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Integrated In Vitro and In Vivo Evaluation of the Antidiabetic, Thrombolytic, and Antioxidant Activities of the Methanolic Extract of *Mimosa pudica* Whole Plant

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ABSTRACT

Background: *Mimosa pudica* L. is a medicinal plant whose use is widespread in many countries for the treatment of various conditions using its antidiabetic, thrombolytic, and antioxidant properties. Nevertheless, the scientific data about the pharmacological potential of this medicinal plant are insufficient. Therefore, the goal of this study was to assess the biological activities of the methanolic extract of *Mimosa pudica*.

Materials and methods: Antidiabetic effect of the extract was evaluated by conducting oral glucose tolerance test (OGTT) on glucose-loaded mice. The mice were divided into several groups which were administered either 250 or 500 mg/kg of the methanolic extract of *Mimosa pudica* or metformin (100 mg/kg) as the positive control drug. Thrombolytic activity was determined by conducting clot lysis test in vitro using streptokinase as the positive control and distilled water as the negative control. The antioxidant activity was estimated by conducting DPPH free radical scavenging activity test using ascorbic acid as the standard.

Results: Methanolic extract showed dose dependent antihyperglycemic activity in OGTT. In particular, administration of 500 mg/kg extract resulted in 57.69% decrease in plasma glucose level in 240 min while metformin decreased plasma glucose level by 81.23%. Extract also displayed good thrombolytic activity providing clot lysis in the amount of 41.17 ± 1.23% while positive control produced 87.67 ± 1.62% of clot lysis and negative control 5.30 ± 0.42%. In addition, DPPH free radical scavenging test indicated the dose dependent free radical scavenging activity of the extract (IC_{50} value 22.6 µg/mL) while ascorbic acid possessed IC_{50} value of 9.6 µg/mL.

Conclusions: The methanolic extract of the whole plant of *Mimosa pudica* demonstrated significant antidiabetic, thrombolytic, and antioxidant activities. These properties are effective in dose dependent manner.

Keywords: Antidiabetic Activity; Antioxidant Activity; DPPH Assay; *Mimosa pudica;* Methanolic Extract; Oral Glucose Tolerance Test.

1. Introduction

Medicinal plants still function as one of the important sources of therapeutic drugs and play a vital role in disease prevention and cure. Up to 80% of the world's population uses herbal medicine as their basic source of health care, which is more affordable, accepted and easily available especially in developing nations [1]. Natural compounds include a variety of phytochemicals that have antioxidant, anti-diabetic, anti-inflammatory, antimicrobial and cardioprotective activity, hence making them good candidates for the preparation of safe and effective drugs. Therefore, scientific validation of medicinal plants with traditional uses has become an important subject in pharmaceutical and biomedical research [2].

Mimosa pudica L. (Family: Fabaceae) or sensitive plant or touch-me-not plant is a perennial creeping herb, widely distributed throughout tropical and subtropical regions. This plant is well recognized for its very fast thigmonastic reaction of leaf to any external stimulus and has been used in Ayurvedic, Unani, and other traditional medicinal practices. Different plant parts such as the entire plant, roots, leaves, and seeds are known for the treatment of diabetes, diarrhea, dysentery, wounds, inflammations, urinary infections, hemorrhoids and liver diseases [3].

Studies on phytochemical analysis reveal that the secondary metabolites in *Mimosa pudica* include flavonoids, phenolics, tannins, alkaloids, glycosides, terpenoids, steroids, saponins, coumarins, and mimosine – an amino acid not incorporated into protein structure [4]. The said compounds possess strong antioxidant properties through their abilities to scavenge ROS, inhibit lipid peroxidation, and protect cells against oxidative injury. Oxidative stress is known to be one of the major causes of development of diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and cancers. Hence, plants possessing strong antioxidant activities have been widely studied for their possible medicinal use [5].

Diabetes mellitus is characterized by chronic hyperglycemia due to reduced insulin production, insulin resistance, or both. It has been reported that the prevalence of diabetes is increasing worldwide and hence poses a huge public health challenge. The persistently high glucose level in the body results in increased oxidative stress, vascular impairment, inflammation, and metabolic abnormalities leading to micro and macrovascular complications. Even though many chemical antidiabetic drugs are available and effective in lowering plasma glucose levels, they can have side effects after continued use and at times may even fail in their treatment function. Hence, finding alternative agents which can improve glucose homeostasis with minimal side effects becomes necessary [6]. Prior studies have revealed that methanolic extracts of *Mimosa pudica* have antihyperglycemic activity due to improved glucose metabolism and enhanced antioxidant mechanisms [7].

Nevertheless, thrombotic conditions still remain one of the major causes of morbidity and mortality from cardiovascular diseases. Clot formation within the vessels leads to myocardial infarction, ischemic stroke, pulmonary embolism and deep vein thrombosis. Even though Streptokinase and other thrombolytic drugs exhibit effectiveness, they suffer from certain drawbacks including bleeding disorders, hypersensitivity reactions and high price. That is why medicinal plants possessing natural fibrinolytic or thrombolytic properties are now subject to investigation and are being explored as alternatives or additional treatments. There were a number of phytochemicals such as flavonoids and phenolic compounds that were known to regulate platelets aggregation, fibrinolysis, endothelium functions and oxidative stress, thus exhibiting cardiovascular protective effects [8].

Mimosa pudica has been proved to possess antimicrobial, anti-inflammatory, wound healing, hepatoprotective, antidiabetic, antioxidant and anticancer properties during various pharmacological experiments. Nevertheless, there has not been much attention paid to the plant itself. Most of the studies have focused on some particular property or some particular part of the plant whereas studies involving assessment of the plant extract as a whole, particularly antihyperglycemic, thrombolytic and antioxidant activities, were not conducted properly yet. Nevertheless, despite promising preclinical data, there is still need for additional experimental validation before the drug can be used therapeutically [9].

That is why the goal of this study was to evaluate pharmacological properties of the methanolic extract of the whole plant of *Mimosa pudica*. It involved assessment of the antidiabetic activity using OGTT in glucose-loaded mice, thrombolytic activity was assessed in vitro through clot lysis test and antioxidant activity was evaluated using DPPH free radical scavenging assay. Thus, the results obtained will help to provide scientific background for

traditional medicinal application of *Mimosa pudica* and help to find out natural bioactive compounds that could be used pharmaceutically.

1.1. Study Objectives

The research study will focus on the determination of the pharmacological properties of the methanolic extract of the whole plant of *Mimosa pudica*. This study will specifically aim at the following:

1) Determination of the antidiabetic properties of the methanolic extract of the whole plant of *Mimosa pudica* through the oral glucose tolerance test (OGTT) on glucose-loaded mice.

2) Determination of the thrombolytic properties of the methanolic extract through an in vitro clot lysis test, with streptokinase as the control substance.

3) Determination of the antioxidant properties of the methanolic extract through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test.

4) Comparative study of the pharmacological properties of the methanolic extract with the standard substances.

2. Literature Review

Natural sources still form a considerable source of bioactive compounds for new drug discovery. A large number of phytochemicals obtained from medicinal plants have been demonstrated to exhibit significant antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and cardioprotective properties. In the recent years, there has been an increasing scientific interest in confirming traditional medicinal plants using pharmacological and phytochemical studies especially of those having diverse medicinal applications [3].

Mimosa pudica L. (Fabaceae), also known as the sensitive plant, is traditionally used in various health conditions such as diabetes, wounds, diarrhea, inflammation, hemorrhoids, infections of urinary tract, and microbial infections throughout Asia, Africa, and South America. Pharmacological research on this plant has been performed in order to confirm its medicinal uses as well as to determine the bioactive compounds that are behind its biological activities [4].

Phytochemical studies show that the sensitive plant has different classes of secondary metabolites such as flavonoids, phenolics, tannins, alkaloids, terpenoids, glycosides, steroids, saponins, and the non-protein amino acid called mimosine. The mentioned substances are characterized by their capability of antioxidant activity through scavenging the reactive oxygen species, preventing the lipid peroxidation process, and increasing the body's endogenous defense mechanism against oxidative damage. Phenolics and flavonoids have shown their diverse pharmacological actions due to their good reducing power and radical scavenging ability [5].

There are some experimental researches that show the antidiabetic property of *Mimosa pudica.* Animal studies have shown that extracts of the plant improve glucose tolerance and decrease blood glucose level and reduce oxidative stress related to diabetes mellitus. All these beneficial effects are connected with better glucose uptake, regulation of carbohydrate metabolism enzymes, protection of pancreatic β-cells and antioxidant effect. However,

inconsistency in extraction solvent, parts of plant used, dose and experimental animal model led to controversial results and emphasizes the need for validation in standardized way [7].

The antioxidant activity of *Mimosa pudica* was studied by such in vitro assays as DPPH, ABTS, FRAP and nitric oxide scavenging assays. In all these studies the dose dependent free radical scavenging activity was observed, what supports the hypothesis that this plant can reduce oxidative stress-related damage. Since the oxidative stress plays a significant role in diabetes, cardiovascular diseases, neurodegenerative diseases and chronic inflammation, antioxidant compounds extracted from medicinal plants still have perspectives as pharmaceutical preparations [5].

In addition to their antioxidative and anti-diabetic effects, medicinal plants are also being studied in relation to their effects on cardiovascular health. Polyphenolic compounds and flavonoids found in plants have been found to have an inhibitory effect on platelet aggregation, to improve endothelial health, and to improve fibrinolysis thus reducing chances of developing blood clots. While there are some medicinal plants which have proved effective in promoting thrombolysis, there are not many studies done on thrombolytic effects of *Mimosa pudica*, and therefore more research needs to be done [3].

Though many papers have been published regarding the pharmacology of *Mimosa pudica*, there is still a lack of investigations that have simultaneously studied the antidiabetic, thrombolytic and antioxidant properties of this plant with the help of the same methanolic extract of the whole plant. The majority of publications have focused on one biological property or investigated certain plant organs, which has made it difficult for the comparisons to be made. Furthermore, comprehensive pharmacological researches, which are able to show several biological activities at once, are rather scarce [9].

Therefore, the main purpose of this paper is to conduct the comprehensive study of the pharmacological potential of methanolic extract of the whole plant of *Mimosa pudica*. Antidiabetic activity will be estimated according to the OGTT test, thrombolytic activity will be measured with the help of the clot lysis assay, and the antioxidant activity will be estimated by the DPPH free radical scavenging test. These tests are relevant to the experimental work performed in the manuscript.

3. Materials and Methods

3.1. Chemicals and Reagents

The solvent used in preparing the plant extract was methanol. Metformin was used as the positive control for glucose lowering activity, whereas Streptokinase acted as the positive control in the thrombolytic test. Distilled water acted as the negative control. DPPH free radicals were used in the experiment, and ascorbic acid was used as the standard antioxidant [10].

3.2. Experimental Animals

Swiss Albino Mice (*Mus musculus*), weighing between 25 and 35 g, and aged between 6 to 8 weeks were used to study analgesic activity. These mice were purchased from a breeding center, Jahangirnagar University, Savar, Dhaka, Bangladesh. These mice were kept under controlled conditions of temperature 25±2°C and humidity 55-65%

with a 12h light/dark cycle. The animals were kept under laboratory conditions for a week before the experiment. All experiments performed in this study adhere to all ethical protocols of lab oratory animals [11].

3.3. Extraction of Crude Extract (Cold Extraction Method)

Dried whole plants of *Mimosa pudica* were collected from the Kurigram district during the spring season, and were authenticated by an expert botanist. The plant materials were washed in distilled water to remove dust particles and other impurities and dried in the shade at room temperature until a constant weight was attained. Then the plant materials were pulverized into a coarse powder using a grinding machine. About 250 g of powdered plant materials were extracted with 1000 mL of methanol (ratio of solvent to plant material 4:1) in a clean and dry glass beaker. The beaker was tightly closed and kept at room temperature for 12 days for extraction. During the process, intermittent shaking was done. The mixture was filtered first with clean cotton cloth and later with Whatman No. 1 filter paper. The filtrate was evaporated using reduced pressure through rotary evaporation to obtain crude methanolic extract [12]. The extraction of 250 g of dried plant powder in methanol produced 58.5 g of crude extract, which corresponds to a yield of 23.4% (w/w).

3.4. Oral Glucose Tolerance Test (OGTT)

The antidiabetic effect of the methanolic leaf extract of *Mimosa pudica* was determined through oral glucose tolerance test (OGTT) using glucose-loaded mice. The animals were starved for 16 hours prior to the test and had access only to water. Three groups of mice (n = 3 per group) were used as described below: Group I (Control): Distilled water (10 mL/kg); Group II (Standard): Metformin hydrochloride (100 mg/kg); Group III (Test): Methanolic leaf extract of *Mimosa pudica* (500 mg/kg) [9]. Glucose solution was given to the mice 30 minutes after treatment. Five different blood samples were taken from the mice at different time intervals (0, 60, 120, 180, 240 minutes). Plasma glucose concentration was estimated through blood glucose estimation using glucometer [10].

3.5. Thrombolytic activity

Five individuals were chosen; they reported absence of any anticoagulants or oral contraceptives intake. 1 mL of their blood was collected and placed in weighed sterile microcentrifuge tube, left to clot at 37^0 C for 45 minutes, and the clot weight was measured after removal of serum [13]. Extract of plant material was made by dissolving 100 mg of plant material in 10 mL of distilled water and filtering. The concentrations of the extracts were varied from 2-10 mg/mL. For each clot, 100 µL of the extracts was added. Positive control was made by using streptokinase and negative control was made by using distilled water. All tubes were incubated at 37^0 C for 90 minutes [14]. The percentage of clot lysis was calculated as:

$$\text{Clot Lysis}(\%)=\frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}}\times 100 \qquad (1)$$

3.6. Antioxidant Activity Evaluation Using the DPPH Free Radical Scavenging Test

An experiment was carried out on the antioxidant activity of the methanolic leaf extract of *Mimosa pudica* using the DPPH scavenging test, ascorbic acid being used as the reference substance. Five different concentrations of the methanolic extract (1, 5, 10, 50, and 100 mg/mL) were made together with five different concentrations of the

ascorbic acid reference substance. The fresh DPPH was dissolved in methanol. To each test or standard solution (2 mL), 3 mL of DPPH solution was added, and the resulting solutions were allowed to stand in the dark for 30 minutes. The absorbance values were taken at 517 nm. The percentage of inhibition of DPPH free radicals by samples was determined by following equation:

$$\text{Inhibition of DPPH free radicals (\%)} = \frac{(Ao - At)}{Ao} \times 100 \qquad (2)$$

Where Ao and At are the absorbance of control (DPPH solution without sample) and test sample or standard solution, respectively [15].

4. Results and Discussion

This present study investigated the pharmacological effects of the methanolic extract of the whole plant of *Mimosa pudica* in terms of antidiabetic, thrombolytic, and antioxidant activities. From the data obtained, it can be concluded that the tested extract had significant biological effects in all experiments performed which point to the existence of biologically active components capable of producing multiple biological activities.

4.1. Antidiabetic Activity

According to the results of the oral glucose tolerance test (OGTT), the methanolic extract of *Mimosa pudica* caused a significant decrease in plasma glucose levels of glucose-loaded mice in a dose-dependent manner. The higher dose (500 mg/kg) produced a more pronounced effect than the lower one (250 mg/kg) in terms of lowering blood glucose levels. Despite the relatively weak effect compared to that of metformin, the extract proved its ability to improve glucose tolerance, which proves its use as an antidiabetic agent. The antihyperglycemic activity of the methanolic extract is summarized in Table 1 and shown in Figure 1 and Figure 2.

Since the observed decrease in plasma glucose levels gradually increased during the whole 240 minutes of experiment performance, it may be concluded that the extract might increase glucose metabolism, reduce glucose absorption in intestines, induce insulin secretion, or improve its action. Since OGTT was used instead of the animal model of diabetes, the results do not refer to the treatment of the disease but only to the improvement of glucose tolerance.

Table 1. Effect of Methanolic Extract of *Mimosa pudica* on Plasma Glucose Levels in Glucose-Loaded Mice during the Oral Glucose Tolerance Test (OGTT).

Group	Dose	0 min	60 min	120 Min	180 min	240 Min	% lessening of Plasma glucose level
Control (Water)	10 mL/kg	24.75	24.03	22.6	21.68	20.94	-
Standard (Metformin)	100 mg/kg	26.96	20.88	14.8	10.65	6.67	81.23
Mimosa pudica Extract	250 mg/kg	26.63	23.50	22.00	18.93	16.57	37.79
	500 mg/kg	25.42	21.86	18.40	15.29	10.75	57.69

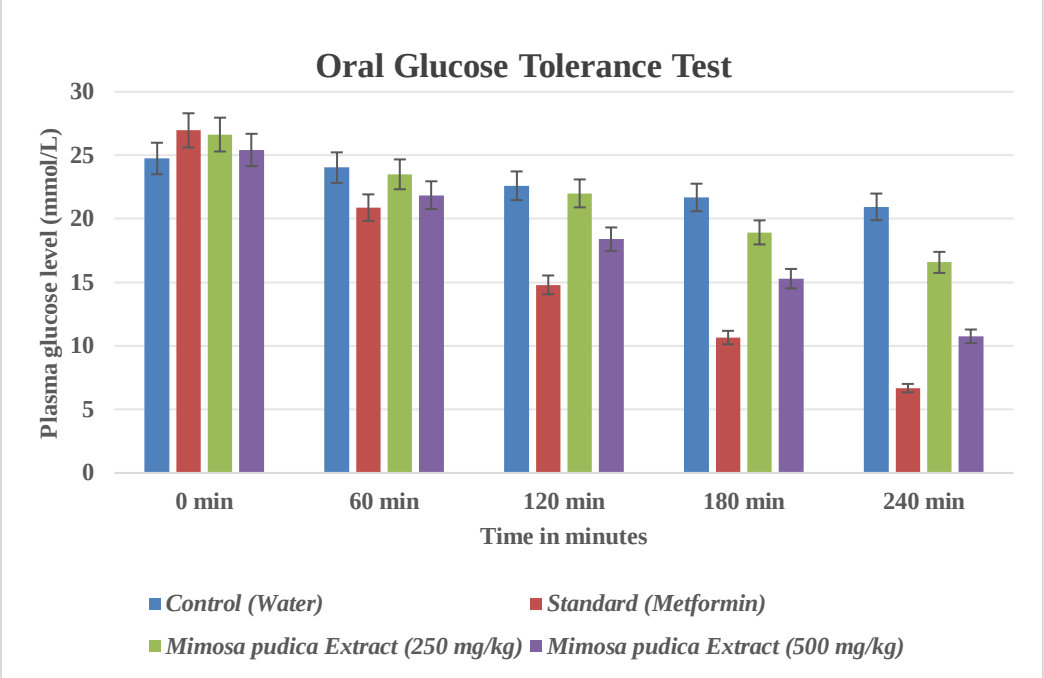


Figure 1. Effect of Methanolic Extract of *Mimosa pudica* on Plasma Glucose Levels During the Oral Glucose Tolerance Test (OGTT) in Mice.

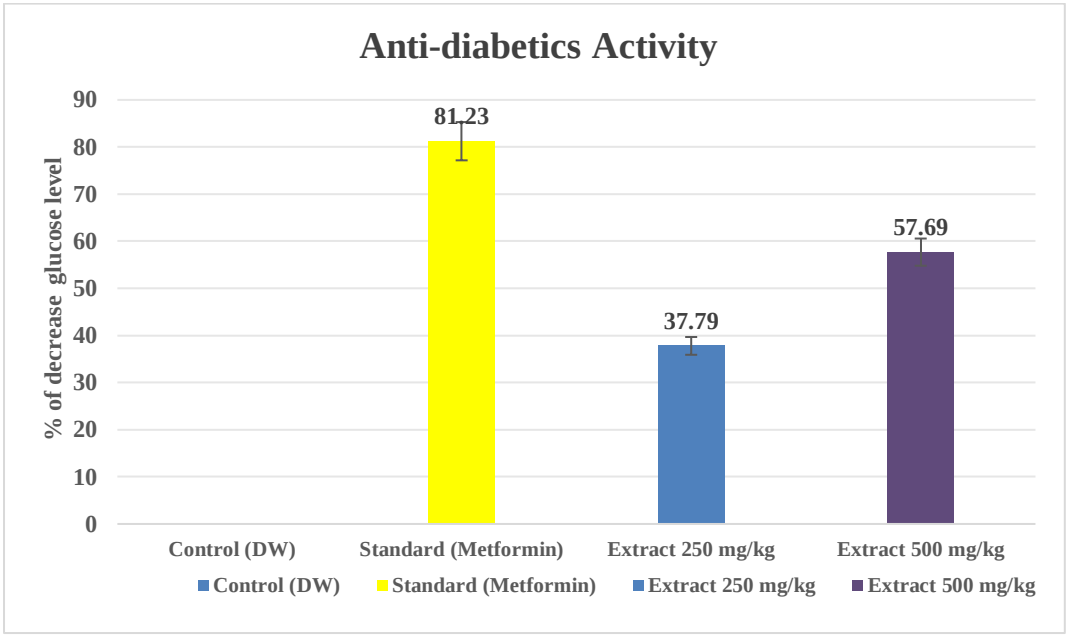


Figure 2. Comparative Percentage Reduction in Blood Glucose Levels by Methanolic Extract of *Mimosa pudica* and Metformin.

4.2. Thrombolytic Activity

The methanolic extract displayed a moderate level of thrombolytic activity, producing 41.17 ± 1.23% of clot lysis as compared with 87.67 ± 1.62% by streptokinase, while the negative control had minimal clot dissolution. From these results calculated by Equation 1, it can be concluded that the extract is effective at producing some level of fibrinolytic activity, but it is significantly lower compared with the reference drug, results are shown in Table 2 and Figure 3.

The presence of activity of clot lysis indicates that there are substances that can promote fibrinolysis or dissolution of clots. However, without further investigation, the exact mechanism of this activity cannot be established. Nevertheless, the results obtained show that *Mimosa pudica* might contain substances which have beneficial action on the cardiovascular system. Further research involving fractionation and mechanism-based assays should be conducted to find out the active ingredients causing this effect.

Table 2. In Vitro Thrombolytic Activity of the Methanolic Extract of *Mimosa pudica* *D*etermined by Clot Lysis Assay (n = 3).

Sample	% of Clot Lysis (Mean ± SD)
Negative control (Distilled water)	5.3 ± 0.42
Positive control (Streptokinase)	87.67 ± 1.62
Mimosa pudica extract	41.17 ± 1.23

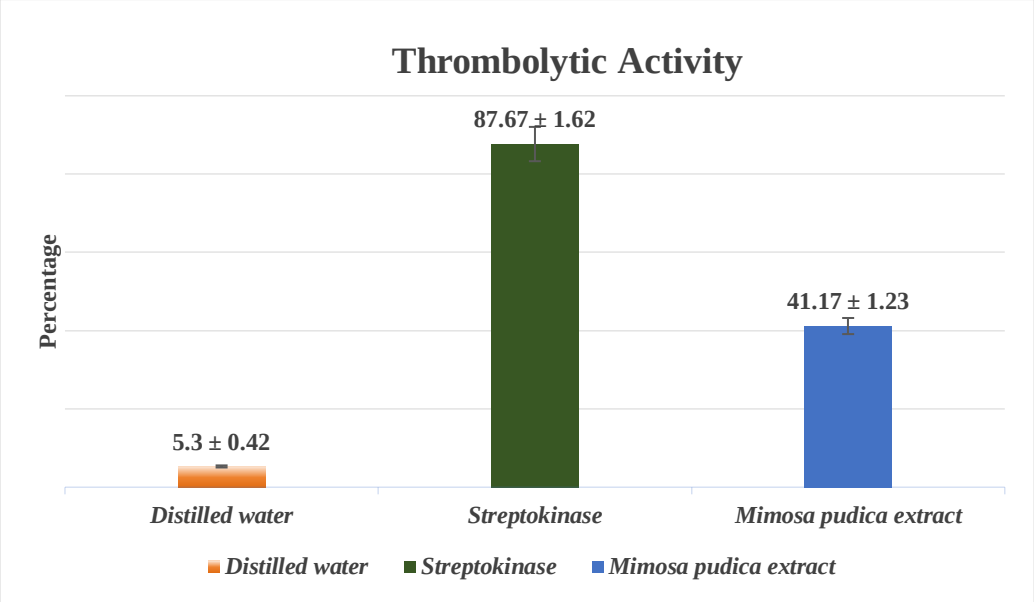


Figure 3. Comparative Clot Lysis Activity of the Methanolic Extract of *Mimosa pudica*, Streptokinase, and Distilled Water.

4.3. Antioxidant Activity

It was found that the extract shows concentration-dependent antioxidant activity when tested in DPPH free radical scavenging assay. With the increase of extract concentration, the percent inhibition of radicals was also increasing, which means that the extract has the ability to inhibit DPPH radicals. It was calculated using Equation 2 and found that the extract has relatively high antioxidant activity, but it was lower compared with the activity of ascorbic acid, with the IC50 of 9.6 µg/mL compared with 22.6 µg/mL in the case of the extract. The results are shown in Table 3 and Figure 4.

From the concentration-dependent nature of the antioxidant activity of the extract, it can be concluded that it has the ability to donate hydrogen or electron to neutralize free radicals. This activity can explain the antihyperglycemic effect observed in the OGTT since oxidative stress has a great effect on diabetes pathogenesis.

Table 3. DPPH Free Radical Scavenging Activity of Methanolic Extract of *Mimosa pudica* and Ascorbic Acid at Different Concentrations.

Concentration (µg/ml)	DPPH Control	Standard (Ascorbic acid)		*Mimosa pudica* Extract	
	Absorbance (nm)	Absorbance (nm)	% of Inhibition	Absorbance (nm)	% of Inhibition
1	1.870	1.524	18.5	1.820	2.67
5		1.331	28.82	1.436	23.21
10		1.205	35.56	0.895	52.14
50		0.351	81.23	0.517	72.35
100		0.152	91.87	0.221	88.18
500		0.114	93.9	0.119	93.64
IC_{50}			22.6		9.6

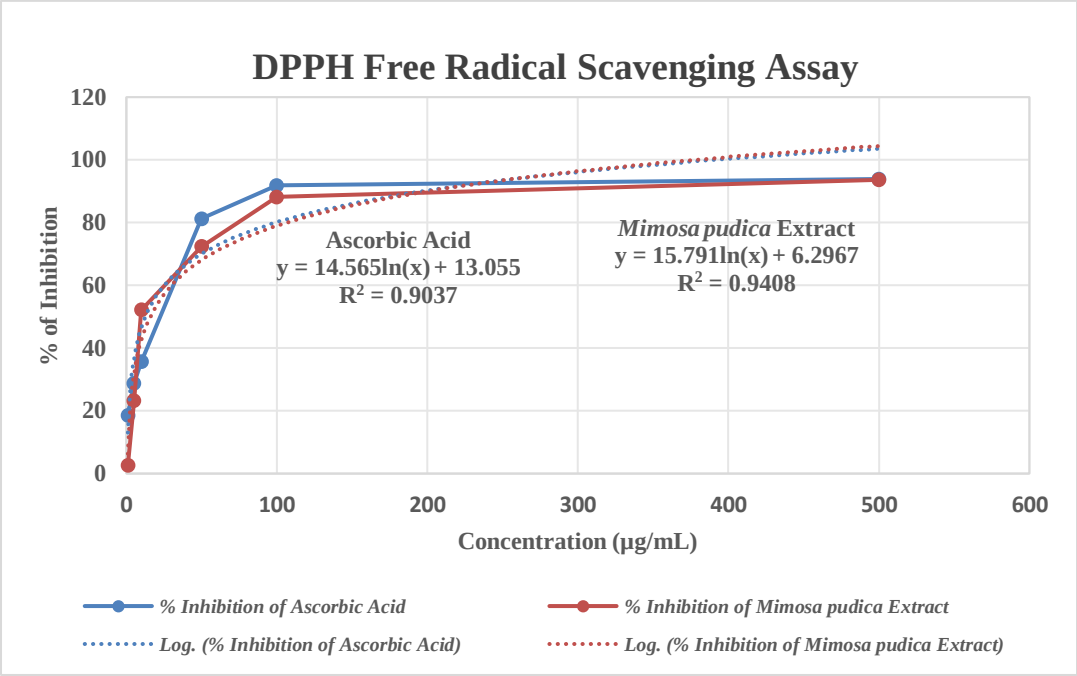


Figure 4. DPPH Free Radical Scavenging Activity of Methanolic Extract of *Mimosa pudica* and Ascorbic Acid at Different Concentrations.

4.4. General Interpretation

From all the experiments carried out, it is evident that the methanolic extract of the entire plant of *Mimosa pudica* contains diverse pharmacological actions. These activities include improving glucose tolerance, having moderate thrombolytic action, and effective free radical scavenging ability. The dose-dependent antihyperglycemic activity and concentration-dependent antioxidant activity confirm the biological activities of the extract. Although the extract fails to have as much effectiveness as the standard drugs used in the study, its concurrent biological activities in the three pharmacological tests suggest that the *Mimosa pudica* plant can be an excellent natural source of biologically active compounds.

Nonetheless, there are a number of limitations to consider in this research. First, the active phytochemical components responsible for the pharmacological activities were not established, no mechanisms studies were done, and preliminary pharmacological activities only were done. Future research work should involve phytochemical analysis, isolation of the active compounds, determination of toxicity and mechanisms of action, and chronic animal experiments to evaluate the potential of *Mimosa pudica* to act as a therapeutic drug before use in humans.

5. Conclusion and Further Studies

5.1. Conclusion

This study shows that the methanolic extract of the entire plant of *Mimosa pudica* is effective in pharmacological activities such as antidiabetic, thrombolytic, and antioxidant activities. In the oral glucose tolerance test, it was found to enhance glucose tolerance in a dose-dependent manner, where the 500 mg/kg dose had more antihyperglycemic activity than 250 mg/kg. Also, it showed moderate thrombolytic activity resulting in notable clot lysis as compared to the standard streptokinase. Moreover, in the DPPH free radical scavenging test, it exhibited concentration-dependent antioxidant activity, which is an indication of its good capability of scavenging free radicals.

The synergistic biological effects noted in this investigation provide scientific basis for the medicinal utilization of *Mimosa pudica* and suggest that this plant may be an important natural source of pharmacologically active chemicals with medicinal uses. While the pharmacological activity was not as potent as the commercial drugs used as positive controls, the extract proved to be highly effective in all the assays.

Taken together, the data presented above clearly demonstrate the therapeutic value of the methanolic extract of the whole plant of *Mimosa pudica* as a promising drug candidate for the treatment of hyperglycemia, oxidative stress, and thrombotic disorders. More research, including the isolation and identification of pharmacologically active constituents, detailed mechanism of action studies, complete safety and efficacy evaluations are necessary to explore the potential pharmaceutical applications of this natural compound.

5.2. Future Studies

In order to confirm and elaborate on the results obtained in this study, the following experiments should be done:

1. Isolate and identify the pharmacologically active chemical compounds.
2. Explain the molecular mechanism of action of the antidiabetic, and antioxidant effects of the extract.
3. Test the extract in chronic diabetic and thrombosis animal models.
4. Perform comprehensive toxicity and safety evaluation studies in order to determine the therapeutic dosages.
5. Develop an herbal formulation and conduct clinical trials.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contributions

Md. Mahbubol Alam, Fahamida Binta Anower, and Most Ashrafia Sultana took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing and editing. Mayesha Farzana, Ananya Sarker, Umma Taznin Urme, Mst Sadia Afrin Sairin, and Tanvir Hossain were analyzed data. Md. Mahbubol Alam and Fahamida Binta Anower were involved in the design of the manuscript. Md. Mahbubol Alam reviewed the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

Informed Consent

Not applicable for this study.

Declaration of Artificial Intelligence

No artificial intelligence tools were used for this study.

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