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PHARMACOLOGICAL AND PHYTOCHEMICAL SCREENINGS OF *BIDENS SULPHUREA CAV.*

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ABSTRACT

In the present study, the methanol extract of *Bidens sulphurea* Cav. (Asteraceae) was screened for its phyto-constituents and pharmacological activities. Phytochemical investigation for secondary metabolites revealed the presence of reducing sugar, glycosides, tannins, flavonoids, saponins and gums. In the antimicrobial activity test by disc diffusion method, the extract at the dose of 500 µg/disc strongly inhibited the growth of *Staphylococcus aureus* and *Salmonella paratyphi*. Same dose of the extract found to be active against almost all the tested fungi. The crude extract of the plant showed the presence of strong analgesic, antipyretic and antifungic activity; moderate anti-inflammatory, amylase inhibition activity and mild membrane stabilization and clotlysis activities. It was also found that the extract has a positive neuropharmacological activity on Swiss mice.

Keywords: *Bidens sulphurea*, methanol extract, phytochemical, pharmacological activities.

INTRODUCTION

Although natural products have been used by civilization since ancient times, only in recent decades has there been growing research into alternative therapies and the therapeutic use of natural products, especially those derived from plants. Herbal preparations are frequently

used not only in rural areas in developing countries but also in developed countries in human and veterinary medical practices.

There are about 200 species from *Bidens* and many of them have reported ethnobotanical and ethnomedical uses leading to the traditional medical systems. Several members of this genus have been reported to possess antibacterial, antidysenteric, anticancer, antipyretic, anti-inflammatory, antimicrobial, antimalarial, diuretic, hepato-protective and hypotensive activities. The study aims at the evaluation of the different chemical groups, antimicrobial and toxic responses as well as the pharmacological potential of the crude methanol extracts and to search logical evidence for their folk uses and further exploitation. In order to the above assertion, the medicinal plants must be subjected to extensive study. For this instances *Bidens sulphurea* Cav. (Family: Asteraceae) was subjected for chemical and pharmacological investigations.

MATERIALS AND METHODS

Plant collection and identification

For the investigation, the leaves of *B. sulphurea* was collected from Chittagong, Bangladesh in the month of March and was identified by the taxonomist, Forest Research Institute (FRI); Chittagong, Bangladesh.

Extraction

Leaves of the plant were collected and dried (temperature not exceeding 35 to 50°c), then the dried leaves were subjected to course grinding. The powdered material was subjected to hot extraction with methanol (absolute) by the Soxhlet apparatus. The extraction was carried out for about 20 h and the extract was filtered through a cotton plug followed by Whatman filter paper no. 1.The extract was then concentrated by using a rotary evaporator. Yield was 11%.

Required chemicals/reagents and their sources

Nutrient agar medium (Hi Media Laboratories, India)
Nutrient broth medium (Hi Media Laboratories, India)
Azithromycin (Square Pharmaceuticals Ltd., BD)
Fluconazole (Square Pharmaceuticals Ltd., BD)
Diclofenac sodium (Square Pharmaceuticals Ltd., BD)
Acetyl salicylic acid (Zenith Pharmaceuticals Ltd., BD)
Acarbose (Pacific Pharmaceuticals Ltd., BD)

Streptokinase (Dong Kook)

Paracetamol (GSK Pharmaceuticals Ltd., BD)

Phytochemical screening

The methanol extract of *B. sulphurea* was subjected for the identification of secondary metabolites (**table 1**).

Pharmacological screening

Antimicrobial screening

The antibacterial and antifungal action of the crude extract was tested by the disk diffusion method. [1] The tests were conducted against 4 Gm (+) and 7 Gm (-) species of pathogenic bacteria and 7 fungi supplied by the Microbiology Lab., Department of Pharmacy, BGC Trust University, Chittagong, Bangladesh. The test organisms were maintained on nutrient agar slopes and were sub-cultured. Azithromycin and fluconazole were taken as standards for antibacteric and antifungic tests respectively. The antimicrobial activity of the test agents was expressed by measuring the diameter of zone of inhibition expressed in millimeters (mm).

Minimum inhibitory concentration determination

'Serial tube dilution technique' [2] was selected for the evaluation of the minimum inhibitory concentration of the extract.

Screening of *in-vitro* anti-inflammatory activity

The present study was developed by certain modification of the method claimed by Shinde et al, (1999) [3] on the basis of *in-vitro* determination of the inflammatory and membrane instability caused by the control group and comparing it with the positive control group. Egg albumin was reconstituted as 5% v/v aqueous solution with iso-saline.

Anti-inflammatory activity was measured by measuring the absorbance of the treatment groups and converting it into total inhibition of protein denaturation. Diclofenac sodium at the final concentration of 200, 100, 50, 25 and 12.5 µg/ml used as reference drug for determination of absorbance and extract of *B. sulphurea* at the final concentration of 400, 200, 100, 50 and 25 µg/ml treated similarly for determination of absorbance. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Assessment of *in-vitro* membrane stabilization activity

The erythrocyte membrane resembles to lysosomal membrane and as such the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. [3] To prepare the erythrocyte suspension, whole blood was obtained using syringes. The more the membrane in-stabilization effects, the more it will cause haemolysis and the measured absorbance will be higher. For standard (0.1 mg/ml), acetyl salicylic acid was dissolved in distilled water. For test sample, aqueous crude extract at the dose of 1 mg/ml and 0.5 mg/ml were prepared.

For **hyposolution-induced haemolysis** the percentage inhibition of membrane stabilization was calculated using the following equation:

% inhibition of haemolysis = $100 \times (OD_1\text{-}OD_2/OD_1)$

where, OD_1 = Optical density of hypotonic – buffered saline solution alone (control) and OD_2 = Optical density of test sample in hypotonic solution.

For **heat-induced haemolysis** the percentage inhibition of membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of haemolysis} = 100 \times [1- (OD_2\text{-}OD_1/ OD_3\text{-}OD_1)]$$

where,OD_1 = Optical density of unheated test sample, OD_2 = Optical density of heated test sample, OD_3 = Optical density of heated control sample.

Assessment for anti-amylase activity

The α-amylase inhibiting activity was measured using the starch-iodine method by Komaki et al, (2003). [4] Acarbose at the dose of 50 µg/ml was taken as standard. The percentage inhibition was calculated by comparing to the control which did not have the extract.

% inhibition of enzyme activity = (A-C) × 100/ (B-C)

where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of control (no extract).

Assessment for thrombolytic activity

In our study 100 mg of methanol extract was used as experimental drug 5 ml of blood samples were collected from volunteer and distributed into five separate pre-weighed (W_1) micro-centrifuge tubes. The blood specimen were centrifuged at 2500 rpm for five minutes and then incubated for 45 minutes at 37°c. After clotting of blood, serum was decanted and removed. Then weight of clotted blood (ΔW) was taken by subtracting the pre-weight (W_1) from the weight of clot containing tube (W_2) as - $\Delta W = W_2 – W_1$. Then 100 µl extract of *B. sulphurea* was added to the clot containing tubes. Similarly 100 µl of streptokinase was added to clot of standard tubes and 100 µl of water was added to clot of blank tubes those were used as positive and negative control respectively. Then all the tubes were incubated at 37°c for 90 minutes and weighed again for getting the weight variation among the pre weight and final weight (W_3) that was achieved for clot lyses (thrombolysis). [5]

Lysis of clot was measured by the released lysed fluid from the reaction vessel of the test groups. Percent lysis was calculated from the initial clot wt (gm). Activity lysis, was compared with the vehicle (DW) group.

Assesment of antipyretic activity

Antipyretic activity of drug was measured by slightly modifying the method described by. [6] Swiss mice were fasted overnight with water *ad-libitum* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the animals' dorsum region. Seventeen hours after the injection, the rectal temperature of each mouse was measured using a digital thermometer. Only mice that showed an increase in temperature of at least 1.33˚F were used for the experiment. Test samples were administered orally and the temperature was measured at 1, 2 and 3 hr after drug administration. Each group used 3 mice. Paracetamol was used as standard. Antipyrexia activity of the test samples was determined by the inhibition of thermal raise method and gradually decrease of temperature of the test animals represent the fever healing activity.

Screening of analgesic activity

The analgesic activity of the crude extractives was determined by Formaldehyde induced writhing method. [7] Diclofenac-Na was used as standard. Young Swiss mice of either sex, average weight 18-25 gm were used for the experiment.

Screening of neuropharmacological activity

The Open Field Test (OFT), described by Cícero Francisco et al, (2008), [8] The Hole Cross Test (HCT), described by File and Wardill (1975) [9] and The Light Dark Test (LDT), described by Rogoz et al., (2003) [10] were adopted for the evaluation of neuropharmacological activity of the crude extract on Swiss albino mice (18-25 gm body weight) of either sex which were housed in 12 hrs dark-light and provided *Ad-libitum* and normal drinking water. For the present study two doses (250 and 500 mg/kg B.W.) of the methanol crude extract of *B. sulphurea* were taken. For vehicle only DW was administered to the experimental animal.

The findings of the experiments are based on the primary data obtained from the tests. All the experiments were performed in duplicate and replicated at least three times. Data are expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

From the data depicted on table 1, it should be mentioned that the methanolic extract of *B. sulphurea* contains reducing sugar, glycosides, tannins, flavonoids, saponins and gums.

In the antibacterial sensitivity test, it was observed that the plant extract produced significant zone of inhibition. The highest zone of inhibition was produced by *Bidens sulphurea* (15 mm) against *Salmonella paratyphi* and *Staphylococcus aureus* at the dose of 50µg/µ1. Then followed by 14.67 13.67 and 8.67 mm by *Shigella dysenteriae*, *Shigella sonnei* and *Vibrio cholerae*, *Salmonella typhi* respectively. But the extract produced no inhibition to the other tested bacteria [table 2]

In the antifungal sensitivity test, it was observed that the plant extract produced significant zone of inhibition against all the clinical species (with some exceptions). The *B. sulphurea* extract (50 µg/µ1) produced highest zone of inhibition (19.67 mm) against the *Blastomyces dermatitidis*. Then followed by 11.33, 11, 10.33 and 9.67 mm *Candida albicans, Cryptococcus neoformans, Aspergillus niger* and *Pityrosporum ovale* respectively. But the extract produced no inhibition to the other tested fungi [table 2]

From the table 3, it was depicted that crude methanol extract inhibited the growth of *Sa. paratyphi* and *Vi. cholerae* significantly at the dose of 64 µg/ml, then followed by *St. aureus,*

Sh. dysenteriae, *Sh. sonnei*, *Sa. paratyphi* by 128 µg/ml. But the extract was found to be inactive against other bacterial strains.

The growth of the species of fungi, *Bl. dermatidis* and *Cr. neoformans* was inhibited by 64 µg/ml of crude methanol extract. Then followed by *As. niger*, *Ca. albicans* and *Pi. ovale* by 128 µg/ml. The extract was found to be inactive against other fungal strains. [table 3]

During the experiment for *in-vitro* anti-inflammatory test, the crude methanol extract of *B. sulphurea* showed inhibition of protein denaturation 189.03% by 400 (µg/ml), 130.09% by 200 (µg/ml), 39.41% by 100 (µg/ml), 10.75% by 50 (µg/ml) and 3.95% by 25 (µg/ml). On the other hand, solution of Diclofenac-Na showed inhibition of protein denaturation 934.98% by 200 (µg/ml), 692.775% by 100 (µg/ml), 282.252% by 50 (µg/ml), 89.5985% by 25 (µg/ml) and 1.28205% by 12.5 (µg/ml). IC_{50} of the stanadrd, diclofenac-Na and extract were 8.25 and 107.59 µg/ml respectively. A dose response relationship was observed during the experiment [table 4]

Test extract both 1 mg/ml and 0.5 mg/ml inhibited hypotonic solution-induced haemolysis of RBCs by 36.36% and 27.27% respectively whereas, the standard; aspirin showed 52.27% [table 5 (a)]

On the other hand, test extract both 1 mg/ml and 0.5 mg/ml inhibited heat-induced haemolysis of RBCs by 373.78% and 305.94% respectively whereas, the standard; aspirin showed 52.27% [table 5 (b)].

Extract consecutive dose from 25-400 µg/ml produced a dose graded inhibition of amylase activity. Mild to moderate inhibitory activity was found by the extract in comparison to the standard, acarbose (79.8%). IC_{50} of methanol extract was 772.53 µg/ml [table 6].

During the *in-vitro* thrombolytic activity addition of 100 µl streptokinase (SK), a positive control (30,000 I.U.) to the clots along with 90 min of incubation at 37°c, showed 67.83% clot lysis. Clots when treated with 100 µl DW (negative control) showed only negligible clot lysis (2.47%).The mean difference in clot lysis percentage between positive and negative control was very significant ($p<0.05$, $p<0.5$). After treatment of clots with 100 µl of *B. sulphurea* negligible clot lysis, i.e., 12.29% and 10.57% by 1.0 and 0.5 mg/ml respectively, were obtained but mean of percentage of clot lysis was more than DW alone [table 7].

In the yeast powder induced pyresis in Swiss mice, the crude methanol extract (500 mg/kg) of *B. sulphurea* significantly ($p<0.001$, $p<0.01$, $p<0.02$) decreased pyretic temperature of the experimental animals. Temperature reduction in the 1st, 2nd and 3rd hours was 1.40, 2.37 and 2.8˚F respectively. Standard, paracetamol at the dose of 150 mg/kg also reduced pyretic temperature in 1st, 2nd and 3rd hours by 1.33, 2.1 and 2.57˚F respectively while the vehicle group (DW) showed an insignificant reduction of fever temperature [table 8].

In the formalin induced analgesic activity test, the methanol extract of *B. sulphurea* at the doses of 500 mg/kg and 250 mg/kg showed the inhibition of writhing by 80.73% and 71.56% respectively in comparison to the positive control, diclofenac sodium (83.49%). Extract increased latency period 7.33 and 5.67 min by 500 mg/kg and 250 mg/kg respectively in comparison to the positive control, diclofenac-Na at 3.67 min [table 9].

In the neuropharmacological studies, it was observed that there was a reduced field cross, hole cross and light residence when compared to the standards [table 10a, 10b and 10c].

Table 1. Phytoconstituents found in the methanol extract

Phytoconstituents	Consequences
Reducing sugar	++
Steroids	++
Glycosides	++
Tannins	++
Alkaloids	-----
Flavonoids	++
Saponins	+
Gums	+
+ = Presence; - = Absence	

CONCLUSION

The extract of the leaves of *Bidens sulphurea* Cav. is claimed to have anti-microbial, anti-inflammatory, membarane stabilizing, thrombolytic, alpha-amylase inhibiting, antipyretic, analgesic and neuropharmacological activity. The traditionl use of the plant was the basis of present investigation; further investigation is needed before isolation and characterization of the active principles.

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CONFLICT OF INTEREST

No conflict of interest.

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