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# Evaluation of Antinociceptive and Antioxidant Activities of Whole Plant Extract of *Bacopa monniera*

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## ABSTRACT

*Bacopa monniera* commonly known as Brahmi grows in Bangladesh. The objective of the present study was to investigate the antinociceptive and antioxidant activities of ethanol extract of the whole plant of (*B. monniera*). The antinociceptive potential of the plant was determined using acetic acid induced writhing method. The results showed 39.79% ($p<0.05$) inhibition of writhings in the experimental animals when the plant extract was given intraperitoneally (i.p.) at 250 mg $kg^{-1}$ b.wt. However, the extract produced maximum 56.14% ($p<0.05$) acetic acid induced writhing inhibition in mice at the dose of 500 mg $kg^{-1}$ b.wt. This was also found to be comparable to the standard drug, Diclofenac-Na at 25 mg $kg^{-1}$ b.wt. which inhibited 76.52% ($p<0.05$) of writhing reflex. The plant extract showed a dose dependent antinociceptive activity in acetic induced writhing model in mice. It was also found that the obtained p-values calculated by student's t-test were statistically significant. In order to determine the antioxidant activity of *B. monniera*, the extract was also assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The plant extract showed significant DPPH free radical scavenging effect compared to the standard antioxidants such as ascorbic acid and Butylated Hydroxy Anisole (BHA). The $IC_{50}$ values of the extract, ascorbic acid and BHA were 79.84, 9.45 and 14.15 μg $mL^{-1}$, respectively. Thus, the present study confirmed the antinociceptive and antioxidant activities of the ethanol extract of the whole plant of *B. monniera*. Finally, it is suggested to do further research for isolation and identification of the chemical structures of the phytochemical compounds responsible for antinociceptive and antioxidant activities of *B. monniera*.

**Key words:** *Bacopa monniera*, antinociceptive, antioxidant activities, DPPH, ethanol

## INTRODUCTION

Antioxidants are chemical compounds that can slow or prevent the oxidation of other compounds through a chain of reactions (Jospeh *et al*., 2010). These chain reactions can be terminated by antioxidants through the elimination of reactive free radical intermediates (Sies, 1997). It is reported that oxidative stress are developed due to low quantity of antioxidant in the cells which damages or kills the cells resulting in cancer and coronary diseases (Jospeh *et al*., 2010). The human body having endogenous antioxidant defense mechanism is not able to stop

completely the oxidative stress in the body (Willcox *et al*., 2004). The phytochemical compounds are one of the most important and huge sources of natural antioxidants (Al-Mustafa and Al-Thunibat, 2008). However, medicinal plants, foods or antioxidant supplements may be utilized to reduce oxidative stress in the body (Malekirad *et al*., 2011; Adesegun *et al*., 2007). The plants might show their antioxidant activities against oxidative damage due to the presence of phenolic, flavonoid and tannins constituents but there is still a demand to find more information on the antioxidant potential of plant species (Frankle and Meyer, 2000; Rahman *et al*., 2011). Moreover, several free radicals play crucial roles to induce short-term algesia (Chung, 2004). It is reported that the plants are also used traditionally as analgesics (Calixto *et al*., 2000; Almeida *et al*., 2001). Naturally occurring pain killers are still required to substitute conventional steroid or non steroidal anti-inflammatory drugs having their severe adverse effects (Alam *et al*., 2012).

*B. monniera* Linn. belonging to the family of Scrophulariaceae is commonly known as Brahmi and has important applications in Ayurveda system. The plant is also referred to as *Bacopa monnieri, Herpestis monniera* and water hyssop (Nandave *et al*., 2007; Mukherjee and Dey, 1996). Several phytochemical compounds such as brahmine and herpestine, saponins d-mannitol, hersaponin, betulinic acid, stigmasterol, beta-sitosterol, bacosides A and B were isolated from the plant (Kapoor, 1990). The plant possesses significant antiulcerogenic (Sairam *et al*., 2001) and cardioprotective activities (Nandave *et al*., 2007) and has wide applications in the treatment of anxiety, depression, mental fatigue (Bhattacharya and Ghosal, 1998; Singh and Singh, 1981), epilepsy (Ganguly and Malhotra, 1967), bronchitis and asthma (Channa *et al*., 2003; Dar and Channa, 1997). Moreover, *in vitro* research has revealed the anticancer activity of *Bacopa* saponin fractions against sarcoma-180 cells. This might be due to inhibition of DNA replication in the cancerous cell line (Elangovan *et al*., 1995). Literature review indicated that no studies have so far been done for antinociceptive and antioxidant potentials of the ethanol extract of the whole plant of *B. monniera*. Thus, the present study was aimed to evaluate the antinociceptive and antioxidant potentials of the ethanol extract of the whole plant of *B. monniera*.

## MATERIALS AND METHODS

**Collection and identification of plant material:** The whole plant of *B. monniera* was collected in the month of September, 2009 from the hilly areas adjacent to the University of Chittagong, Bangladesh. The plant was taxonomically classified and identified scientifically by Department of Botany, University of Chittagong, Bangladesh.

**Preparation of plant extract:** The fresh plants were washed with water immediately after collection. The collected plants were chopped into small pieces, air dried at room temperature for about 10 days and ground into powder form which was stored in an airtight container. The 900 g powder was macerated in 2.5 L pure ethanol for 5 days at room temperature with occasional stirring and then mixture was filtered with Whatman No. 1 filter paper. The obtained filtrate was concentrated under reduced pressure below 50°C through rotatory vacuum evaporator (Bibby RE200, Sterlin Ltd., England). The concentrated filtrate was collected and allowed to air dry for complete evaporation of ethanol. The whole process was repeated three times and finally, 50 g of blackish-green colored, concentrated extract was obtained (yield approx. 5.55% w/w) which was kept in refrigerator at 4°C before use.

**Test animals and drugs:** Young Swiss-albino mice, either sex of 3-4 weeks of age weighing 20 g, were used for *in vivo* pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental conditions at animal house of Pharmacy Department of BGC Trust University Bangladesh, Chittagong and fed with rodent diet and water ad libitum. The standard drug Diclofenac-Na was used for this study and obtained from Square Pharmaceuticals Ltd., Bangladesh as a gift. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and Butylated Hydroxy Anisole (BHA) were obtained from Sigma Chemical Co., USA. Ethanol and Tween 80 were of analytical grade and purchased from Merck, Germany.

***In vitro* assay for antioxidant activity of plant extract:** The antioxidant activity of *B. monniera* extract was assessed in comparison to standard antioxidant ascorbic acid depending on the scavenging effect of DPPH free radical. The DPPH free radical scavenging activity of the ethanol extract of the plant was performed according to the method described by Chang *et al.* (2001). The radical scavenging activity was expressed as the percentage inhibition (% inhibition) and calculated as per following equation:

$$\text{Inhibition (\%)} = \frac{A_{(blank)} - A_{(sample)}}{A_{(blank)}} \times 100$$

where, $A_{(blank)}$ is the absorbance of the control (containing all reagents except the test compound), and $A_{(sample)}$ is the absorbance of the experimental sample with all reagents. $IC_{50}$ value (the concentration of sample required to scavenge 50% of DPPH free radical) was calculated from the plot of inhibition (%) against the log concentration of the extract. Here, ascorbic acid and BHA were used as standards.

**Antinociceptive activity of the plant extract:** The antinociceptive activity of the crude ethanol extract of *B. monniera* was studied using acetic acid induced writhing model in mice (Whittle, 1964). The percentage inhibition of writhing was calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhing (control)-Mean No. of writhing (test)}}{\text{Mean No. of writhing (control)}} \times 100$$

**Statistical analysis:** The results of the experiment were expressed as Mean±Standard Deviation (SD). All determinations were carried out in triplicate and average of the results was noted. Student's t-test (GraphPad Software) was used to determine a significant difference between the control and experimental groups where p values of less than 5% ($p<0.05$) was chosen as the level of significance.% inhibition was plotted against log concentration and $IC_{50}$ (Inhibition Concentration 50) value was calculated by linear regression analysis.

## RESULTS

**DPPH free radical scavenging activity:** It was found from the study results that the plant extract exhibited 86.62% inhibition of DPPH free radical scavenging activity at 100 μg $mL^{-1}$ whereas, ascorbic acid and BHA showed 99.34 and 90.38% inhibition, respectively at the same concentration. DPPH free radical scavenging activity of the crude ethanol extract of *B. monniera*

was increased with the increase of concentration of the extract (Fig. 1). Moreover, $IC_{50}$ value of the extract, ascorbic acid and BHA was calculated by linear regression analysis. It was found that $IC_{50}$ value of the extract was 79.84 μg $mL^{-1}$ with regression coefficient value ($R^2$) of 0.988 as shown in Fig. 2. On the other hand, Fig. 3 showed the $IC_{50}$ value of ascorbic acid (9.45 μg $mL^{-1}$) and the regression coefficient value ($R^2$) was 0.963 whereas, Fig. 4 revealed the $IC_{50}$ value of BHA ($IC_{50}$: 14.15 μg $mL^{-1}$) with $R^2$ value of 0.946. From the above results, it was found that all of the results showed linearity and $IC_{50}$ value of the standards and the extracts increased in the following order: Ascorbic acid <BHA <ethanol extract of *B. monniera*. The $IC_{50}$ value of the extract was found to be significant and to be comparable to that of ascorbic acid and BHA.

**Antinociceptive activity of the plant extract:** Antinociceptive activity of ethanol extract of the whole plant of *B. monniera* was done using acetic acid induced writhing model in mice. The extract produced 39.79 and 56.14% ($p<0.05$) writhing inhibition in mice at the dose of 250 and 500 mg $kg^{-1}$ body weight, respectively which were comparable to Diclofenac-Na (76.52% writhing inhibition, $p<0.05$) at the dose of 25 mg $kg^{-1}$ body weight as shown in Table 1.

The above findings may play an important role in the field of phytochemistry and pharmacology and this may be helpful for the future scientists to identify novel analgesics and antioxidants.

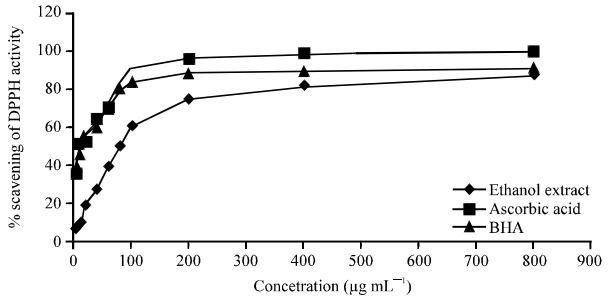


Fig. 1: DPPH free radical scavenging activity (% inhibition) of the ethanol extract of *B. monniera,* ascorbic acid and BHA. The values are the average of triplicate experiments and are represented as Mean±SD

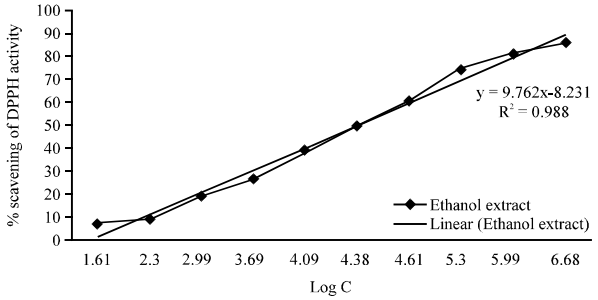


Fig. 2: $IC_{50}$ value of the ethanol extract of *B. monniera*. The values are the average of triplicate experiments and are represented as Mean±SD

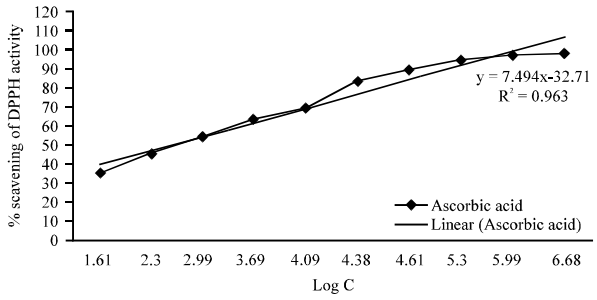


Fig. 3: $IC_{50}$ value of ascorbic acid. The values are the average of triplicate experiments and are represented as Mean±SD

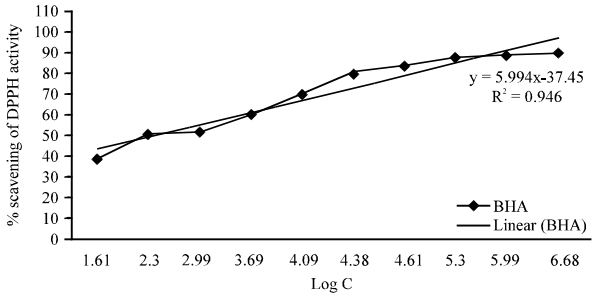


Fig. 4: $IC_{50}$ value of BHA. The values are the average of triplicate experiments and are represented as Mean±SD

Table 1: Effect of ethanol extract of *B. monniera* on acetic acid-induced writhing in mice

| Animal groups | Treatment | Writhing (Mean±SD) | Inhibition of writhing (%) | p-value |
|---|---|---|---|---|
| Control (n = 5) | 1% Tween solution in water | 33.67±0.58 (100%) | - | - |
| | (10 mL $kg^{-1}$ b.wt.) | | | |
| Positive control (n = 5) | Diclofenac sodium | | | |
| | (25 mg $kg^{-1}$ b.wt.) | 7.67±0.33 (23.48%) | 76.52 | <0.0001 |
| Test Group 1 (n = 5) | Ethanol extract | | | |
| | (250 mg $kg^{-1}$ b.wt.) | 19.67±0.58 (60.21%) | 39.79 | <0.0001 |
| Test Group 2 (n = 5) | Ethanol extract | | | |
| | (500 mg $kg^{-1}$ b.wt.) | 14.33±0.58 (43.86%) | 56.14 | <0.0001 |

Values are expressed as Mean±SD, SD: Standard deviation, n: No. of mice, *Significant at 5% significance level, *p <0.05 vs. control

## DISCUSSION

The antinociceptive activity of the plant extract was tested by acetic acid induced writhing method in mice which is usually used for investigation of antinociceptive activities (Koster *et al.*, 1959). Acetic acid produces analgesia and liberates endogenous compounds which in turn stimulate the pain nerve endings (Taesotikul *et al.*, 2003). Moreover, acetic acid increases the levels of prostaglandins such as $PGE_2$ and $PGF_{2\alpha}$ in the peritoneal fluid which are responsible for pain sensation (Deraedt *et al.*, 1980) and the analgesic activity can be achieved by the inhibition of prostaglandin productions (Gupta *et al.*, 2007). Few sympathetic nervous system mediators are also liberated by acetic acid (Hokanson, 1978). The ethanol extract of *B. monniera* showed significant

inhibition of writhing reflex which was comparable to the standard drug, Diclofenac-Na. Bhaskar and Jagtap (2011) also reported the antinociceptive activity of aqueous extract of *B. monniera* at the dose of 160 mg $kg^{-1}$ b.wt. They also suggested that the aqueous extract of *B. monniera* worked on the endogenous adrenergic, serotonergic and opioidergic systems for analgesic activity. The present study results also provide evidence of antinociceptive activity of the ethanol extract of *B. monniera* at the dose of 500 mg $kg^{-1}$ b.wt.

On the other hand, antioxidant activity of the plant extract was tested by DPPH free radical scavenging method. Yamaguchi *et al.* (1998) and Mokbel and Hashinaga (2006) reported that the antioxidants showed DPPH scavenging activities due to their proton donating abilities. The study results revealed that the scavenging effect (% inhibition of DPPH activity) of the extract and standards (ascorbic acid and BHA) was dependent on concentration. The scavenging power of the extract and standards increased with increasing of concentration. The DPPH free radical scavenging power of the extract and standards decreased in the following order: Ascorbic acid (99.34%)>BHA (90.38%)>ethanol extract of *B. monniera* (86.62%) with $IC_{50}$ of 79.84, 14.15 and 79.84 μg $mL^{-1}$, respectively. However, oxidative free radical scavenging activity of *B. monniera* was reported by Bhattacharya *et al.* (2000). The plant extract has also possessed abilities to scavenge superoxide anion and hydroxyl radical as antioxidant activities (Tripathi *et al.*, 1996). The results of the present study showed again its significant antioxidant potentials of the ethanol extract of the whole plant of *B. monniera* and the present study supported the results of the previous studies done on the same plant by the other researchers.

## CONCLUSION

Finally, it is concluded that the ethanol extract of the whole plant of *B. monniera* has significant antinociceptive and antioxidant activities. Although, the antinociceptive and antioxidant activities are relatively lower than those of ascorbic acid and BHA, the plant is supposed to be an important source of active phytochemical compounds for pharmacological efficacy. Thus, it is recommended for the future scientists to isolate, purify and elucidate structures of the active phytochemical compounds responsible for antinociceptive and antioxidant activities of *B. monniera*. Additionally, *in vivo* experiments for the pharmacological activities of the plant must be carried out to confirm the appropriateness of the results obtained from *in vitro* experiments.

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