Mediterranean Journal of Basic and Applied Sciences (MJBAS)
Volume 10, Issue 4, Pages 26-41, October-December 2026

Exploring the Therapeutic Potential of the Methanolic Extract of *Mimosa pudica* Whole Plant: Cytotoxic, Analgesic, Anti-Inflammatory, Phenolic, and Flavonoid Properties

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DOI: Under Assignment

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Article Received: 14 August 2026 *Article Accepted: 24 October 2026* *Article Published: 26 October 2026*

ABSTRACT

This study was conducted to determine the cytotoxicity, analgesic activity, anti-inflammatory activity, and the presence of phenolic and flavonoids in the methanolic extract of the whole plant of *Mimosa pudica*. Cytotoxicity of the extract was analyzed through the use of brine shrimp lethality bioassay with vincristine sulfate as the standard drug, while the analgesic activity was assessed through the acetic acid-induced writhing assay using Swiss albino mice. Anti-inflammatory activity was tested using the carrageenan-induced paw edema test. The total flavonoid content and total phenolic content of the methanolic extract of M. pudica were determined through the use of the spectrophotometric method using quercetin and gallic acid as standard drugs, respectively. The methanolic extract exhibited concentration-dependent cytotoxicity against brine shrimp nauplii, giving an LC50 value of 80 µg/mL compared with vincristine sulfate which had an LC50 of 1.57 µg/mL. In the acetic acid-induced writhing assay, the extract inhibited the writhing at 37.21% and 62.79% inhibition at doses of 250 and 500 mg/kg, respectively, while diclofenac sodium inhibited the writhing at 88.37% at a dose of 100 mg/kg. In the carrageenan-induced paw edema test, the extract gave 26.35% inhibition at a dose of 500 mg/kg in contrast to diclofenac sodium which gave 52.78% inhibition at 10 mg/kg. Total flavonoid content of the extract was determined to be 172.50 ± 10.00 mg QE/g extract, while the total phenolic content was 123.25 ± 1.50 mg GAE/g extract. These results show that the methanolic extract of the whole plant of *Mimosa pudica* has cytotoxic, analgesic, anti-inflammatory activities, and phenolic and flavonoid contents.

Keywords: Anti-Inflammatory Activity; Analgesic Activity; Cytotoxicity; Flavonoids; *Mimosa pudica*; Whole-Plant Extract.

1.0. Introduction

Secondary metabolites from medicinal plants are valuable in terms of their diverse structures and possible uses as pharmaceutical agents. There has been an increased research effort directed at investigating the bioactive components from traditionally used medicinal plants using phytochemical and pharmacological approaches to provide evidence of the biological activity of plants and the identification of plants with medicinal value. One example of a medicinal plant under extensive investigation is *Mimosa pudica* L., which is famous for its wide range of pharmacological activities and traditional uses. Some reviews on the biological activities of different parts of *Mimosa pudica* include reports of antioxidant, antimicrobial, anti-inflammatory, antinociceptive, antidiabetic, hepatoprotective, and other biological activities [1,2].

Mimosa pudica L. or the sensitive plant or touch-me-not plant is well known for its rapid leaf-folding ability in response to mechanical stimuli. Besides the unique ability of this plant to fold its leaves, *Mimosa pudica* is traditionally used in ethnomedicine in treatment of certain disorders like gastrointestinal diseases, dysentery, wounds, inflammation, diabetes, and other illnesses. Different types of phytochemicals have been isolated from *Mimosa pudica*, which include phenols, flavonoids, tannins, terpenoids, steroids, and other phytochemicals of biological significance. Moreover, mimosine is another important bioactive component found in this medicinal plant. These phytochemical constituents have been implicated in the various pharmacological activities of *Mimosa pudica* [2,3].

Evaluation of cytotoxic potential of plants is one of the main objectives of pharmacological screening of medicinal plants. Brine shrimp lethality test (BST) is a commonly used technique in the assessment of the general cytotoxic potential of crude plant extracts and natural products. Brine shrimp lethal concentration (LC_{50}) value can be used as an indicator of presence of biologically active or toxic components in the plant material under study. Brine shrimp lethality test (BST) has previously been used in the preliminary assessment of cytotoxic activity of medicinal plant extracts [4,5].

Pain and inflammation have been considered as one of the main targets in pharmacological studies, with medicinal plants being among the main sources of natural compounds for analgesia and anti-inflammation. The acetic acid-induced writhing test is one of the most widely used methods to test peripheral analgesia. Another experiment that is considered to be a gold standard to evaluate acute inflammation is the carrageenan-induced paw edema. The use of these two models provides additional information about the possible analgesic and anti-inflammatory effects of plant extract [6]. Antinociceptive and anti-inflammatory activities of *Mimosa pudica* were described previously; therefore, the rationale of conducting experimental investigation of this species was justified [1–3].

Phenolics and flavonoids are some of the important plant secondary metabolites that exhibit wide ranges of biological properties. It therefore explains why determination of the total phenolic content (TPC) and total flavonoid content (TFC) has been found useful in characterizing the phytochemistry profile of medicinal plant extracts [7].

Despite the number of pharmacological properties attributed to *Mimosa pudica*, biological activities of plant extracts may be affected by various factors including the plant part used, extraction solvents, geographical location of the plant, extraction procedures and even phytochemical constituents. Various reviews have therefore stressed the need for more research on characterization of the active constituents and the mechanism of action of this plant extract in order to increase the scientific evidence on the medicinal potential of this plant [1-3].

In this regard, studies conducted using the whole plant will give an overview of both the phytochemical and pharmacological properties of this plant which cannot be achieved by studies on isolated plant parts. This research was therefore carried out to evaluate the cytotoxic, analgesic and anti-inflammatory properties of the methanolic extract of the whole plant of *Mimosa pudica* and the total content of phenolic and flavonoids.

1.1. Study Objectives

The main objective of the research is to study the pharmacological and phytochemical nature of the methanolic extract from the whole plant of *Mimosa pudica*. The specific objectives of the research are:

1. To carry out a study on the cytotoxic effects of the methanolic whole-plant extract of *Mimosa pudica* using the brine shrimp lethality test.

2. To study the analgesic effects of the methanolic whole-plant extract using the acetic acid induced writhing test in Swiss albino mice.

3. To study the anti-inflammatory effects of the methanolic whole-plant extract using the carrageenan induced paw edema test.

4. To quantify the total flavonoid content of the methanolic whole-plant extract using quercetin as the reference standard.

5. To quantify the total phenolic content of the methanolic whole-plant extract using gallic acid as the reference standard.

6. To develop the preliminary relationship between phytochemical makeup and pharmacological effects of the methanolic whole-plant extract of *Mimosa pudica*.

2.0. Literature Review

Earlier studies have identified *Mimosa pudica* as a phytochemically rich medicinal plant comprising various types of secondary metabolites. In their review on the importance of phytochemical and pharmaceutical aspects of *Mimosa pudica*, Muhammad et al. have found out that the plant is rich in mimosine, tannins, steroids, flavonoids, triterpenes, and glycosylflavones. The authors stressed that the biological activities shown by the plant are due to synergistic actions of these chemicals [1]. Similar work was done by Kumar in which he has focused on phytochemical and pharmacological aspects of *Mimosa pudica* and stated that alkaloids, phenols, and flavonoids play a crucial role in the biological activities [2].

In addition, Patro et al. studied the presence of phenols and flavonoids along with the antioxidant property of *Mimosa pudica*. They were able to determine the amount of total phenolics and flavonoids and showed its antioxidant activity under different conditions [8]. Quite recently, Gandhi et al. carried out an intensive study on *Mimosa pudica* leaves regarding phytochemical quantification and volatile compound characterization, and concluded that the plant has complex chemical composition responsible for its various biological activities [9]. All these studies suggest that phenol and flavonoid contents can act as good markers of *Mimosa pudica* biological potential.

The pharmacological properties of *Mimsosa pudica* have been investigated using in vitro and in vivo models. According to Muhammad et al., there is strong evidence in favor of the following pharmacological effects associated with various parts of the plant and extracts: antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antinociceptive, anticonvulsant, antidiarrheal, antiparasitic, antimalarial, and hypoglycemic effects [1]. In turn, Kumar described a wide range of pharmacological effects and emphasized the importance of isolating and characterizing the substances that provide such effects [2]. In a recent review by Ugbogu et al., ethnomedicinal uses, phytochemistry, pharmacological activity, and toxicological aspects of*Mimosa pudica* were assessed. The authors noted that the following biological activities are demonstrated in previous studies: antioxidant, antimicrobial, wound healing, hepatoprotective, antimalarial, anti-inflammatory, antidiabetic, and anticancer effects [10]. It is necessary to note that the presented evidence is heterogeneous since different plant parts, methods of extract preparation, models of experiment, and doses were used in the mentioned studies.

Cytotoxic screening is often used as the first approach when looking for active medicinal plants. The use of *Artemia salina* brine shrimp lethality test is rather simple model that can be used for evaluating cytotoxicity of plant extracts. There are numerous studies which proved its applicability for screening natural products and crude extracts prior to further cellular or molecular assays. Reviewing previous researches, one may conclude that the cytotoxic and anticancer potential of *Mimosa pudica* is associated with bioactive secondary metabolites such as mimosine and phenols and flavonoids [1,2]. According to Kumar, there are reported cases of anticancer potential of *Mimosa pudica*, although insufficient data was obtained for determining its exact mechanisms and toxicological properties [2]. Similarly, Ugbogu et al. list anticancer potential of *Mimosa pudica* among its pharmacological characteristics and state that further experimental studies are needed [10].

Pharmacological property such as analgesic activity is quite thoroughly studied for *Mimosa pudica*. Literature contains information about the antinociceptive effect of extracts of different plant organs, which may indicate that some of the substances found in *Mimosa pudica* can influence pain and inflammatory pathways. Muhammad et al. list antinociceptive activity among the pharmacological activities of *Mimosa pudica* [1]. Kumar provides information on analgesic and anti-inflammatory activity of *Mimosa pudica* as well as discusses its possible phytochemical background [2].

Inflammatory reaction is a rather complicated biological process which involves a number of mediators and pathways. Therefore, plant phenolic and flavonoids compounds gained attention due to their possible anti-inflammatory activity. Anti-inflammatory activity of *Mimosa pudica* has been found by several previous researchers [11].

Phenolic and flavonoid components are usually associated with the antioxidants and pharmacological properties of the medicinal plants. In the case of *Mimosa pudica*, previous studies showed the presence of these components and the possible role of them in the biological actions of crude extracts [1,8,9]. In one study, Patro et al. isolated these phytoconstituents from *Mimosa pudica* and demonstrated that there was antioxidant activity in it; thus, the importance of these phytoconstituents in pharmacological studies on *Mimosa pudica* could be stated [8].

Despite numerous studies on the ethnopharmacology, phytochemistry, and pharmacological actions of *Mimosa pudica*, some shortcomings should be pointed out. Previous studies mostly focused on different parts of plants, including leaves, roots, seeds, and flowers. Only a few studies examined pharmacological and phytochemical actions of *Mimosa pudica* as a methanolic whole plant extract simultaneously. In addition, the differences in extraction media, plant material, geographical origin, model, and dose could interfere with the results of previous studies.

3.0. Materials and Methods

3.1. Chemicals and Reagents

The extraction solvent of choice was methanol. Vincristine sulfate acted as the reference standard during the brine shrimp lethality bioassay, whereas diclofenac sodium served as the standard drug in the analgesic and anti-inflammatory assays. Acetic acid and carrageenan were used to induce pain and inflammation, respectively.

Gallic acid and quercetin were used as reference standards for the determination of TPC and TFC, respectively. All other chemicals and reagents used in the current work were of analytical grade [7].

3.2. Experimental Animals

Swiss albino mice (*Mus musculus*) of both sexes, weighting 25-35 g and having an age of 6-8 weeks, were used in the analgesic and anti-inflammatory assays. The experimental animals were purchased from the breeding colony at Jahangirnagar University, Savar, Dhaka, Bangladesh. They were maintained in standard polypropylene cages under controlled conditions of temperature (25 ± 2°C) and relative humidity (55-65%) with 12-hour cycles of day and night. Standard laboratory chow and water were provided ad libitum. The mice were acclimatized to the laboratory environment for one week before performing the experiments. The procedures followed all the required ethical guidelines regarding the care and use of laboratory animals [7].

3.3. Brine Shrimp Nauplii

The eggs of brine shrimp (*Artemia salina*) were procured from Fish Aquarium, Katabon, Dhaka, Bangladesh. The artificial seawater was prepared using 38 g of sea salt dissolved in 1 liter of distilled water thoroughly. The seawater so prepared was used for the hatching of brine shrimp eggs and maintenance of the nauplii [7].

3.4. Preparation of Crude Extract (Cold Extraction Method)

Mimosa pudica plant was collected and identified by an expert botanist. The collected plant samples were washed with distilled water to remove dust and other contaminations and were shade dried at room temperature to achieve constant weight. The dried samples were mechanically ground into powder form. Cold extraction method using methanol as the solvent was used to extract phytochemicals from the powder. The plant material was added to methanol in a clean and dry glass container that was tightly sealed and kept at room temperature for the suitable duration with intermittent shaking. The mixture was first filtered using clean cotton cloth followed by filtration using Whatman No. 1 filter paper. The filtrate was then evaporated using a rotary evaporator to obtain the crude methanolic extract. The obtained extract was then dried and stored at 4°C in a clean glass container. The obtained methanolic extract was then used in the brine shrimp lethality, analgesic, anti-inflammatory, total phenolic content, and total flavonoid content tests. The uploaded data specifically refers to methanolic extract of *Mimosa pudica* [12].

3.5. Lethal Brine Shrimp Bioassay

In order to determine the cytotoxic effect of methanolic extract of whole plant of *Mimosa pudica*, a lethal brine shrimp (*Artemia salina*) bioassay was performed. Brine shrimp eggs were incubated in artificial seawater under optimal conditions with proper aeration and light for 24-48 hours to allow their hatching. The hatched nauplii were then collected and used for lethal bioassay. Serial dilutions of methanolic extract were made in such a way that its concentration ranges from 0.078 to 160 µg/mL. Ten live nauplii were placed into each test tube containing the required concentrations of extract. Vincristine sulfate was used as the positive control whereas artificial seawater as the negative control. After 24 hours of incubation period, the surviving nauplii were counted and percentage mortality was computed using the given formula. The LC_{50} value was obtained through the concentration-

mortality curve. The LC_{50} values of vincristine sulfate and methanolic extract of M. pudica were found to be 1.57 µg/mL and 80 µg/mL, respectively.

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total number of nauplii}} \times 100 \quad (1)$$

The LC_{50} was calculated based on the percentage mortality vs. log of concentration graph [13].

3.6. Analgesic Activity

The analgesic activity of the methanol extract of the whole plant of *Mimosa pudica* was carried out using the acetic acid-induced writhing test in Swiss albino mice. Each experiment group was made up of five mice (n = 5). These animals were grouped into control group, drug group and extract treated groups. The control group was given distilled water in a dosage of 10 ml/kg body weight, while the drug group was given diclofenac sodium in a dosage of 100 mg/kg body weight. The methanolic extract of *Mimosa pudica* was given at dosages of 250 and 500 mg/kg body weight. Writhing was induced following the administration of acetic acid in the animal intraperitoneally. The number of writhes was recorded, and the percentage inhibition of writhes was calculated based on comparison of the mean number of writhes in the treated groups to those of the control group based on the required formula [14].

$$\text{Writhing inhibition (\%)} = 1 - \frac{\text{Writhing mean of control}}{\text{Writhing mean of sample/standard}} \times 100 \quad (2)$$

3.7. Anti-inflammatory activity

To assess the anti-inflammatory activity of the methanolic extract of *Mimosa pudica*, the carrageenan-induced paw edema test on mice was conducted. The experimental animals were categorized into control, standard, and treatment groups. The diclofenac sodium (10 mg/kg) was used as a standard drug, while the methanolic extract of *Mimosa pudica* was used at 500 mg/kg. The paw volume of mice was taken after 0, 60, 120, 180, and 240 minutes of carrageenan administration [15]. The mean paw volumes for each group and each mentioned time were recorded and the percentage of reduction in the paw volume of the control group was calculated. The anti-inflammatory activity in percentage can be obtained from the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1 - T/C) \times 100 \quad (3)$$

Where, T = Treatment Group, and C = Control Group [16].

3.8. Quantitation of Total Phenolic Compounds (TPCs)

The Folin-Ciocalteu reagent method with gallic acid as a standard was used to quantify total phenolics. First, 0.5 mL of the solution of the plant extracts (1 mg/mL) was mixed with 2.5 mL of 10% (v/v) of Folin-Ciocalteu reagent. After five minutes, 2.0 mL of 7.5% (w/v) sodium carbonate solution was added. The mixture was then vortexed and left undisturbed in the dark for 30 minutes at room temperature. The absorbance value was determined at 765 nm using a UV-visible spectrophotometer with the reagent blank. A standard curve was prepared with various concentrations of gallic acid, and the total phenolic compounds were reported in mg of gallic acid equivalents per g of dry extract (mg GAE/g extract) [17].

3.9. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was evaluated by colorimetric method using aluminum chloride and quercetin as reference standard. In detail, 0.5 mL of test extract (1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% (w/v) aluminum chloride solution, 0.1 mL of 1M potassium acetate solution, and 2.8 mL of distilled water. After that, samples were kept at room temperature for 30 minutes. The yellow-colored complex produced was read for absorbance at 415 nm on a UV-visible spectrophotometer with a blank containing only reagents. A calibration curve was established using different concentrations of quercetin solutions, and TFC was quantified as quercetin equivalents (mg/g extract) [17].

3.10. Statistical Analysis

Descriptive statistics was performed on phytochemical analysis (TPC and TFC). Experiments were done in triplicates (n = 3), and results were done in Excel.

4.0. Results and Discussion

This study aimed at determining the biological properties and phytochemistry of methanolic extract of whole plants of *Mimosa pudica*, specifically those associated with cytotoxicity, analgesia, anti-inflammation, phenolics and flavonoids. The findings demonstrate that the extract possesses biologically active properties in all three pharmacological assays but its efficacy is not as great as those of their corresponding reference substances. In general, it can be concluded from the study findings that the methanolic extract possesses biologically active properties.

4.1. Cytotoxic activity

The lethality of the methanolic whole-plant extract of *Mimosa pudica* based on the results of the bioassay in brine shrimp was found to be strongly concentration-dependent. Lower concentrations of the extract were associated with low levels of mortality, while increasing extract concentrations were associated with an increasing number of deaths. All results were calculated using Equation 1, represented in Table 1 and shown in Figure 1. Specifically, the mortality rate was 10% at 20 µg/mL, 30% at 40 µg/mL, 50% at 80 µg/mL, and 70% at 160 µg/mL, which gives the extract an LC50 of 80 µg/mL. On the other hand, the vincristine sulfate had an LC_{50} of 1.57 µg/mL.

The concentration dependence of mortality means that it is an effect of prolonged exposure to the extract. While the LC_{50} is indicative of the presence of cytotoxic activity, it cannot be seen as proof of anticancer activity. Instead, brine shrimp lethality assay can serve as an initial screen for extracts that can be tested further against cancer cell lines using various mechanistic assays. It is to be expected that the cytotoxic activity of vincristine will be higher than that of the crude extract since vincristine is a purified pharmacologically active drug while the extract is a complex mixture of different substances present at various concentrations.

In the regression analysis of the uploaded data, the linearity was found to be better in the case of the standard (R^2 = 0.9878) than in the case of the extract (R^2 = 0.8061). It might mean that the concentration-response relationship for the extract is more complicated, and thus further fractionation and chemical characterization could be helpful in identifying the compounds responsible for lethality.

Mediterranean Journal of Basic and Applied Sciences (MJBAS)
Volume 10, Issue 4, Pages 26-41, October-December 2026

Table 1. Brine Shrimp lethality bioassay of methanolic extract of *Mimosa pudica* (Vincristine sulfate as standard).

Concentration (µg/mL)	Log C	Alive Nauplii (n = 10)			Mortality (%)		LC_{50} (µg/mL)
		Control	Standard	Extract	Standard	Extract	
0.078	-1.108	10	10	-	0	-	Vincristine Sulfate Standard (1.57)
0.156	-0.807	10	10	-	0	-	
0.313	-0.504	10	10	-	0	-	
0.625	-0.204	10	8	-	20	-	
1.25	0.097	10	6	-	40	-	
2.50	0.398	10	3	10	70	0	
5	0.699	10	2	10	80	0	*Mimosa pudica* Extract (80)
10	1.000	10	1	10	90	0	
20	1.301	10	0	9	100	10	
40	1.602	10	0	7	100	30	
80	1.903	10	0	5	100	50	
160	2.204	10	0	3	100	70	

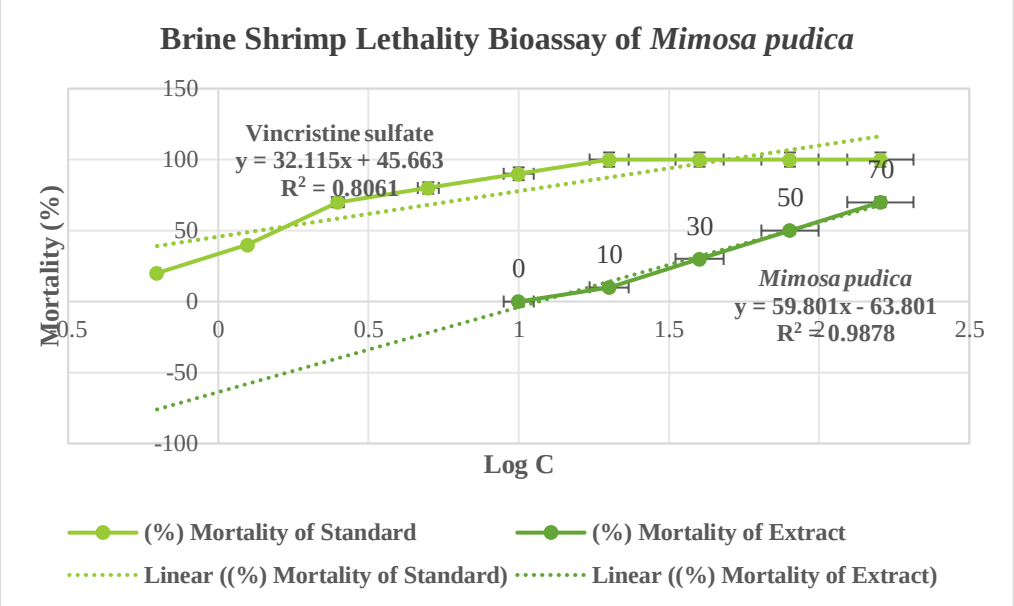


Figure 1. Comparative percentage mortality by methanolic extract of *Mimosa pudica* and Vincristine sulfate.

4.2. Analgesic activity

The methanolic extract showed marked analgesic property against the writhing induced by acetic acid. The control rats showed 43 writhes, but the standard diclofenac sodium-treated animals showed just 5 writhes which translated to 88.37% inhibition, which is calculated using Equation 2. Treatment of *Mimosa pudica* extract at 250 mg/kg caused reduction of writhes to 27 resulting to 37.21% inhibition. Further increase of dose to 500 mg/kg caused reduction of the number of writhes to 16, causing the inhibition to go up to 62.79%.

The dose-response relationship in terms of the increase of the percent inhibition of writhes from 37.21% for 250 mg/kg to 62.79% for 500 mg/kg is an indication of a dose dependent analgesic activity, which are presented in

Table 2 and illustrated in Figure 2. While the level of inhibition achieved in 500 mg/kg dose did not reach the one achieved by diclofenac sodium, the significant reduction in the number of writhes showed that the extract must contain some components capable of affecting the nociception induced by acetic acid.

Acetic acid-induced writhing is an animal model highly sensitive to any compounds affecting peripheral nociceptive processes. But since in this particular report there are no data provided in terms of biochemical or receptor assays, the exact mechanism of action cannot be determined based on the present findings. The analgesic effect of the extract may be due to the cumulative effect of different active constituents found in the crude extract.

Table 2. Effect of the methanolic extract of *Mimosa pudica* extract on acetic acid-induced writhing in swiss albino mice (n = 5).

Group	Dose	Total Number of Writhing	% Writhing Inhibition
Control	10 mL/kg	43	0.00
Standard (Diclofenac Na)	100 mg/kg	05	88.37
Mimosa pudica extract	250mg/kg	27	37.21
	500 mg/kg	16	62.79

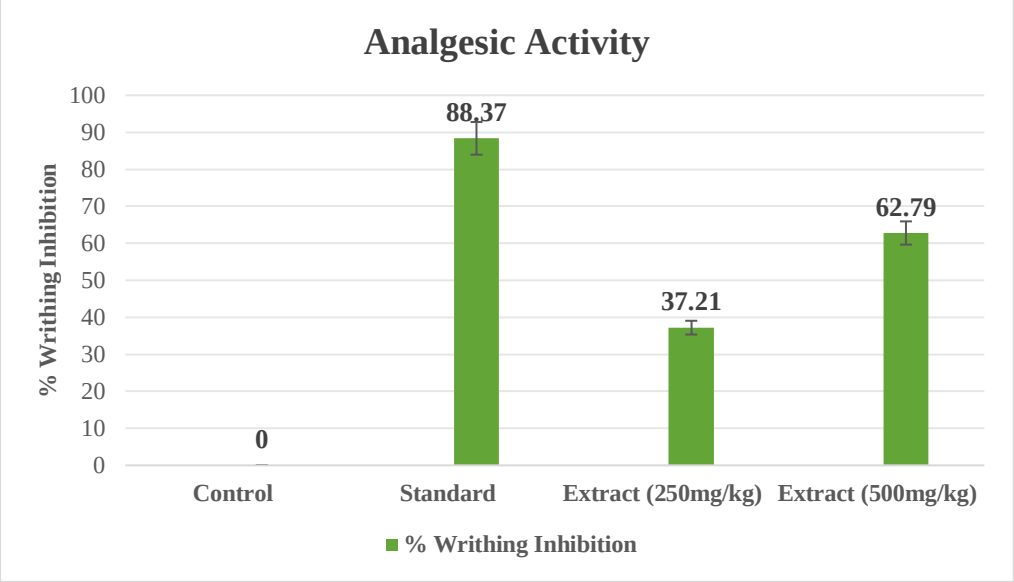


Figure 2. Analgesic activity of the methanolic extract of *Mimosa pudica* in the acetic acid-induced writhing test.

4.3. Anti-inflammatory activity

The experiment on anti-inflammatory evaluation through carrageenan-induced paw edema highlighted the biological activity of the methanolic whole-plant extract. In the control group, paws of mice had the following sizes: 4.40, 4.28, 4.18, 4.10, and 4.02 at 0, 60, 120, 180, and 240 min correspondingly. Treatment with diclofenac sodium 10 mg/kg resulted in a gradual decrease in paw volume and reached 2.29 at 240 min, which corresponds to 52.78% decrease. Treatment with the *Mimosa pudica* extract in 500 mg/kg caused a decrease in paw volume up to 3.13 at 240 min that corresponds to 26.35%. All results were calculated by Equation 3, expressed in Table 3 and represented in Figure 3.

In this case, 26.35% of the reduction in the size of paw is a demonstration of moderate biological activity in terms of the anti-inflammatory effect of the extract in the studied conditions. Nevertheless, it should be noted that the extract showed activity, which is twice lower compared with that of the reference anti-inflammatory agent. Still, this point should be regarded positively because it shows that the biological activity of the extract exists.

It is worth noting that both analgesic and anti-inflammatory activities have been observed simultaneously. 62.79% of inhibition of the acetic acid-induced writhing at the dosage of 500 mg/kg and 26.35% decrease in the size of the paw at the same dosage are indicative of the pharmacological properties of the extract. At the same time, it is impossible to state that analgesic activity is connected with anti-inflammatory effects only.

Table 3. Effect of methanolic extract of *Mimosa pudica* on carrageenan-induced paw edema in mice for anti-inflammatory activity.

Group	0 min	60 min	120 min	180 min	240 min	% Decrease in paw volume
Control	4.40	4.28	4.18	4.10	4.02	0.00
Diclofenac-Na (10mg/kg)	4.85	3.78	3.54	2.92	2.29	52.78
Mimosa pudica (500mg/kg)	4.25	3.82	3.54	3.27	3.13	26.35

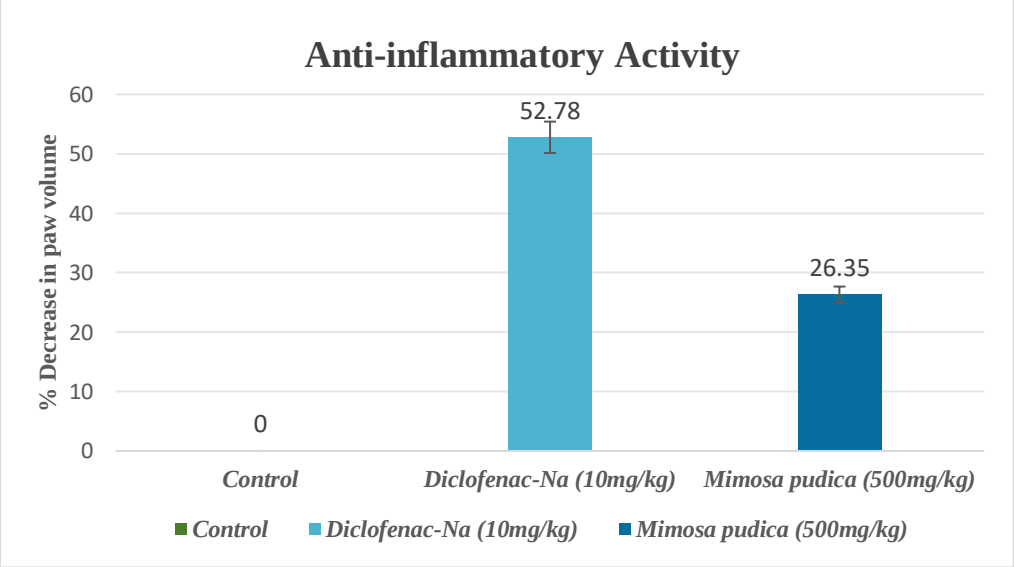


Figure 3. Anti-inflammatory activity of methanolic extract of *Mimosa pudica* in carrageenan-induced paw edema model showing percentage inhibition of paw edema.

4.4. Total flavonoid content

The total flavonoid content in the methanolic extract was high with a value of 172.50 ± 10.00 mg QE/g extract. The flavonoid content was determined using the quercetin calibration curve with the equation y = 0.000151x + 0.3045 and the R^2 value of 0.9833, which indicates a high degree of linearity in the calibrated range, shown in Figure 4 and represented in Table 4. The absorbance of the extract was 0.339 ± 0.002.

The comparatively high content of flavonoids indicates that flavonoid-containing phytochemicals form a considerable quantifiable portion of the methanolic extract. Flavonoids are phytochemicals with varied chemistry

and may have several different biological activities. Therefore, their presence gives a phytochemical background to the pharmacological activities reported in this study. Nevertheless, the total flavonoid content is a summative measure of all flavonoids in a sample and does not reveal individual flavonoids. Therefore, the exact causal relationship between the total flavonoid content measured in this study and the biological activities could not be established.

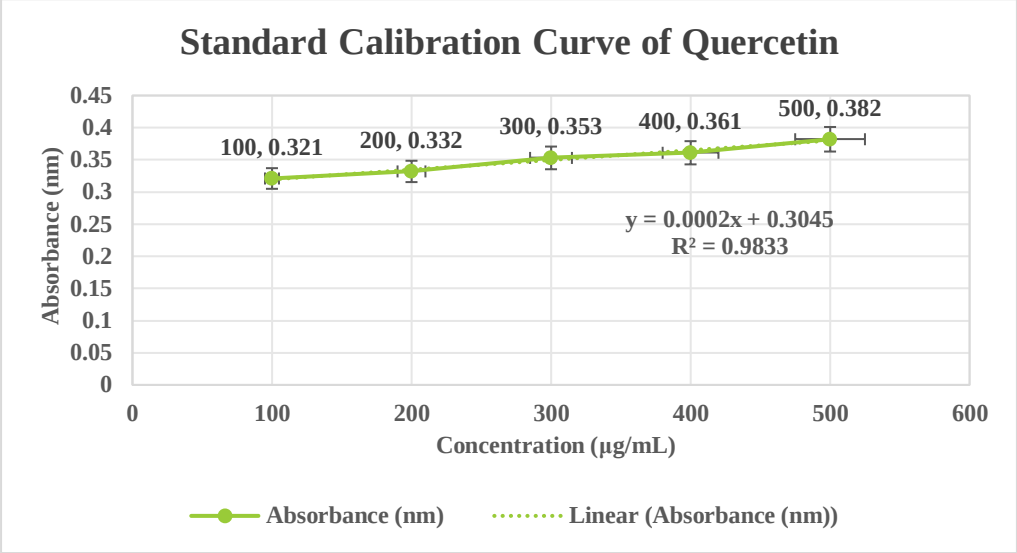


Figure 4. Quercetin calibration curve, UV absorbance of standard at 372 nm, for the determination of total flavonoid content in the methanolic extract of *Mimosa pudica*.

4.5. Total phenolic content

The TPC of the methanolic extract obtained from the entire plant was 123.25 ± 1.50 mg GAE g-1 extract. The calibration curve of gallic acid was y = 0.002045x + 0.8625 with the R^2 value of 0.9774, while the absorbance of the extract was 1.109 ± 0.003. The calibration curve of gallic acid was presented in Figure 5 and shown in Table 4.

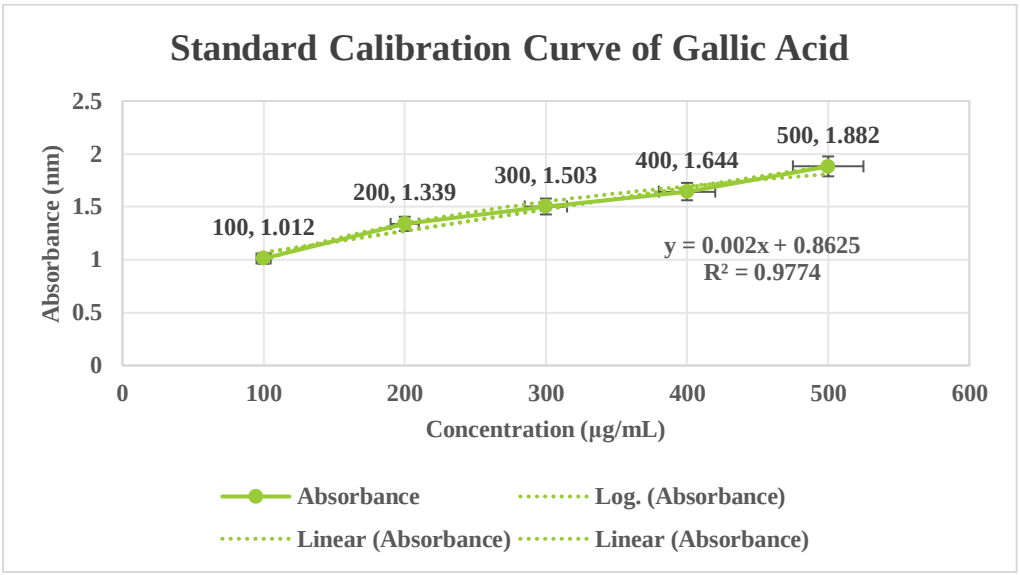


Figure 5. Gallic acid calibration curve, UV absorbance of standard at 271 nm, for the determination of total phenolic content in the methanolic extract of *Mimosa pudica*.

Table 4. Total phenolic and total flavonoid contents of the methanolic extract of *Mimosa pudica* extract (n=3).

Total Flavonoid Contents		**Total Phenolic Content**	
Absorbance (nm) Mean ± SD	Concentration (mg QE/gm extract) Mean ± SD	Absorbance (nm) Mean ± SD	Concentration (mg GAE/gm extract) Mean ± SD
0.339 ± 0.002	172.50 ± 10.00	1.109 ± 0.003	123.25 ± 1.50

It is important to note that the high concentration of total phenolic compounds is essential in the biological activity of the extract since phenolic compounds form one of the most abundant secondary metabolites of plants. The relatively low standard deviation observed in TPC determination signifies repeatability between the three measurements. Nevertheless, the Folin-based test for total phenolic content is not selective enough, thus it only provides bulk analysis of all phenols and cannot separate individual phenolic compounds. Therefore, additional techniques will be needed to determine them.

4.6. Overall interpretation

Together, the findings suggest that the methanolic extract of the whole plant of *Mimosa pudica* has a wide range of preliminary pharmacological activity. The extract shows concentration-dependent cytotoxic activity, dose-dependent analgesic activity, and anti-inflammatory activity in several test systems, thus proving its biological activity. Furthermore, the extract possesses significant amounts of total phenolic compounds and flavonoids, serving as phytochemical grounds for further research.

Nevertheless, the activities of the crude extract are lower compared to the respective reference standards – vincristine sulfate for cytotoxic activity and diclofenac sodium for analgesic and anti-inflammatory activities. It is crucial to note this difference in order to interpret the data correctly since the present research suggests only preliminary pharmacological properties of the extract.

5.0. Conclusion and Further Studies

5.1. Conclusion

This study reveals that the methanolic extract of the whole plant of *Mimosa pudica* displays certain pharmacological and phytochemical characteristics. The cytotoxic effect of the extract was shown to be dependent on the concentration, with the LC_{50} value of 80 µg/mL. The extract also had a dose-dependent analgesic activity with 37.21% and 62.79% inhibition of acetic acid-induced writhing in doses of 250 and 500 mg/kg, respectively. In addition, the extract decreased the paw edema induced by carrageenan, resulting in a 26.35% reduction of paw volume in a dose of 500 mg/kg. Moreover, the extract contained significant amounts of total flavonoids (172.50 ± 10.00 mg QE/g extract) and total phenolics (123.25 ± 1.50 mg GAE/g extract).

Overall, this study demonstrates that the methanolic extract of the whole plant of *Mimosa pudica* displays certain cytotoxic, analgesic and anti-inflammatory activities and is a valuable source of phenolics and flavonoids. At the same time, the observed biological activities were lower than those of the corresponding reference standards. Thus, the presented data must be considered preliminary, and further research is needed.

5.2. Further Studies

Further investigations are required to validate the pharmacological potential of the methanolic whole-plant extract of *Mimosa pudica* and to identify its active constituents and mechanisms.

1) Phytochemical profiling: Identify and quantify bioactive compounds using advanced analytical techniques.

2) Compound isolation: Isolate and characterize the major phenolic, flavonoid, and other active constituents.

3) Mechanistic studies: Investigate the molecular mechanisms underlying cytotoxic, analgesic, and anti-inflammatory effects.

4) Cytotoxicity validation: Evaluate the extract against relevant human cancer cell lines with normal-cell controls.

5) Safety assessment: Conduct acute, subacute, and chronic toxicity studies to establish its safety profile.

6) In vivo validation: Confirm the observed analgesic and anti-inflammatory activities using additional validated animal models.

7) Drug development: Explore standardization and formulation approaches for potential pharmaceutical applications.

Declaration

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, Fatema Akhter, Most Ashrafia Sultana, and Fahamida Binta Anower were took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing and editing. Halima Akter, Afrin Farzana, and Jakia Jahan Jui analyzed data. Md. Mahbubol Alam was involved in the design of the manuscript. Fatema Akhter 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.

Acknowledgement

Not applicable for this study.

Declaration of Artificial Intelligence

No AI tools were used in this study.

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