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**Original Research Article**

# Evaluation of phytochemical and pharmacological activities of *Bacopa monnieri* (L.)

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## ABSTRACT

**Background:** *Bacopa monnieri* (L.) locally called Brahmi of Scrophulariaceae family has a long history for numerous therapeutic purposes like memory enhancing, antianxiety and antiepileptic agent. The aim of this study was to investigate phytochemical screening, antibacterial, cytotoxic, analgesic and neuropharmacological activities of *B. monnieri*.
**Methods:** The antibacterial activity was performed by disc diffusion method. Brine shrimp lethality bioassay was carried out to determine cytotoxic potential. Acetic acid induced writhing method was employed for the assessment of analgesic activity of *B. monnieri* extracts. The neuropharmacological activity was determined by hole cross, open field and thiopental sodium induced sleeping time test using Swiss Albino mice as experimental animal.
**Results:** Phytochemical screening revealed that *n*-hexane, dichloromethame and methanolic extracts contained reducing sugar, alkaloids, flavonoids, steroids and saponins. The sample showed comparable antimicrobial and cytotoxic activity. In analgesic activity test, methanol soluble extract showed highest activity compared to standard drug, Diclofenac sodium. The neuropharmacological activity of three extracts showed moderate activity as compared with standard drug, Diazepam and Thiopental sodium.
**Conclusions:** Our exploration suggests that *B. monnieri* contains bioactive compounds and it should be studied further for isolation and purification of such novel compounds.

**Keywords:** *Bacopa monnieri*, Antibacterial, Cytotoxicity, Analgesic, Neuropharmacological

## INTRODUCTION

The therapeutic use of plants continued with the progress of civilization and development of human knowledge. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants. Surprisingly, this large quantity of modern drugs comes from less than 15% of the plants which are known to have been investigated pharmacologically out of the estimated 500,000 species of higher plants growing on earth and more than 20,000 medicinal plants have been identified. Phytochemical and pharmacological studies of some of these plants have already resulted in the discovery and development of many important drugs.[1] *Bacopa monnieri* is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. It is referred to as *Bacopa monnieri*, *Herpestis monniera*, water hyssop and Brahmi. It is popularized as memory enhancing agent (Branulia). Traditionally, it was used as a brain tonic to enhance memory development and to provide relief to patients with anxiety or epileptic disorders.[2]

## METHODS

### *Chemicals and drugs*

Diclofenac sodium (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), acetic acid (Merck, Germany), diazepam

(Square Pharmaceuticals Ltd., Dhaka, Bangladesh), thiopental sodium (Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh) and tween-80 (India).

***Plant materials and extraction***

The whole plant of *Bacopa monnieri* (L.) was collected from the Bagerhat in January 2012 and was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 36147). The dried plant was then ground to coarse powder using high capacity grinding machine. 60.57 g plant powder was extracted with 300 ml of *n*-hexane, 300 ml of dichloromethane and 300 ml of methanol by a soxhlet apparatus. The crude extract was preserved in refrigerator. Different fraction of extracts was designed as *n*-HSE = *n*-hexane soluble extract; DCMSE = Dichloromethane soluble extract; MSE = Methanol soluble extract.

***Test animal***

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-27 g, were collected from the animal research branch of International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: 24.0±1.0˚C, relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle). The newly bought mice were given a week rest to get over the food and water restrictions incurred during transit and to get themselves adapted with the new environment of the laboratory, before being employed in any experiment.[3]

***Phytochemical screening***

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups such as alkaloids, reducing sugar, tannins, gums, flavonoids, saponins and steriods by standard method.[4]

***Antibacterial assay***

In antibacterial assay, 5 discrete media in petridishes were prepared for 5 bacterial strains. 100 mg of test samples were dissolved in 4 ml of ethanol and concentrations became 250 µg/10 µl. Ethanol was used as negative control where ciprofloxacin was taken as positive control. In each petridish standard antibiotic discs (ciprofloxacin 30 µg/disc) and blank discs (one for sample and one for negative control) were applied. Dose of sample and negative control were 250 µg/disc and 10 µl/disc, respectively. The plates were then inverted and kept in refrigeration for about half hours at 4°C to diffuse plant materials into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 16-18 hours. After proper incubation, the antibacterial activity was determined by measuring the diameter of zone of inhibition in terms of millimeter with calibrated digital slide calipers.[5]

***Brine shrimp lethality bioassay***

Brine shrimp lethality bioassay was carried for the determination of cytotoxic property of the *n*-hexane, dichloromethane and methanol soluble fractions of the crude extract. The eggs of brine shrimp (*Artemia salina* Leach) were hatched in a tank with 24 hours oxygen supply facility at $37^0$C temperature. Dimethyl sulfoxide (DMSO) is taken as negative control. All the test samples of 4 mg were taken and dissolved in 100 µl of DMSO to get stock solutions. Then 50 µl of solution was taken in each test tube containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781 µg/ml) were prepared by serial dilution.

Vincristine sulphate was used as the positive control. 0.4 mg of vincristine sulphate was dissolved in 100 µl of DMSO to get an initial concentration of 40 µg/ml from which serial dilutions are made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125 µg/ml. Then the positive control solutions are added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water. 30 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii were used for negative control groups. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.[6]

***Analgesic properties***

The acetic acid induced writhing method is an analgesic behavioural observation assessment method that demonstrates a noxious stimulation in mice. Test samples and control were given orally by means of a feeding needle. 30 minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) 0.2 ml was administered intraperitoneally to each of the animals of a group. After an interval of 15 minutes, this was given for absorption and number of squirms (writhing) was counted for 5 minutes. Diclofenac sodium was used as standard drug.[3]

***Neuropharmacological properties***

The animals were divided into negative control, positive control, and test groups containing five mice each for hole cross, open field and thiopental sodium induced sleeping time test.

The test groups received bark and leaves extracts of *B. monnieri* at the doses of 250 and 500 mg/kg body weight orally whereas control and positive control groups recei-

ved vehicle (1% tween 80 in water) and the standard drug diazepam (1 mg/kg body weight), respectively in all test.

In hole cross test the number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60 and 90 min after oral administration of the test drugs and the standard.[7] The floor of an open field of half square meter was divided into a series of squares each alternatively coloured black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 minutes at 0, 30, 60 and 90 minutes after oral administration of the test drugs and the standard.[8] In thiopental sodium induced sleeping time test sample, positive control and control were administered as hole cross and open field test. Thirty minutes later, thiopental sodium (40 mg/kg) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep i.e. time between the loss and recovery of righting reflex.

### *Statistical analysis*

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; $p < 0.05, 0.001$ were considered to be statistically significant.

## RESULTS

The result of different phytochemical groups of *n*-hexane, dichloromethane and methanol soluble extracts of *B. monnieri* (L.) is presented in Table 1. Different partitionate of the plant extracts of *B. monnieri* were tested for antibacterial activity against both gram positive and gram negative bacteria and compared with positive control, ciprofloxacin (30 µg/disc) as given in Table 2. In brine shrimp lethality bioassay, $LC_{50}$ were 2.02, 4.8 and 4.02 µg/ml for *n*-hexane, dichloromethane and methanol soluble fractions of crude extracts, respectively where for vincristine sulphate (positive control) it was 0.33 µg/ml as shown in Table 3. DMSO has no effects on brine shrimp nauplii. The different solvent soluble fractions showed moderate to mild activity compared with the standard drug. A dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of *n*-hexane, dichloromethane and methanolic soluble extracts of *B. monnieri*. In analgesic activity test, MSE showed inhibition of writhing of 37.37% and 46.46% at the dose of 250 and 500 mg/kg body weight, respectively as in Table 4 that was comparable to Diclofenac Sodium (62.22% of inhibition). The neuropharmacological activities of the extracts determined by hole cross, open field and thiopental induced sleeping time test is shown in Table 5, 6 and 7, respectively.

**Table 1: Result of different phytochemical group test of *B. monnieri* extracts.**

| Extract | Gums | Alkaloids | Steroids | Flavonoids | Tannins | Saponins | Reducing sugar |
|---|---|---|---|---|---|---|---|
| **Methanol** | - | + | + | + | - | + | + |
| **Dichloromethane** | - | + | + | + | - | + | + |
| ***n*-Hexane** | - | + | + | + | - | + | + |

Here, + = Presence, - =Absence

**Table 2: Antibacterial activity of the different fractions of *B. monnieri* extracts.**

| Bacterial Strains | Negative Control | Diameter of zone of inhibition (mm) (Dose/disc) | | | |
|---|---|---|---|---|---|
| | | *n*-HSE | DCMSE | MSE | (Positive control) Ciprofloxacin |
| ***Bacillus cereus*** | 0 | 11 | 10 | 11 | 25 |
| ***Staphylococcus aureus*** | 0 | 12 | 14 | 10 | 30 |
| ***Escherichia coli*** | 0 | 8 | 0 | 12 | 30 |
| ***Klebsiala granulomatis*** | 0 | 9 | 15 | 11 | 28 |
| ***Aromonus hydrophila*** | 0 | 13 | 12 | 8 | 30 |

## DISCUSSION

To get preliminary idea about the active constituents present in different fractions of *B. monnieri* extracts, phytochemical screening was performed that showed the presence of several important phytochemical constituents like reducing sugar, flavonoids, alkaloids, steroids and saponins. It has been reported that different phytochemicals are responsible for antibacterial, cytotoxic, analgesic and neuropharmacological activities.[9] The dichloromethane soluble fraction showed highest antibacterial activity compared with ciprofloxacin against *Klebsiella granulomatis*. This rest of the fractions also showed comparable antibacterial activity except

DCMSE against *Escherichia coli*. Among the chemical constituents alkaloids and flavonoids may be responsible for antibacterial property.[10] The samples showed comparable cytotoxic activity in brine shrimp lethality bioassay. The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, steroid and flavonoid present in *B. monnieri* extract.[11] Reducing sugar and flavonoids may be responsible for the analgesic activity of *B. monnieri* extract.[12,13] The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 4th observation period (90 min). Both hole cross and open field tests showed that the depressing activities of the extracts was evident from the 2nd observation period in the test animals at the doses of 250 and 500 mg/kg body weight. The results obtained in our present study, indicate that the extracts might have depressant action on the CNS.[14-20]

**Table 3: Effects of *n*-HSE, DCMSE and MSE on brine shrimp nauplii.**

| Conc. (C) (µg/ml) | Log C | % Mortality | | | $LC_{50}$ (µg/ml) | | | Vincristine Sulfate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n*-HSE | DCMSE | MSE | *n*-HSE | DCMSE | MSE | Conc. (C) (µg/ml) | Log C | % Mortality | $LC_{50}$ (µg/ml) |
| **400** | 2.301 | 100 | 100 | 100 | 2.02 | 4.8 | 4.02 | 40 | 1.602 | 100 | 0.33 |
| **200** | 2 | 90 | 100 | 100 | | | | 20 | 1.301 | 100 | |
| **100** | 1.698 | 80 | 90 | 90 | | | | 10 | 1.000 | 90 | |
| **50** | 1.397 | 70 | 80 | 80 | | | | 5 | 0.698 | 80 | |
| **25** | 1.097 | 70 | 80 | 70 | | | | 2.5 | 0.397 | 70 | |
| **12.5** | 0.796 | 60 | 70 | 70 | | | | 1.25 | 0.096 | 50 | |
| **6.25** | 0.495 | 50 | 70 | 60 | | | | 0.625 | -0.204 | 40 | |
| **3.125** | 0.194 | 50 | 70 | 50 | | | | 0.3125 | -0.505 | 30 | |
| **1.563** | -0.107 | 40 | 60 | 50 | | | | 0.15625 | -0.806 | 30 | |
| **0.781** | -0.409 | 40 | 50 | 40 | | | | 0.078125 | -1.107 | 20 | |

**Table 4: Results of acetic acid induced writhing test of different fractions of *B. monnieri*.**

| Administered Substance | Dose | *n*-HSE (Mean±SEM) | DCMSE (Mean±SEM) | MSE (Mean±SEM) | % of Inhibition (*n*-HSE) | % of Inhibition (DCMSE) | % of Inhibition (MSE) |
|---|---|---|---|---|---|---|---|
| **Control** | 0.10 ml | 49.5 ± 1.51 | 49.5 ± 1.51 | 49.5 ± 1.51 | 0 | 0 | 0 |
| **Positive control** | 25 mg/kg | 18.7± 1.23 | 18.7 ± 1.23 | 18.7 ± 1.23 | 62.22 | 62.22 | 62.22 |
| **Group-1** | 250 mg/kg | 45.6 ± 1.20 | 35.7 ± 0.96 | 31 ± 1.32 | 7.88 | 27.88 | 37.37 |
| **Group-2** | 500 mg/kg | 36.4 ± 1.52 | 29.7 ± 1.22 | 26.5 ± 1.41 | 26.46 | 40 | 46.46 |

**Table 5: Effect of different fractions of *B. monnieri* by hole cross test on mice.**

| Group | Dose | *n*-HSE | | | | DCMSE | | | | MSE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 0 min | 30 min | 60 min | 90 min | 0 min | 30 min | 60 min | 90 min |
| **Control** | 0.2 ml | 17 ± 1.97 | 17.8 ± 1.92 | 17.6 ± 2.28 | 17.2 ± 1.56 | 17 ± 1.97 | 17.8 ±1.92 | 17.6 ± 2.28 | 17.2 ± 1.56 | 17 ± 1.97 | 17.8 ± 1.92 | 17.6 ± 2.28 | 17.2 ± 1.56 |
| **Positive control** | 1 mg/kg | 15.4 ±0.9 | 6 ± 0.94 | 2 ± 0.79 | 1.6 ± 1.04 | 15.4 ± 0.91 | 6 ± 0.94 | 2 ± 0.79 | 1.6 ± 1.04 | 15.4 ±0.91 | 6 ± 0.94 | 2 ± 0.79 | 1.6 ± 1.04 |
| **Group 1** | 250 mg/kg | 14.5 ±1.3 | 3.75 ± 0.58 | 2 ± 1.01 | 1.50 ± 0.97 | 14.75 ±1.32 | 8.25 ±0.58 | 4.75 ± 1.01 | 9.75 ± 0.97 | 17.75 ±1.32 | 8.5 ± 0.58 | 2.25 ± 1.01 | 9.25 ± 0.97 |
| **Group 2** | 500 mg/kg | 17 ± 0.83 | 3.75 ± 0.67 | 1 ± 0.770 | 1 ± 1.01 | 16.25 ±0.83 | 7 ± 0.67 | 8.75 ± 0.77 | 11.50 ± 1.02 | 11.75 ±0.83 | 7 ± 0.67 | 3.75 ± 0.77 | 8.5 ± 1.02 |

**Table 6: Effect of different fractions of *B. monnieri* by open field test on mice.**

| Group | Route | *n*-HSE | | | | DCMSE | | | | MSE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 0 min | 30 min | 60 min | 90 min | 0 min | 30 min | 60 min | 90 min |
| **Control** | Oral | 121.6 ±5.35 | 117.8 ± 3.94 | 116.6 ± 2.61 | 110.8 ± 7.53 | 121.6 ± 5.35 | 117.8 ± 3.94 | 116.6 ± 2.61 | 110.8 ± 7.53 | 121.6 ± 5.35 | 117.8 ± 3.94 | 116.6 ± 2.61 | 110.8 ± 7.53 |
| **Positive control** | I.P. | 118.4 ±7.04 | 67.8 ± 5.63 | 41.6 ± 3.65 | 19.6 ± 2.61 | 118.4 ± 7.04 | 67.8 ± 5.63 | 41.6 ± 3.65 | 19.6 ± 2.61 | 118.4 ± 7.04 | 67.8 ± 5.63 | 41.6 ± 3.65 | 19.6 ± 2.61 |
| **Group 1** | Oral | 69 ± 7029 | 42 ± 9.71 | 30 ± 5.15 | 22.35 ± 4.59 | 86.25 ± 7.29 | 44 ± 9.71 | 14 ± 5.15 | 48.35 ± 9.19 | 69.25 ± 7.29 | 42.50 ± 9.71 | 22 ± 5.15 | 28.25 ± 4.59 |
| **Group 2** | Oral | 55.75 ±8.32 | 35.50 ± 8.21 | 27.25 ± 5.04 | 19 ± 3.64 | 88.75 ± 8.32 | 39.75 ± 8.21 | 13.25 ± 5.04 | 17.75 ± 3.64 | 59.25 ± 8.32 | 40.50 ± 8.21 | 32.50 ± 5.04 | 37 ± 3.64 |

Values are expressed as Mean ± SEM (n=5); I.P. = Intraperitoneally

**Table 7: Effect of different fractions of *B. monnieri* by thiopental sodium induced sleeping time test on mice.**

| Group | Dose (mg/kg) | Route | *n*-HSE | | DCMSE | | MSE | |
|---|---|---|---|---|---|---|---|---|
| | | | Onset of sleep (min) | Duration of sleep (min) | Onset of sleep (min) | Duration of sleep (min) | Onset of sleep (min) | Duration of sleep (min) |
| **Control** | 0.2 ml | Oral | 6.50 ± 0.65 | 47 ± 5.07 | 6.50 ± 0.645 | 47 ± 5.07 | 6.50 ± 0.645 | 47 ± 5.07 |
| **Positive control** | 40 mg/kg | I.P. | 3.75 ± 0.48 | 91.50 ± 4.65 | 3.75 ± 0.479 | 91.50 ± 4.65 | 3.75 ± 0.479 | 91.50 ± 4.65 |
| **Group-1** | 250 mg/kg | Oral | 1.50 ± 0.03 | 23.75 ± 1.75 | 1.46 ± 0.021 | 43 ± 2.48 | 1.41 ± 0.022 | 16.75 ± 1.18 |
| **Group-2** | 500 mg/kg | Oral | 1.96 ± 0.14 | 77.75 ± 4.11 | 2.50 ± 0.021 | 83 ± 2.90 | 2.19 ± 0.035 | 86 ± 1.68 |

## CONCLUSION

It can be concluded that *B. monnieri* (L.) possesses significant antibacterial and mild cytotoxic potential. It showed moderate analgesic and CNS depressant potential. The findings of the observation also provide future support and reinforce the traditional use of the plant in different medical disorders. Hence, further studies are suggested to pinpoint the compounds found in the plant extracts of *B. monnieri* and to better understand the mechanism of such actions scientifically.

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