147–153.
25. Aladesanmi AJ, Sofowora A, Leary JD. Preliminary biological and phytochemical investigation of two Nigerian medicinal plants. *Int J Crude Drug Res* 1986; 24:147–153.
26. Al-Saleh FS, Gamal El-Din AY, Abbas JA, Saeed NA: Phytochemical and biological studies of medicinal plants in Bahrain: family Chenopodiaceae. *Int J Pharmacogn* 1997; 35: 38–42. <http://dx.doi.org/10.1076/phbi.35.1.38.13266>
27. Mahmoud MJ, Jawad AL, Hussain AM, Al-Omari M, Al-Naib A. In vitro antimicrobial activity of *Salsola rosmarinus* and *Adiantum capillus-veneris*. *Int J Crude Drug Res* 1989; 27:14–17.
28. Quarengi MV, Tereschuk ML, Baigori MD, Abdala LR. Antimicrobial activity of flowers from *Anthemis cotula*. *Fitoterapia* 2000; 71:710–712. [http://dx.doi.org/10.1016/S0367-326X\(00\)00229-X](http://dx.doi.org/10.1016/S0367-326X(00)00229-X)
29. Rauha JP, Remes S, Heinonen M, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol* 2000; 56:3–12. [http://dx.doi.org/10.1016/S0168-1605\(00\)00218-X](http://dx.doi.org/10.1016/S0168-1605(00)00218-X)
30. Singh RK, Nath G. Antimicrobial activity of *Elaeocarpus sphaericus*. *Phytother Res* 1999; 13:448–450. [http://dx.doi.org/10.1002/\(SICI\)10991573\(199908/09\)13:5<448::AID-PTR480>3.3.CO;2-Y](http://dx.doi.org/10.1002/(SICI)10991573(199908/09)13:5<448::AID-PTR480>3.3.CO;2-Y)
31. Tarle D, Dvorzak I. Antimicrobial activity of the plant *Cirsiumoleraceum* (L.) Scop. *Acta Pharm Jugosl* 1990; 40:569–571.
32. Torrenegra RD, Ricardo AA, Pedrozo JP, Fuentes OC. Flavonoids from *Gnaphalium gracile* H.B.K. *Int J Crude Drug Res* 1989; 27:22–24.
33. Ramalingam RT, Krishnan K, Venkatesan GK. Antibacterial activity of saponin isolated from the leaves of *Solanum trilobatum* Linn. *Journal of pharmacy Research* 2009; 2(2):273-276.
34. Ahmed M, Shikha HA, Sadhu SK, Rahman MT, Datta BK. Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. *Pharmazie* 2001; 56(8):657-660. PMid:11534346
35. Derardt R, Jouguet S, Delevalceee F, Falhout M. Release of prostaglandins E and F in an allogenic reaction and its inhibition. *Eur. J. Pharmacol.* 1987; 51:17-24.
36. Zakaria Z, Ghani ZD, Nor RN, Gopalan HK, Sulaiman MR. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of *Dicranopteris linearis* leaves in experimental animal models. *J. Nat. Med.* 2008; 62:179-187. <http://dx.doi.org/10.1007/s11418-007-0224-x> PMid:18404320
37. Ferdous M, Rouf R, Shilpi JA, Uddin SJ. Antinociceptive activity of the ethanolic extract of *Ficus racemosa* Linn. (Moraceae). *Orient. Pharm. Exp. Med.* 2008; 8:93-96. <http://dx.doi.org/10.3742/OPEM.2008.8.1.093>
38. Sreejayan RMNA. Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol.* 1997; 49:105-7. <http://dx.doi.org/10.1111/j.2042-7158.1997.tb06761.x> PMid:9120760
39. Attele AS, Wu JA, Yuan C. Analgesic effects of different acupuncture stimulation frequencies in humans. *Biochem. Pharmacol.* 1999; 58:1685-1693. [http://dx.doi.org/10.1016/S0006-2952\(99\)00212-9](http://dx.doi.org/10.1016/S0006-2952(99)00212-9)

Cite this article as:

Siraj Md. Afjalus, Salahuddin Malik, Hossain Emrul, Safi Sanjana, Yasmin Farjana. Evaluation of neuropharmacological, antibacterial and antinociceptive activity of methanolic extract of the bark of *Cynometra ramiflora* Linn. (leguminosae). *Int. J. Res. Ayurveda Pharm.* 2013; 4(2):192-197

Source of support: Nil, Conflict of interest: None Declared