

THE PHARMA INNOVATION - JOURNAL

Evaluation of antioxidant and cytotoxic activity of methanolic extract of *Mimosa pudica* leaves

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In this study methanolic extract of *Mimosa pudica* (Mimosaceae) leaves was investigated to evaluate its antioxidant and toxic properties. Antioxidant activity was evaluated using total phenol content, total antioxidant capacity, total flavonoid contents and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. The methanolic crude extract showed moderate antioxidant activity such as total flavonoid content was found to be 63.8 mg QE/ g and total phenol content was 42.9 mg GAE/g. The total antioxidant capacity was expressed as ascorbic acid equivalents (AAE) and for leaf it was 5.038 mg/g AAE. DPPH scavenging activity was measured by comparing with ascorbic acid. Both leaf and ascorbic acid IC₅₀ values were 126.71 and 20.13µg/ml, respectively. On the other hand, the cytotoxic activity of methanolic extract of leaf was evaluated using brine shrimp lethality bioassay. LC₅₀ value of the methanolic leaf extract of *Mimosa pudica* was found to be 282.3495 µg/ml and its showed lethality in a dose reliant conduct. In brine shrimp lethality bioassay, *Mimosa pudica* leaf showed significant source of anticancer compounds.

Keyword: *Mimosa pudica*, Antioxidant, DPPH Scavenging activity, Cytotoxicity, Brine shrimp lethality bioassay.

1. Introduction

The beginning of the existence on earth, human being had a prime concern of staying healthy and free from disease. The knowledge of the medicinal properties of plants and their uses in different disease were transmitted verbally through generation to generation and this knowledge begun to expand which contributes to the use of medicinal plant as a remedy for disease. This study is based on ethno-botanical knowledge of plant *Mimosa pudica*. The leaves of the *Mimosa pudica*

have been reported useful traditionally for treating various types of disease like piles, diarrhea, persistent dysentery and convulsion of children [1]. Antioxidants obtained from natural source are very much promising due to its better efficacy with less adverse effects. Antioxidants prevent cell damage significantly scavenging the free radicals and reactive oxygen species (ROS) developed in various diseases like cancer, hepatic failure, diabetes mellitus inflammation, renal failure,

atherosclerosis etc. [2,3,4,5]. Cytotoxic activities of natural and synthetic compounds from diverse source were reported [6,7]. Therefore, in our present investigation was directed toward evaluating the antioxidant and cytotoxic potential of the methanolic extract of *Mimosa pudica* leaves.

2. Material and Method

2.1 Identification of plant and Preparation of extract

Mimosa pudica leaves were collected in January from Nikunja, Dhaka, Bangladesh and botanically identified simultaneously authenticated. The fresh leaves of *Mimosa pudica* were washed with water after collection and air dried at room temperature for about 25 days, dried leaves were powdered and 300 g were submerged in 1000 ml methanol containing air-tight containers for seven days, with occasional shaking and stirring. Then the extract was filtered and dried on an electrical water bath at 50 °C.

2.2 Chemicals and Reagent

DPPH (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, quercetin, and gallic acid were obtained from Sigma Chemical Co (MO, USA). Folin-ciocalteu reagent (FCR) and Griess reagent were purchased from Merck, Germany. All other chemicals and reagents were of analytical grade.

2.3 Determination of Total phenols

The content of total phenolic compounds was determined by Folin-Ciocalteu reagent [8]. 1.0 ml of leaf extract (10 µg/µl) or standard of different concentrations (50, 100, 150, 200 and 250 µg/ml) were taken in different test tubes and 5 ml of Folin-Ciocalteu (diluted 10 fold) and 4 ml of Sodium carbonate reagent was added to the test tubes. The test tubes were incubated for 30 minutes at 20 °C to completing the reaction. The absorbances of the solutions were measured at 765 nm using a UV-Vis spectrophotometer against distilled water as blank. Total phenolic compounds in leaf extracts were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound. The result was calculated from the

regression equation of the calibration curve ($y=0.009x + 0.058$; $R^2=0.997$).

2.4 Determination of flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination [9]. 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV/Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 12.5 to 100 µg/ml in Methanol.

2.5 Determination of total antioxidant capacity

The assay is depending on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [10]. The antioxidant activity was expressed as the number of equivalents of ascorbic. The leaf extract (0.3 ml) was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for 90 minutes and the mixture was cooled to room temperature, the absorbance of the solution was measured at 695 nm against an appropriate blank. Total antioxidant capacity of leaf extract was measured from the calibration curve constructed by using ascorbic acid standard solutions.

2.6 DPPH scavenging capacity assay

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured by the method developed by Manzorro *et al.* (1998) [11]. 0.2 ml of leaf extract and standard of each concentration (500, 200, 100, 50, 10, 5 µg/ml) were mixed with 2 ml of DPPH solution (0.5 mM). The reaction was carried out at room temperature in a dark place for 30 minutes; the absorbance was measured at 517 nm. IC₅₀ values (concentration of samples required to scavenge 50% of free radicals) were calculated from the regression equation, developed by plotting

concentration of samples versus percentage inhibition of free radicals. Positive control was used as Ascorbic Acid.

2.7 Cytotoxic screening:

Brine Shrimp lethality bioassay was applied for determination of general toxic property of the plant extract [12, 13, 14]. The required amount of leaf extracts were mixed with a specific amount of dimethyl sulfoxide (DMSO) and prepared different concentrations (500 µg/ml to 1µg/ml). Ten brine shrimp (nauplii) were taken in vials containing 5 ml of artificial sea water (25 g salt per liter of water). Then samples were added to previously marked vials with a micropipette.

After 24 hours; the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The percent (%) of lethality of the brine shrimp (nauplii) was calculated from this data for each concentration. Positive control was used as Vincristine sulphate in this study.

3. Results and Discussion

The chemical constituents present in a plant are directly responsible for its therapeutic and other pharmacological properties and the constituents of the plant which are detected during this investigation should have some direct relationship with local medicinal uses.

Table 1: Antioxidant activity of the *M. pudica* leaves.

Sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH free radical scavenging activity (IC ₅₀ µg/ml)	Total antioxidant capacity (mg AAE/g)
Ascorbic Acid			20.13 ± 0.24	
Leaf	42.9 ± 0.22	63.8 ± 0.63	126.71 ± 0.34	5.038 ± 0.31

Total phenolics in the methanolic leaf extracts were determined by the Folin-Ciocalteu assay. The content of phenolics was found to be 42.9 mg GAE/g in each extracts (Table 1). The antioxidant capacity of the phenolic compounds is mainly due to their redox properties, which can play a major role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [15].

Flavonoid content can be calculated from the regression equation of the calibration curve ($y = 0.005x - 0.005$) and is expressed as quercetin equivalents (QE). The flavonoid content was determined as 63.8 mg QE/g for leaf extract (Table 1).

The total antioxidant capacities of the methanolic extracts of the plant leaves were determined from the calibration curve established by ascorbic acid at 695 nm. The regression line was $y=0.006x+0.0101$ and $R^2=0.991$. The ascorbic

acid equivalents (AAE) for leaf extract were 5.038 mg/g.

In DPPH scavenging assay, IC₅₀ values of the methanolic extracts of *M. pudica* were found to be 126.71 µg/ml and IC₅₀ value for ascorbic acid was 20.13 µg/ml (Table 1). DPPH reading is not very close to the Ascorbic acid. It is because the leaves extract of the plant is in crude form and it is quite possible to be a potent antioxidant after bioactive compound isolation.

In brine shrimp lethality bioassay, after 24 hours the leaves extract showed significant lethality. Median lethal concentration or LC₅₀ values were found 282.3495 µg/ml for leaves extract, whereas the reference standard vincristine sulphate exhibited LC₅₀ values of 0.45 µg/ml. It is clearly evident from the above findings that the leaves of *M. pudica* have moderate antioxidant activity and cytotoxic property. Future isolation of the active

principles from this leaves of the plant can assist to open up exciting new therapeutic potentiality.

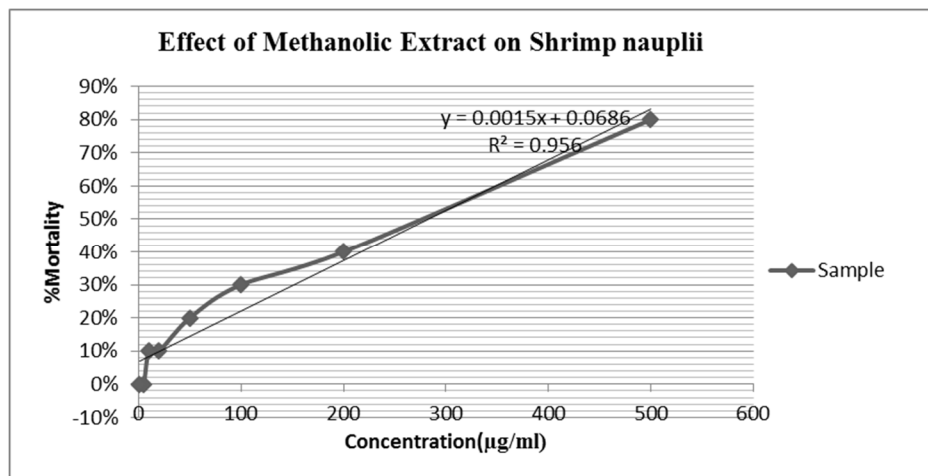


Fig 1: Graphical representation of cytotoxic activity of crude extracts on shrimp nauplii of *Mimosa pudica* leaves by brine Shrimp lethality bioassay method.

4. Conclusion

The results of the investigation do not express that which chemical compound is responsible for activity and further studies will be directed to explore the lead compound responsible for antioxidant & cytotoxic activity of the plant leaves.

5. Acknowledgement

Authors are grateful to the authority of the Department of Pharmacy, School of Science, Primeasia University to perform these investigations.

6. References

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