IJPSR (2014), Vol. 5, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 November, 2013; received in revised form, 10 February, 2014; accepted, 09 March, 2014; published 01 April, 2014

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY AND TOTAL TANNIN CONTENT FROM THE LEAVES OF *BACOPA MONNIERI* (LINN.)

Hemayet Hossain*[1], Abdullah Al-Mansur [1], Sanjida Akter [2], Umme Sara [3], Md. Ranzu Ahmed [4] and Abu Anis Jahangir [1]

BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research [1], Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh
Department of Chemistry, Jahangirnagar University [2], Dhaka-1315, Bangladesh
Department of Pharmacy, The University of Asia Pacific [3], Dhaka-1205, Bangladesh
Department of Chemistry, Bangladesh University of Health Sciences [4], Dhaka-1216, Bangladesh

Keywords:

Bacopa monnieri, Anti-inflammatory, Carrageenan, Histamine, Total tannins

Correspondence to Author:

Md. Hemayet Hossain

Senior Scientific Officer, Chemical Research Division, BCSIR Laboratories, Dhaka, Bangladesh

E-mail: hemayethossain02@yahoo.com

ABSTRACT: The methanolic leaf extract of *Bacopa monnieri* Linn. (Family: Scrophulariaceae) was evaluated for its anti-inflammatory activity, and total tannins content in different *in-vivo* and *in-vitro* methods. The anti-inflammatory activity was studied using carrageenan and histamine-induced rat paw edema test at different doses (200 and 400 mg/kg body weight) of the methanol extract. At the dose of 400 mg/kg body weight, the extract showed a significant anti-inflammatory activity both in the carrageenan and histamine-induced oedema test models in rats showing 62.73% and 61.99% reduction in the paw volume comparable ($P<0.01$) to that produced by the standard drug indomethacin (67.08% and 70.76%) at 5 h respectively. The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was also statistically significant ($P<0.05$; $P<0.01$) compared favorably with the indomethacin treated animals at 1, 2, 3 4 and 5 h in both models. The total tannins content was calculated high and significant in methanol extract (105.90 mg of tannic acid equivalent per g of dry extract). Acute toxicity test showed that the plant part might be safe for pharmacological uses. Therefore, the obtained results tend to suggest the acute anti-inflammatory activity of the methanol extract of *Bacopa monnieri* leaves and thus provide the scientific basis for the traditional uses of this plant part as a remedy for pain, and inflammations.

INTRODUCTION: *Bacopa monnieri* (L) Penn. (*B. monnieri*) commonly known as 'Brahmi' is an ancient and renowned medicinal plant and has important applications in Ayurveda system [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(4).1246-52

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1246-52

Several phytochemical compounds such as brahmine and herpestine, saponins d-mannitol, hersaponin, betulinic acid, stigmasterol, ß-sitosterol, bacosides A and B were isolated from the plant [2].

The plant possesses significant antiulcerogenic [3] and cardioprotective activities [4] and has wide applications in the treatment of anxiety, depression, mental fatigue [5, 6], asthma [7], antinociceptive, antidiarrhoeal and antioxidant activities [8, 9, 10]. Moreover, *in vitro* research has revealed the

anticancer activity of *B. monnieri* saponin fractions against sarcoma-180 cells. This might be due to inhibition of DNA replication in the cancerous cell line [11].

Chronic inflammatory diseases remain one of the world's major health problems [12]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [13]. Non-steroidal anti-inflammatory drugs (NSAID) are among the most commonly prescribed drugs due to their consistent effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders. However, their use is associated with adverse effects at the level of digestive tract, ranging from dyspeptic symptoms, gastrointestinal erosions and peptic ulcers to more serious complications, such as over bleeding or perforation [14].

Now-a-days attention is being focused on the investigation of the efficacy of plant-based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies [15]. Therefore to overcome the toxicity of NSAID, the development of new anti-inflammatory drugs is still necessary and the natural product such as medicinal plants could lead in discovering new anti-inflammatory drugs with less undesirable effects.

Since no literature is currently available to substantiate anti-inflammatory activity, and total tannins content of the leaf extract of *B. monnieri*, the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the above pharmacological and tannins contents of the methanolic extract that also confirm its use in folk remedy for inflammation, pain.

MATERIALS AND METHODS:

Collection and identification of plant materials: *B. monnieri* (leaves) were collected from Karamjal, Sundarban Khulna, Bangladesh in August, 2012. The collected samples were then identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A specimen copy was deposited to Bangladesh National Herbarium for identification & the accession number was DACB-32605.

Preparation of methanolic extract: The plant materials (leaves) of *B. monnieri* were freed from any of the foreign materials. Then the plants were air-dried under shed temperature followed by drying in an electric oven at 40ºC. The dried plant materials were then ground into powder. About 400g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300 ml of 95% methanol. The container with its contents was sealed and kept for a period of 14 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) which was concentrated with rotary evaporator at bath temperature not exceeding 40^{o}C to have gummy concentrate of extract (yield approx. 2.77%).

Test Animals: For the screening of acute toxicity activity male rats of Wister strain weighing 175-202 g were used. The animals were housed under standard Laboratory (at Pharmacology Laboratory of BCSIR, Chittagong) conditions maintained at 25±1°C and under 12/12 h light/dark cycle and feed with Balanced Trusty Chunts and water *ad libitum*. All experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for the use and care of experimental animals.

Chemicals & drugs: Carrageenan, histamine phosphate, indomethacin, tannic acid, and folin-ciocalteu phenol reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, tween 80, sodium carbonate were of analytical grade and bought from E. Merck, India Ltd.

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, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent and reducing sugars with Benedict's reagent [16, 17, 18].

Acute toxicity test: The acute toxicity of *B. monnieri* methanolic extract was determined in male rats of Wister strain according to the method of Hilaly *et al* [19] with slight modifications. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered (dissolved in 1% tween 80 in normal saline) 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 72 h for signs of acute toxicity. The number of deaths within this period was recorded.

Anti-inflammatory activity:

1. **Carrageenan-induced oedema test:** Carrageenan induced rat hind paw oedema was used as the animal model of acute inflammation. In this experiment, the rats were divided into five groups of five animals each. The different groups of animals were administered with 1% Tween 80 in normal saline (10 ml/kg, p.o.); *B. monnieri* (200 and 400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). Acute inflammation was induced in all the five groups by sub plantar injection of 0.1 ml of carrageenan (1% suspension in Tween 80) in the right paw of rats 30 min after the oral administration of the tested materials. The paw volume was measured with micrometer screw gauze at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract [20, 21]. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

Percentage inhibition of inflammation = $[(V_c - V_t)/V_c] \times 100$

Where V_c is the average degree of inflammation by the control group, and V_t is the average degree of inflammation by the test group.

2. **Histamine-induced oedema test:** This test was studied using the paw oedema model induced by 0.1% histamine into the sub plantar region of the right hind paw of the rats [13, 22]. The different groups of animals were administered with 1% Tween 80 in normal saline (10 ml/kg, p.o.); *B. monnieri* (200 and 400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). Acute inflammation was induced in all the five groups by sub plantar injection of 0.1 ml of histamine with (1% suspension in Tween 80) in the right hind paw of the rats 30 min after the oral administration of the tested materials. The paw volume was measured with micrometer screw gauze at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula for carrageenan-induced paw oedema.

Determination of total tannins content: The tannins were determined using the Folin-Ciocalteu Phenol reagent as reported by Amorim *et al* [23,24]. Briefly, 1.0 ml of the sample extract was added with 7.5 ml of distilled water and added 0.5 ml of Folin-Ciocalteu Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water.

The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is read against a blank. The results of tannins were expressed in terms of tannic acid in mg/g of dry extract.

Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve $y=4.5692x-0.2538$, $R^2=0.9953$.

Statistical Analysis: Data were presented as mean ± Standard deviation (S.D). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., U.S.A.).

The results obtained were compared with the control group. *P* values < 0.05 were considered to be statistically significant.

RESULTS:

Chemical group test: Results of different chemical tests on the methanolic extract of *B. monnieri* leaves showed the presence of alkaloid, reducing sugars, saponins, tannins, and flavonoids (**Table 1**).

TABLE 1: RESULTS OF DIFFERENT GROUP TESTS OF METHANOLIC EXTRACT OF *B. MONNIERI* LEAVES

Phytoconstituents	Methanol extract of *B. monnieri*
Alkaloid	+
Reducing sugars	+
Tannins	+
Gums	-
Flavonoids	+
Steroid	-

+: Positive result; - : Negative result; ++: significantly positive

Acute toxicity test: In acute toxicity study, oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg p.o.) of the methanol extract of *B. monnieri* 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 72 h of administering the extract to the animals. Therefore, *B. monnieri* extract was safe up to a dose of 3200 mg/kg of body weight.

Anti-inflammatory activity:

1. **Carrageenan-induced paw oedema:** The anti-inflammatory effect of the methanolic extract of the leaves of *B. monnieri* using carrageenan induced oedema tests is expressed in (Table 2). In this test, the positive control (Indomethacin) significantly ($p<0.05$; $p<0.01$) decreased the paw edema at 1h to 5 h after carrageenan injection compared to saline with inhibition 52.43% to 67.08%. A maximum oedema paw volume of 1.66 ± 0.09 mm was observed in the control rats, 4 h after the carrageenan injection.

Rats with the extract at 400 mg/kg body weight significantly decreased ($p<0.05$; $p<0.01$) the carrageenan-induced oedema paw volume from 1 h to 5 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The inhibition percentage of the oedema paw volume by the 400 mg/kg body weight of the extract was also found statistically significant compared to the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The highest reduction in the paw volume by the 400 mg/kg body weight was 62.73% was comparable to that of the indomethacin (67.08%) at 5 h.

TABLE 2: EFFECT OF METHANOL EXTRACT OF *B. MONNIERI* LEAVES AND INDOMETHACIN ON CARRAGEENAN-INDUCED OEDEMA PAW VOLUME IN MALE WISTAR RATS

Treatment	Doses (mg/kg)	Right hind paw volume (mm)				
		1 h	2 h	3 h	4 h	5h
Vehicle	2 ml/kg	1.03 ± 0.07	1.31 ± 0.05	1.49 ± 0.03	1.66 ± 0.09	1.61 ± 0.06
Indomethacin	10	0.49 ± 0.08* (52.43)	0.62 ± 0.09* (52.67)	0.70 ± 0.07* (53.02)	0.59 ± 0.06** (64.45)	0.53 ± 0.04** (67.08)
Extract	200	0.94 ± 0.05* (8.74)	1.09 ± 0.07* (16.79)	1.14 ± 0.03* (23.49)	1.19 ± 0.04* (28.31)	1.03 ± 0.08* (36.02)
Extract	400	0.58 ± 0.09* (43.69)	0.69 ± 0.06* (47.32)	0.76 ± 0.09* (48.99)	0.69 ± 0.07* (58.33)	0.60 ± 0.06** (62.73)

Values in brackets denote percentage inhibition of the oedema paw volume. Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnett's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; $n = 5$.

Histamine-induced paw oedema: Table 3 showed the anti-inflammation effect of the methanolic extract of *B. monnieri* leaves using histamine-induced paw oedema tests. In the histamine-induced oedema test, a maximum oedema paw volume of 1.75 ± 0.07 mm was observed in the control rats, 4 h after the histamine injection. Rats pre-treated with the extract at 400 mg/kg body weight significantly decreased ($p<0.05$; $p<0.01$) the histamine-induced oedema paw volume from 1h to 4 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight.

The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was also statistically significant ($p<0.05$; $p<0.01$) compared favorably with the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The maximum reduction in the paw volume by the 400 mg/kg body weight was 61.99% compared to the indomethacin (70.76%) at 5 h.

Total tannins content: The amount of total tannins content was calculated as significant in the methanolic crude extract of *B. monnieri* (41.07 ± 2.87 mg/g of gallic acid equivalent) (**Table 4**).

TABLE 3: EFFECT OF METHANOL EXTRACT OF *B. MONNIERI* LEAVES AND INDOMETHACIN (STANDARD DRUG) ON HISTAMINE-INDUCED OEDEMA PAW VOLUME IN MALE WISTAR RATS

Treatment	Doses (mg/kg)	Right hind paw volume (mm)				
		1 h	2 h	3 h	4 h	5h
Vehicle	2 ml/kg	1.15 ± 0.09	1.41 ± 0.07	1.59 ± 0.06	1.75 ± 0.07	1.71 ± 0.08
Indomethacin	10	0.51 ± 0.05** (55.65)	0.62 ± 0.08* (56.03)	0.66 ± 0.09* (58.49)	0.59 ± 0.05* (66.28)	0.50 ± 0.06** (70.76)
Extract	200	0.96 ± 0.08* (16.52)	1.06 ± 0.04* (24.82)	1.11 ± 0.09* (30.89)	1.16 ± 0.05* (33.71)	1.01 ± 0.07* (40.93)
Extract	400	0.59 ± 0.06** (48.69)	0.70 ± 0.09* (50.35)	0.75 ± 0.06* (52.83)	0.73 ± 0.08* (58.28)	0.65 ± 0.06** (61.99)

Values in brackets denote percentage inhibition of the oedema paw volume. Values are expressed as mean±SD; Values are calculated as compared to control using one way-ANOVA followed by Dunnett's Test; * indicates $P < 0.05$; ** indicates $P < 0.01$ vs. control; n = 5.

TABLE 4: TOTAL TANNINS CONTENT OF THE METHANOL EXTRACT OF *B. MONNIERI* LEAVES

Extract	Avg. absorbance at 765 nm	Total phenolic content
		mg of tannic acid equivalent (TAE) per g of dry extract
Methanol extract of *B. monnieri* leaves	0.2301 ± 0.08	105.90 ± 6.87

The values are expressed as mean ± standard deviation (n=3).

DISCUSSION: Carrageenan-induced oedema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, all of which also cause pain and fever [25]. Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [26].

Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [13, 27]. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [28, 29], the results of this study are an indication that *B. monnieri can* be effective in acute inflammatory disorders.

The extract also exhibited pronounced reduction in the oedema produced by histamine. This result tends to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. The antihistaminic effect of the extract increased with increase in the dose of the extract.

Histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability [30, 31, 32]. Since the extract effectively suppressed the oedema produced by histamine, it showed that the extract exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins. This study has shown that the methanol extract of the leaves of *B. monnieri* possessed a significant anti-oedematogenic effect ($P<0.01$) on paw oedema induced by carrageenan and histamine compared favorably with the standard drug (indomethacin) in treated rats.

Non-steroidal anti-inflammatory drugs (NSAID) such as indomethacin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation-mediating agent prostaglandin E_2 (PGE_2) from arachidonic acid [33]. Therefore, the pattern of anti-inflammatory activity exhibited by this extract was similar to that of indomethacin.

Tannins and Flavonoids, commonly found in plants have been reported to have significant antioxidant activity [34, 35, 36]. Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity [37, 38]. Phytochemical components, especially phenolic compounds (such as flavonoids, phyenyl propanoids, phenolic acids, tannins etc.) are very important components for the free radical

scavenging and antioxidant activities of plants. Polyphenols are generally of the chemical patterns; phenolic groups react as hydrogen donors and neutralize the free radicals[39]. In the present study the total amount of tannin compounds was calculated as quite high in the methanol extract of *B. monnieri* leaves. The result of present study revealed that the presence of high concentration of tannin components in the extract might cause the high inhibition value of the extract.

CONCLUSION: Since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine, the leaves of *B. monnieri* exhibited acute anti-inflammatory activity. The potential of the extract as acute anti-inflammatory activity, free radical scavenging agents may be due to the presence of phytoconstituents like saponins, tannins, and flavonoids and might be responsible for its activity. Again, no mortality was recorded in the acute toxicity test; it showed that the plant might be safe for use. Therefore, it can be revealed that the methanolic extract of *B. monnieri* leaves possess acute anti-inflammatory activity as well as tannin contents and justify its use as a traditional folk remedy for inflammation, pain. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

ACKNOWLEDGEMENT: We are grateful to Dr. Md. Tofazzal Hossain, Member Science & Technology, BCSIR & Ex. Director BCSIR Laboratories Dhaka, for his inspiration during this research work.

REFERENCES:

1. Anonymous: In the wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi, India. 1998; 2: 116-118.
2. Kapoor LD: CRC Handbook of Ayurvedic Medicinal Plants. CRC Press Inc., Boca Raton, USA. 1990.
3. Sairam K, Rao CV, Babu MD and Goel RK: Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. Phytomedicine 2001; 8:423-430.
4. Nandave M, Ojha SK, Joshi S, Kumari S and Arya DS: Cardioprotective effect of *Bacopa monneira* against isoproterenol-induced myocardial necrosis in rats. International Journal of Pharmacology 2007; 3:385-392.
5. Bhattacharya SK and Ghosal S: Anxiolytic activity of a standardized extract of *Bacopa monniera*: An experimental study. Phytomedicine 1998; 5:77-82.
6. Singh RH and Singh L: Studies on the anti-anxiety effect of the medhya rasayana drug, Brahmi (*Bacopa monniera* Wettst)-Part I. J. Res. Ayur. Siddha 1981; 41:138-148.
7. Dar A and Channa S: Relaxant effect of ethanol extract of *Bacopa monniera* on trachea, pulmonary artery and aorta from rabbit and guinea-pig. Phytotherapy Research 1997; 11:323-325.
8. Subrata KB, Joysree D, Anusua C, Utpal KK and Hemayet H: Evaluation of Antinociceptive and Antioxidant Activities of Whole Plant Extract of *Bacopa monniera*. Research Journal of Medicinal Plant 2012; 6(8): 607-614.
9. Hemayet H, Sariful IH, Shubhra KD, Arpona H and Arif A: Evaluation of Analgesic, Antidiarrhoeal and Cytotoxic activities of Ethanolic Extract of *Bacopa monnieri* (L). British Journal of Pharmaceutical Research 2012; 2(3):188-196.
10. Shah M, Behara YR and Jagadeesh B: Phytochemical Screening and *in vitro* Antioxidant Activity of aqueous and hydroalcoholic extract of *Bacopa monnieri* Linn. International Journal of Pharmaceutical Sciences and Research 2012; 3(9): 3418-3424.
11. Elangovan V, Govindasamy S, Ramamoorthy N and Balasubramaanian K: *In vitro* studies on the anticancer activity of *Bacopa monnieri*. Fitoterapia 1995; 66: 211-215.
12. Yesilada EO, Ustun, Sezik E, Takishi Y, Ono Y and Honda G: Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1α, interleukin-1ß and tumor necrosis factor-α. Journal of Ethnopharmacology 1997; 58: 59-73.
13. Perianayagam JB, Sharma SK and Pillai KK: Anti-inflammatory activities of Trichodesma indicum bark extract in experimental animals. Journal of Ethnopharmacology 2006; 104: 410-414.
14. Corrado B, Marco T, Colucci R, Fornai M, Antonioli L, Ghisu N and Tacca MD: Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-inflammatory therapy. Pharmacology Research 2009; 59: 90-100.
15. Muthu C, Ayyanar M, Raja N and Ignacimuthu S: Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine 2006; 2: 43-54.
16. Evans WC: Trease and Evan's Pharmacognosy, 3rd edn. University Press, Cambridge, 1989: 546-547.
17. Kanchan, Chauhan PK, Jaryal M, Kumari K and Singh M: Phytochemical and *in vitro* antioxidant potential of aqueous leaf extracts of *Brassica juncea* and *Coriandrum sativum*. International Journal of Pharmaceutical Sciences and Research 2012; 3(8): 2862-2865.
18. Hemayet H, Shahid-Ud-Daula AFM, Ismet AI, Tarek A, Subrata B and Utpal K: Antinociceptive and Antioxidant potentials of Crude Ethanol Extract of the Leaves of *Ageratum Conyzoides* Grown in Bangladesh. Pharmaceutical Biology 2013; 51(7): 893-898.
19. Hilaly JE, Israili ZH and Lyoussi B: Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. Journal of Ethnopharmacology 2004; 91: 43-30.
20. Mequanint W, Makonnen E, Urga K: In vivo anti-inflammatory activities of leaf extracts of *Ocimum lamiifolium* in mice model. Journal of Ethnopharmacology 2011; 134, 32-36.
21. Hemayet H, Shahid-Ud-Daula AFM, Jahan IR, Nimmi I, Maruf KMR and Hassan MM: Evaluation of Anti-Inflammatory activity and determination of Total Flavonoids and Tannin contents of *Lagenaria siceraria* Root. International Journal of Pharmaceutical Sciences and Research 2012; 3(8): 2679-2685.
22. Hemayet H, Ismet AJ, Sariful IH, Jamil AS, Shubhra KD and Arpona H: Anti-inflammatory and antioxidant activities of ethanolic leaf extract of *Brownlowia tersa* (L.)

Kosterm. Oriental Pharmacy and Experimental Medicine 2013; 13:181–189.
23. Amorim ELC, Nascimento JE, Monteiro JM, Peixoto Sobrinho, Araujo TAS and Albuquerque UAP: A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology. Funct Ecosyst Commun 2008; 2: 88-94.
24. Hemayet H, Musfizur H, Ismet AJ, Ishrat N and Amirul I: Antidiarrhoeal, nitric oxide scavenging and total tannin content from the bark of *Ceriops decandra* (Griff.) Ding Hou. International Journal of Pharmaceutical Sciences and Research 2012; 3(5): 1306-1311.
25. Asongalem EA, Foyet HS, Ekoo S, Dimo T and Kamtchouing P: Anti-inflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. Journal of Ethnopharmacology 2004; 95: 63-68.
26. Ozaki Y: Anti-inflammatory effects of *Curcuma Xanthorrhiza* Roxb, and its active principle. Chemical and Pharmaceutical Bulletin 1990; 38:1045-1048.
27. Kaushik A, Kaushik JJ, Das A, Gemal S and Gaim D: Preliminary studies on anti-inflammatory activities of *Diplazium esculentum* in experimental animal models. International Journal of Pharmaceutical Sciences and Research 2011; 2(5): 1251-1253.
28. Mossai JS, Rafatullah S, Gala AM and Al-Yahya M: Pharmacological studies of *Rhus retinorrhaea.* International Journal of Pharmacognosy 1995; 33: 242-246.
29. Sawadogo WR, Boly R, Lompo M and Some N: Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. International Journal of Pharmacology 2006; 2: 267-273.
30. Cuman RKN, Bersani-Amadio CA and Fortes ZB: Influence of type 2 diabetes on the inflammatory response in rat. Inflammation Research 2001; 50: 460-465.
31. Vasudevan M, Gunman KK and Parle M: Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. Journal of Ethnopharmacology 2007; 109: 264-270.
32. Shubhra KD, Arpona H, Sariful IH, Arif A and Hemayet H. Anti-inflammatory and antioxidant activities of ethanolic extract of aerial parts of *Vernonia patula* (Dryand.) Merr. Asian Pacific Journal of Tropical Biomedicine 2013; 3(10): 798-805.
33. Moody JO, Robert VA, Connolly JD and Houghton PJ: Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). Journal of Ethnopharmacology 2006; 104: 87-91.
34. Ismet AJ, Hemayet H, Ishrat N, Siblara I, Hassan K and Amirul I: Evaluation of anti-inflammatory and antioxidant potential from the kernel root of *Xylocarpus mekongenesis* (Lamk.) M. Roem. Oriental Pharmacy and Experimental Medicine 2012; 12 (3): 181-188.
35. Hemayet H, Ismet AJ, Shahidshid-Ud-Daula KM, Rahat M and Moniruzzaman M: Evaluation of Antinociceptive and Antioxidant Properties of the Ethanolic Extract of Root from Bangladesh. Asian Journal of Pharmaceutical and Biological Research 2012; 2(2): 106-112.
36. Hemayet H, Ismet AJ, Sariful IH, Shubhra KD, Arpona H and Arif A: Evaluation of Phytochemical Screening and Antinociceptive Properties of the Ethanolic Leaf Extract of *Trema cannabina* Lour. Advanced Pharmaceutical Bulletin 2013; 3(1): 103-108.
37. Ekuadzi E, Dickson RA and Fleischer TC: Antibacterial, anti-inflammatory and antioxidant properties of *gouania longipetala* hemsl. International Journal of Pharmaceutical Sciences and Research 2012; 3(5): 1300-1305.
38. Kanchan, Chauhan PK, Jaryal M, Kumari K and Singh M: Phytochemical and *in vitro* antioxidant potential of aqueous leaf extracts of *Brassica juncea* and *Coriandrum sativum*. International Journal of Pharmaceutical Sciences and Research 2012; 3(8): 2862-2865.
39. Adu F, Gbedema SY, Brown P, Annan K and Boamah VE: Antibacterial and free radical scavenging activity of *duranta plumieri*, linn. International Journal of Pharmaceutical Sciences and Research 2011; 2(2): 282-287.

How to cite this article:
Hossain H, Al-Mansur A, Akter S, Sara U, Ahmed MR and Jahangir AA: Evaluation of anti-inflammatory activity and total tannin content from the leaves of *Bacopa monnieri* (Linn.). *Int J Pharm Sci Res* 2014; 5(4): 1246-52.doi: 10.13040/IJPSR.0975-8232.5(4).1246-52