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PHYTOCHEMICAL SCREENINGS, MEMBRANE STABILIZING ACTIVITY, THROMBOLYTIC ACTIVITY AND CYTOTOXIC PROPERTIES OF LEAF EXTRACTS OF *MIMOSA PUDICA*

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ABSTRACT

In this present study, the leaf extracts of *Mimosa pudica* were subjected to a comparative evaluation of the membrane stabilization, thrombolytic and cytotoxic activity to brine shrimps (*Artemia salina*). The thrombolytic and membrane stabilizing activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK) and standard anti-inflammatory drug, acetyl salicylic acid (ASA), respectively. The extracts demonstrated significant toxicity to *A. salina* with LC_{50} values ranging from 1.58 to 3.38 µg/ml as compared to standard vincristine sulphate (VS, LC_{50}value 0.92 µg/ml). Preliminary phytochemical investigation suggested the presence of carbohydrates, flavonoids, glycosides and steroids.

Key words: *Mimosa pudica*, thrombolytic activity, membrane stabilization, cytotoxic properties

INTRODUCTION

Mimosa pudica (Fam. Mimosaceae) locally known as Lozzaboti. The plant has very sensitive leaves which fold on touching and has reddish roots. The useful parts of this plant are roots, leaves and flower heads. The whole plant is used medicinally in ayurvedic folk medicine and its photochemical studies revealed the presence of minosine, orientin, isoorientin, β- sterol, D-pinitol, norepinepherine, crocetin, tannins and turgorins. In our country this plant is grown in rainy season and this plant is also called as touch me not plant at the same it should have a various names in different languages like in English sensitive plant. Leaves and stems of the plant have been reported to contain an alkaloid mimosine, leaves also contain mucilage and root contains tannins [1]. *Mimosa pudica* is used for its anti diarrhoeal and anti-convulsant [2]. The plant also contains turgorins, leaves and roots are used in treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. Plant is also used in the treatment of sore gum and is used as a blood purifier [1]. In this study the leaf extracts of *Mimosa pudica* were subjected to a comparative evaluation of the membrane stabilization, thrombolytic cytotoxic activity to brine shrimps.

MATERIALS AND METHODS

***Plant materials*:** The whole plants of *Mimosa pudica* were collected during August, 2012 from Kotbari, Mainamoti, Comilla. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No. 37879).

Preparation of extract: The sun dried and powdered leaves (300 gm) of *Mimosa pudica* was successively extracted in a Soxhlet extractor at elevated temperature using 200 ml of distilled petroleum ether (40-60)°C which was followed by n-hexane, ethanol,

chloroform and methanol. All extracts were filtered individually through filter paper and poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

***Preliminary phytochemical screening*:** One gram of the methanol extract of *Mimosa pudica* was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents [3].

***Streptokinase (SK)*:** Commercially available lyophilized Altepase (Streptokinase) vial (Trade name-S-Kinase from Popular pharmaceutical Ltd.) of 15, 00,000 I.U., was collected and 5 ml 0.9% NaCl was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis.

***Blood sample*:** Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 500 ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

***Thrombolytic activity*:** The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala (2006**)** [4] and slightly modified by Kawsar (2011) [5] using streptokinase (SK) as the standard.

***Membrane stabilizing 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 [6]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced mice erythrocyte haemolysis [7].

To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant3.1% Na-citrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

Hypotonic solution- induced haemolysis: The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0 mg/mL) or acetyl salicylic acid (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 *g* and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

% inhibition of haemolysis = 100 x (OD_1-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.

Heat-induced haemolysis: Isotonic buffer containing aliquots (5 ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54^{o}C for 20 min in a water bath, while the other pair was maintained at (0-5)^o C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

% Inhibition of hemolysis = 100 x [1- (OD_2-OD_1/ OD_3-OD_1)]

Where, OD_1 = optical density of unheated test sample, OD_2 = optical density of heated test sample and OD_3 = optical density of heated control sample

***Brine shrimp lethality bioassay*:** Brine shrimp lethality bioassay [8, 9] technique was applied for the determination of general toxic properties of the plant extractives. DMSO solutions of the samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 1 mg of each of the pet - ether, n-hexane, ethanol, chloroform and methanol soluble fractions were dissolved in DMSO and solutions of varying concentrations (100, 50, 25, 12.50, 6.25, 3.125, 1.563, µg/ml) were obtained by serial dilution technique using DMSO. Vincristine sulphate was used as positive control.

RESULTS AND DISCUSSION

***Preliminary phytochemical screening*:** In preliminary phytochemical screening, the methanol extract of leaf of *Mimosa pudica* demonstrated the presence of carbohydrates, flavonoids, glycosides and steroids **(table 1)**.

***Thrombolytic activity*:** As a part of discovery of cardio-protective drugs from natural sources the extractives of *Mimosa pudica* were assessed for thrombolytic activity and the results are presented in **table 2**. Addition of 100 µl Streptokinase (30,000 I.U.), standard to the clots along with 90 minutes of incubation at 37°C, showed 77.42% clot lysis. Clots when treated with 100 µl sterile distilled water (control) showed only negligible clot lysis (3.215%). In this study, the methanol soluble fraction of methanol extract of *Mimosa pudica* revealed highest thrombolytic activity 68.14%, whereas ethanolic, chloroform, pet ether, n-Hexane extract of *Mimosa pudica* (68.14%, 50.08%, 45.82 %, 36.63%, 29.72%) displayed moderate thrombolytic activities.

***Membrane stabilizing activity*:** The leaf extracts of *Mimosa pudica* at concentration of 1.0 mg/mL, were tested against lysis of human erythrocyte membrane induced by hypotonic solution as well as heat, and compared with the standard acetyl salicylic acid (ASA) (0.10 mg/ml) **(table 3)**. For hypotonic solution induced haemolysis, at a concentration of 1.0 mg/ mL, the methanolic extract (ME) inhibited 73.56% haemolysis of RBCs as compared to 92.13% produced by acetyl salicylic acid (0.10 mg/mL). On the other hand, during heat induced condition different organic soluble materials of *Mimosa pudica* demonstrated 54.03%, 57.06%, 79.71% and 2.85% inhibition of hemolysis of RBCs, respectively whereas ASA inhibited 74.24%.

Cytotoxicity assessment: In the present bioactivity study the five crude extracts and pure compounds showed positive results indicating that the test samples are biologically active. The methanol, ethanol, petroleum ether, chloroform and n-hexane extract of the dried bark of were subjected to brine shrimp lethality bioassay following the procedure which has been utilized by Meyer (1982) [8]. The lethality of the extractives to brine shrimps was determined and the results are given in **table 4**. Vincristine Sulphate (VS) was used as positive control and the LC_{50} was found as 0.927µg/ ml. Compared with the negative control, VS (positive control) gave significant mortality and the LC_{50} values of the different extractives were compared with negative control. The LC_{50} values of methanol, ethanol, chloroform, petroleum ether and n-hexane and were found to be 3.09, 2.089, 3.311, 3.389, 1.585 µg/ml respectively (**table 4**).

CONCLUSION

On the Basis of above result and available reports, all five leaf extracts of *Mimosa pudica* had potent cytotoxic effects. The phytochemical screening revealed the presence of carbohydrates, glycosides, flavonoids and steroids. In percent mortality of Brine Shrimp nauplii produced by the extracts of *Mimosa pudica* indicates the presence of cytotoxic principles in these extracts. After thrombolytic and membrane stabilizing activity significant results are shown in the present study.

Table 1: Analysis of phytochemicals leaf extracts of *Mimosa pudica*

Phytochemicals	Result
Carbohydrates	+
Flavonoids	+
Glycosides	+
Steroids	+

+ = Presence

Table 2: % Clot lysis by different extracts of *Mimosa pudica*

Samples	% of Clot Lysis
Methanolic Extract	68.14 ± 3.77
Ethanolic Extract	50.08 ± 2.07
Chloroform Extract	45.82 ± 2.48
Pet Ether Extract	36.63 ± 3.16
n-Hexane Extract	29.72 ± 2.37
Control	3.215 ± 0.51
Streptokinase	77.42 ± 1.6

Values are expressed as mean ± S.D. (n=6)

Table 3: Effect of extractives of *Mimosa pudica* on hypotonic solution & heat induced hemolysis of erythrocyte

Samples	Concentration (mg/mL)	% Inhibition of Haemolysis	
		hypotonic solution	heat induced
Hypotonic solution (Control)	50 mM	—	—
Acetyl salicylic acid	0.1	92.13± 0.012	74.24± 0.027
Methanol Extract	1	73.56 ± 0.021	54.03± 0.006
Ethanol Extract	1	74.04 ± 0.026	57.06± 0.007
Chloroform Extract	1	71.01 ± 0.021	79.71± 0.075

Values are expressed as mean ± S.D. (n=6)

Table 4: LC_{50} and LC_{90} values of the five extracts of *Mimosa pudica* and Standard

Test Samples	Regression Line	R^2	LC_{50}	LC_{90}
Vincristine sulphate	y = 29.79x + 64.62	0.927	0.927	6.31
Methanol extract	y = 26.1x+54.22s	0.889	3.09	85.113
Ethanol extract	y = 29.65x+36.03	0.976	2.089	60.256
Chloroform extract	y = 36.77x+36.80	0.917	3.311	32.359
Pet-ether extract	y = 32.03x+34.86	0.813	3.389	77.625
n-hexane extract	y = 29.66x+44.60	0.877	1.585	48.977

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