



RESEARCH ARTICLE

# IN VITRO CYTOTOXIC ACTIVITIES OF METHANOLIC EXTRACT OF *MIMOSA PUDICA*

Srikanta Chowdhury<sup>1\*</sup>, Dibyajyoti Saha<sup>2</sup> and Swati Paul<sup>2</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong, Bangladesh

<sup>2</sup>Department of Pharmacy, BGC Trust University, Chittagong, Bangladesh

\*E-mails: srikanta.cu@gmail.com, saha.dibyajyoti@gmail.com

Tel.: +88-01717868519

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The present research was conducted to investigate the cytotoxic activities of methanolic extract of plant of *Mimosa pudica*. Cytotoxic activity was evaluated using brine shrimp lethality bioassay. For the determination of cytotoxicity, seven different concentrations (80, 100, 200, 400, 600, 800 and 1000  $\mu\text{g/ml}$ ) of methanol extract of *Mimosa pudica* were used.  $\text{LC}_{50}$  value of methanolic extract of *Mimosa pudica* was found to be 2.6621  $\mu\text{g/ml}$ . Methanolic extract of *Mimosa pudica* showed lethality in a dose reliant conduct. More exclusively 0%, 10%, 30%, 50%, 80% and 100% mortality were observed at the concentration of 80, 100, 200, 400, 600, 800 and 1000  $\mu\text{g/ml}$ , respectively. The brine shrimp lethality bioassay results suggest that the plant can be a promising source of anticancer compounds.

**Key words:** Cytotoxicity, Brine shrimp lethality bioassay, *Mimosa pudica*, Mimosaceae.

## INTRODUCTION

In the absence of an efficient primary health care system, traditional medicine occupies a central place in the provision of health care especially among rural communities of developing countries. The strong historical bond between plants and human health is well substantiated by plant species diversity and related knowledge of their use as herbal medicines. Lately, the uses of herbal medicines are increasing rapidly in developed countries too. As therapeutic uses of plants continued with the progress of civilization and development of human knowledge, scientists endeavored to isolate different chemical constituents from plants, put them to biological and pharmacological tests and thus have been able to identify and isolate therapeutically active compounds, which have been used to prepare modern as well as herbal medicines (Nahak and Sahu, 2010). Literature has shown several cases indicating cytotoxic potential of natural and synthetic compounds from diverse sources (Dahiya and Gautam, 2011; Jain *et al* 2011).

*Mimosa pudica* (Chhui-mui or sensitive plant or touch-me-not), is a short lived ever green shrub which can be treated as an annual or perennial herb (Figure 1).

Peculiar movement of leaflets that are sensitive to touch, makes it as an interesting plant (Ghani, 1998; Vaidyaratnam, 2001). Its fem like leaves close up and droop down whenever touched either by hand or by any object, living or non-living. It is due to the specific characteristics of its leaves that mimosa is regarded as a plant of high ornamental value. It grows to height of 5 ft and spreads around 3 ft.

Leaves are bipinnate, sensitive to touch, pinnae 1-2 pairs, leaflets 10-20 pairs, linear, glabrous, 9-12 mm long and 1.5 mm wide. Flowers head small, punduncle up to 2.5 cm long, globose, axillary, pink, purple; calyx, campanulate; petals, crenate towards base. Pods 1.5-2.5 cm long, closely prickly on the sutures. Stems are red-brown prickly. Seeds are bristles on seep pod cling to fur and clothing about 2 mm broad rounded, brown.



**Fig. 1.** *Mimosa pudica* plant

*Mimosa pudica* is common in rather moist waste ground, in lawns, in open plantations, and weedy thickets. It forms a dense ground cover, preventing reproduction of other species. It is a wild land fire hazard when dry. In many places, *Mimosa Pudica* is becoming noxious and can be controlled with various chemical herbicides. *Mimosa Pudica* is also a host to parasites such as cochineals insects, one gets rid of the insects by progressively removing them using a cotton stem soaked with alcohol, but if the insects are too numerous, one must sacrifice the sensitive plant and to not reuse the ground nor the pot on which it was cultured. There have been researches which show *Mimosa pudica* to be a herbal medicine (Ngo Bum *et al* 2004; Balakrishnan *et al* 2006; Chowdhury *et al* 2008; Rajendran *et al* 2009; Rajendran and Krishnakumar, 2010); Muthukumar *et al* 2010; Tamilarasi and Ananthi, 2012) but pharmaceutical companies are still researching its properties and uses. In continuation of such efforts, present investigation was directed toward evaluating the cytotoxic potential of the plant *Mimosa pudica*.

## MATERIALS AND METHODS

### *Plant materials*

The whole plants of *Mimosa pudica* were collected from Chittagong district of Bangladesh. After selection of plants, suitable herbarium sheet for plant with some general information were prepared and the plant was taxonomically identified by Dr. Azizur Rahman, Professor, Department of Botany, University of Chittagong.

### *Extraction*

The fresh plant of *Mimosa pudica* was washed with water immediately after collection. The collected plants were chopped into small pieces, air dried at room temperature for about 10 days,

ground into powder form and stored in an air tight container. About 750 g powder was macerated in 2.5 litre pure methanol for 5 days at room temperature with occasional stirring. After 5 days, methanolic extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50 °C through rotatory vacuum evaporator. The concentrated extracts were collected in a petri dish and allowed to dry for complete evaporation of methanol. The whole process was repeated three times and finally, blackish-green colored, concentrated plant extract was obtained which was kept in refrigerator at 4 °C.

### *Cytotoxic screening*

The cytotoxic activity of plant material was performed by using brine shrimp lethality bioassay (Meyer *et al* 1982; Solis *et al* 1993; Masseur and Nshimo, 1995). Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops were used as the test organisms. Artificial "sea water" was prepared by dissolving 25 g salt per liter of water. Sea water was placed in an unequally divided tank and shrimp eggs added to the larger compartment of the tank, which was darkened by covering it with aluminium foil. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam. 30 mg of each extract were separately dissolved in 3 ml of DMSO and from these, 500, 250, 125, 62.5, 31.25 and 25 µg/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 15 test tubes for each sample. A control containing 5 ml of DMSO solvent was used for each solvent. The final volume of the solution in each test tube was made up to 5 ml with seawater immediately after adding shrimp larvae. The test tubes were maintained under illumination. After 24 h, survivors were counted with the aid of a 3x magnifying glass.

## RESULTS AND DISCUSSION

For the determination of cytotoxicity by brine shrimp lethality bioassay, seven different concentrations (80, 100, 200, 400, 600, 800 and 1000 µg/ml) of *Mimosa pudica* extract were used which is elaborately expressed in the **Table 1**.

**Table 1.** Brine shrimp cytotoxicity of methanolic extract of *Mimosa pudica*

Dose ( $\mu\text{g/ml}$ )	Log dose	Total	Alive	Dead	% Lethality	Actual %	Probit
80	1.90	10	10	0	0	2.5	3.0396
100	2	10	9	1	10	10	3.7183
200	2.30	10	9	1	10	10	3.7183
400	2.60	10	7	3	30	30	4.476
600	2.77	10	5	5	50	50	5
800	2.90	10	2	8	80	80	5.8415
1000	3	10	0	10	100	97.5	6.9604

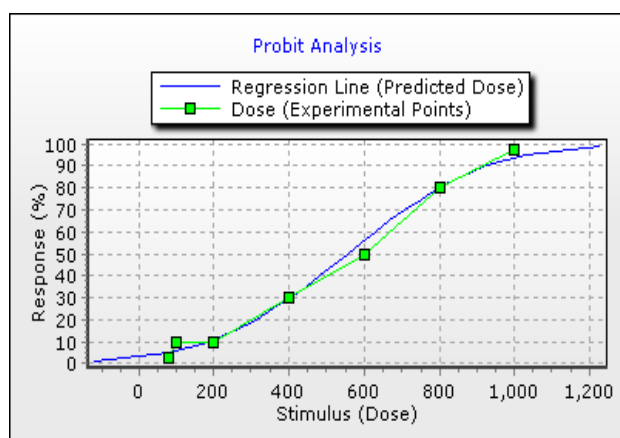
\*Actual percent and probit were calculated using statistical software 'Biostat 2009'

More exclusively, 0%, 10%, 30%, 50%, 80% and 100% mortality were observed at the concentration of 80, 100, 200, 400, 600, 800 and 1000  $\mu\text{g/ml}$ , respectively. The  $\text{LC}_{50}$  values were calculated from probit chart using computer software 'BioStat-2003'. Probits were then,

plotted against corresponding essential oil log concentration and from the plot  $\text{LC}_{50}$  (log concentration 50) value was calculated by regression analysis (**Table 2, Figure 2**).  $\text{LC}_{50}$  value of methanolic extract of *Mimosa pudica* was found to be 2.6621  $\mu\text{g/ml}$ .

**Table 2.** Calculation of  $\text{LC}_{50}$  value, regression equation and confidence limit by probit analysis

$\text{Log}_{10} \text{LC}_{50}$	$\text{LC}_{50}$ ( $\mu\text{g/ml}$ )	95% confidence limit ( $\mu\text{g/ml}$ )	Regression equation	Chi square
2.6621	459.2584	336.9647-620.4807	$Y = -162.45 + 81.119 * X$	4.3272

**Fig. 2.** Regression line for determining  $\text{LC}_{50}$  value of *Mimosa pudica* extract

Brine shrimp lethality bioassay was used to assess the cytotoxicity of *Mimosa pudica* extract. In this cytotoxicity study, the  $\text{LC}_{50}$  value of the extract was found to be 2.6621  $\mu\text{g/ml}$  which indicated that the methanolic aerial part extract of *Mimosa pudica* had little cytotoxic action. This research statement might serve as a preliminary step on this aspect.

## CONCLUSION

The results of the investigation do not reveal that which chemical compound is responsible for aforementioned activity and future studies will be directed to explore the lead compound responsible for cytotoxic activity of this plant.

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