

**ANALGESIC AND CYTOTOXIC ACTIVITIES OF *SIDA RHOMBIFOLIA* LINN.**

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**Summary**

The ethanol extract of dried aerial part of *Sida rhombifolia* Linn. (Family - Malvaceae) was investigated for its possible analgesic and cytotoxic activities in animal models. The extract produced significant ( $P < 0.001$ ) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The crude ethanolic extract also produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* ( $LC_{50} = 40 \mu\text{g/ml}$  and  $LC_{90} = 80 \mu\text{g/ml}$ ). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

**Key Words:** Analgesic activity, cytotoxic activity, *Sida rhombifolia* Linn.

**Introduction**

*Sida rhombifolia* Linn. (English Name: Arrow-leaf Sida, Common Sida; Family: Malvaceae; Synonym: *Sida microphylla* Cav., *Sida ruderata* Macfad.) locally known as 'Lal Berela Atibala' in Bangladesh. It is also known as Bala, Mahabala (India); Ndeni Puaka (Fijian); Herbe à Balais (French); Angosacha (Spanish); Matala Hoatā (Tongan); Cuban Jute, Paddy's Lucerne, Queensland Hemp (Other English Name); Huang Hua Mu (China); Chittamadi (Srilanka); Escobilla (Panama); Mautofu (Samoa) and Petoria-bossie (Africa). It is a short-lived perennial subshrub (woody stem and herbaceous branches) commonly growing to 60 cm, but sometimes reaching 1.5 m in height, distributed throughout Bangladesh<sup>1</sup>. It is also distributed over 70 countries throughout the tropical, subtropical, and warm temperate regions<sup>2-3</sup>. It is native to tropics, probably the Americas, now widespread in tropics and native range obscure. It is very common throughout Australia, India and Sri Lanka, especially in tropical to warm temperate open grassy areas<sup>4</sup>.

*Sida rhombifolia* Linn. is demulcent, diaphoretic, diuretic, emollient, stomachic, tonic, sudorific, appetite and stimulant. Arrowleaf sida has significant medicinal applications for which it is cultivated throughout Bangladesh and India. Leaves and roots are used for piles, gonorrhoea, anti-soud, diuretic, aphrodisiac. Roots of these herbs are held in great repute in treatment of rheumatism<sup>5</sup>. Stems abound in mucilage and are employed as demulcents and emollients both for external and internal use. The herb is also useful in calculous troubles and as a febrifuge with pepper. Mucilage is used as an emollient and for scorpion sting. Australian aborigines use the herb to treat diarrhoea. Leaves are smoked in Mexico and a tea is prepared in India for the stimulation it provides<sup>4</sup>.

Research study showed that the isolated pure compound phenyl ethyl  $\beta$ -D glucopyranoside from the stem of the plant *Sida rhombifolia* has larvicidal activity against common filaria vector, *Culex quinquefasciatus* at different instar under laboratory conditions and the LC values for the isolated compound was 36.22, 43.94 and 50 44.92, 58.34 and 60.40, 63.32 and 70.72, 82.52 ppm for 1st, 2nd, 3rd and 4th instar larvae *Culex quinquefasciatus* at 24 and 48 h post exposure respectively<sup>6</sup>.

Another research study revealed that flavonoids crude extract from *Sida rhombifolia* L. could *in vitro* inhibit the activity of Xanthine Oxidase (XO) (xanthine: oxygen oxidoreductase EC 1.2.3.2) up to 55% and could be anti-gout. The flavonoids crude extract yielded approximately 12% with LC<sub>50</sub> of 501 mg L<sup>-1</sup> and its inhibitory effect from 48 to 71% (100-800 mg L<sup>-1</sup>). And the kinetic study resulted that the type of flavonoids crude extract inhibition was a competitive inhibition with inhibitor affinity ( $\alpha$ ) of 2.32 and  $p < 0.01$ <sup>7</sup>.

From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the analgesic and cytotoxic activities of the ethanol extract of dried aerial part of *Sida rhombifolia* Linn.

## **Materials and Methods**

### **Plant Material**

Aerial part of *Sida rhombifolia* Linn. were collected from Khulna University campus, Khulna, Bangladesh in June 2009 and were authenticated by the experts at National Herbarium (Accession Number: 34402). After collection, aerial parts were sun dried for several days to remove moisture. After drying, the dried aerial parts were cut into small pieces by the help of a sharp knife and then were ground into coarse powder by 'Hammer' mill. About 400 gm of powdered aerial parts was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. And this crude ethanolic extract was used for all phytochemical and pharmacological screening.

### **Animals**

For analgesic activity study, young Swiss-albino mice of either sex, weighing 20-25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), were used. After purchase, the animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 $\pm$ 2.0°C and 12h light-dark cycle) and fed with standard diets and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol. All the experiments were conducted on an isolated and noiseless condition.

**Drugs**

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh).

**Preliminary Phytochemical Analysis**

The ethanol extract of dried aerial part of *Sida rhombifolia* Linn. was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test<sup>1,8</sup>.

**Tests for Reducing Sugar**

Benedict's Test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

**Tests for Tannins**

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

**Test for Flavonoids**

A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

**Test for Saponins**

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

**Test for Gums**

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

**Tests for Steroids**

Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

**Tests for Alkaloids**

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

### **Tests for Glycosides**

A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added. Yellow precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath. Brick-red precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath. Brick red precipitate is considered as an indication for the presence of glycosides.

## **Pharmacological Studies**

### **Analgesic Activity**

Analgesic activity of the ethanolic extract of dried aerial part of *Sida rhombifolia* Linn. was tested using the model of acetic acid induced writhing in mice<sup>9-10</sup>. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control' which received 1% (v/v) Tween-80 solution in water; 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 ethanolic extracts of dried aerial part of *Sida rhombifolia* Linn. at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and the ethanolic extracts were administered orally 30 min prior to the intra-peritoneal injection of 0.7 % acetic acid, then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

### **Cytotoxicity Test**

The brine shrimps used for cytotoxicity test<sup>11</sup> were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Six doses of plant extract (10, 20, 40, 60, 80 and 100 µg/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 µl/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette. The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

### **Statistical Analysis**

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

## Results

### Chemical Group Test

Results of different chemical group tests on the ethanolic extract of dried aerial part of *Sida rhombifolia* Linn. showed the presence of Reducing Sugar, Steroids, Alkaloids, Gums, Flavonoids and Glycosides (Table 1).

**Table 1:** Results of different chemical group tests of the extract of dried aerial part of *Sida rhombifolia* Linn.

| Extract                                                                 | Reducing Sugar | Steroids | Alkaloids | Tannins | Gums | Flavonoids | Glycosides | Saponins |
|-------------------------------------------------------------------------|----------------|----------|-----------|---------|------|------------|------------|----------|
| Ethanolic extract of dried aerial part of <i>Sida rhombifolia</i> Linn. | +              | +        | +         | -       | +    | +          | +          | -        |

Key: + = Presence, - = Absence

### Antinociceptive Activity

Table 2 showed the effect of dried aerial part of *Sida rhombifolia* Linn. on acetic acid-induced writhing model in mice. The extract produced about 43.84% and 69.95% writhing inhibition at the dose of 250 and 500 mg/kg of body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 82.27% at the dose of 25 mg/kg of body weight (Table 2).

### Cytotoxic Activity

In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper  $LC_{50}$  and  $LC_{90}$  were deduced ( $LC_{50} = 40 \mu\text{g/ml}$ ;  $LC_{90} = 80 \mu\text{g/ml}$ ) (Table 3).

**Table 2:** Effect of ethanolic extract of dried aerial part of *Sida rhombifolia* Linn. on acetic acid induced writhing in mice

| Animal Group / Treatment                                | Number of writhes (% writhing) | Inhibition (%) |
|---------------------------------------------------------|--------------------------------|----------------|
| Control<br>1% tween-80 in water, p.o.                   | 20.3±1.76<br>(100)             | ---            |
| Positive control<br>Diclofenac sodium 25 mg/kg,<br>p.o. | 3.6±1.17*<br>(17.73)           | 82.27          |
| Test group-I<br>Ethanolic extract 250 mg/kg,<br>p.o.    | 11.4±1.27*<br>(56.16)          | 43.84          |
| Test group-II<br>Ethanolic extract 500 mg/kg,<br>p.o.   | 6.1±1.89*<br>(30.05)           | 69.95          |

Values are expressed as Mean±S.E.M (n=10), \* $P < 0.001$ , % = Percentage, p.o. = per oral.

**Table 3.** Brine shrimp lethality bioassay of the ethanolic extract of dried aerial part of *Sida rhombifolia* Linn.

| Test sample       | Concentration (µg/ml) | Log (concentration) | Number of alive shrimp | Mortality (%) | LC <sub>50</sub> (µg/ml) | LC <sub>90</sub> (µg/ml) |
|-------------------|-----------------------|---------------------|------------------------|---------------|--------------------------|--------------------------|
| Ethanolic Extract | 10                    | 1.00                | 09                     | 10            | 40                       | 80                       |
|                   | 20                    | 1.30                | 07                     | 30            |                          |                          |
|                   | 40                    | 1.60                | 05                     | 50            |                          |                          |
|                   | 60                    | 1.77                | 03                     | 70            |                          |                          |
|                   | 80                    | 1.90                | 1                      | 90            |                          |                          |
|                   | 100                   | 2.00                | 0                      | 100           |                          |                          |

### Discussion

Analgesic activity of the ethanolic extract of dried aerial part of *Sida rhombifolia* Linn. tested by acetic acid induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algia by liberation of endogenous substances, which then excite the pain nerve endings<sup>12</sup>. The extract produced significant writhing inhibition comparable to standard drug diclofenac sodium. Based on this, it could be concluded that it might possess analgesic activity.

The cytotoxic activity of the ethanol extract of dried aerial part of *Sida rhombifolia* Linn. was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc.<sup>13</sup>. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it could be suggested that the crude ethanolic extract of dried aerial part of *Sida rhombifolia* Linn. possesses analgesic and cytotoxic activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential to confirm its pharmacological properties.

### **Acknowledgements**

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Dr. Asish Kumar Das, Associate professor, Pharmacy Discipline, Khulna University; Ahmed Ayedur Rahman, Assistant professor, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia, for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

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