

PHYTOCHEMICAL SCREENING AND EVALUATION OF IN-VITRO ANTI-INFLAMMATORY, ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES OF MIMOSA PUDICA STEM EXTRACT

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ABSTRACT

Medicinal plants serve as an important source of bioactive compounds with potential therapeutic applications. *Mimosa pudica* L. is a traditionally used medicinal plant reported to possess various pharmacological activities due to the presence of secondary metabolites. The present study was aimed to evaluate the phytochemical constituents and investigate the in vitro anti-inflammatory, antidiabetic and antioxidant activities of ethanolic and hexane stem extracts of *Mimosa pudica*. The stem extracts were subjected to preliminary phytochemical screening. The anti-inflammatory activity was evaluated by protein denaturation assay using egg albumin with diclofenac sodium as the standard drug. The antidiabetic activity was assessed by glucose uptake assay using metformin as the standard drug, and antioxidant activity was determined by hydrogen peroxide scavenging assay using ascorbic acid as the standard antioxidant. The results of phytochemical screening

revealed the presence of various bioactive constituents including flavonoids, phenolic compounds, tannins, alkaloids, saponins and other secondary metabolites. This study supports the use of *Mimosa pudica* in the development of natural formulations for the prevention and treatment of oxidative stress-related diseases and diabetes.

KEYWORDS: *Mimosa pudica*, *invitro* anti-inflammatory, *invitro* antidiabetic, *invitro* antioxidant, glucose uptake assay, hydrogen peroxide scavenging assay, protein denaturation assay.

INTRODUCTION

Medicinal plants have been used for centuries as an important source of therapeutic agents due to their rich content of biologically active phytoconstituents. In recent years, plant-based medicines have gained considerable attention because of their antioxidant, anti-inflammatory, antidiabetic and other pharmacological properties. Oxidative stress and chronic inflammation play important roles in the progression of various metabolic disorders, including diabetes mellitus.

Mimosa pudica L. (Family: Fabaceae), commonly known as the touch-me-not plant, is a medicinal herb widely distributed in tropical regions. Traditionally, different parts of this plant have been used for the management of inflammation, wounds, pain and metabolic disorders. The plant is reported to contain various phytoconstituents such as flavonoids, phenolic compounds, tannins, alkaloids and saponins, which are associated with several pharmacological activities.

Diabetes mellitus is a major metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin secretion, insulin resistance or both. Hyperglycaemia-induced oxidative stress contributes to cellular damage and complications associated with diabetes. Therefore, identifying natural compounds that can improve glucose utilization and reduce oxidative stress is of significant interest.

Inflammation is a protective biological response; however, prolonged inflammation can contribute to tissue injury and disease progression. Protein denaturation is one of the mechanisms involved in inflammatory conditions, and inhibition of protein denaturation is considered an important *in vitro* method for evaluating anti-inflammatory potential.

The antioxidant activity of medicinal plants is mainly attributed to phenolic and flavonoid compounds that can neutralize reactive oxygen species (ROS). Hydrogen peroxide scavenging assay is a commonly used in vitro method to evaluate the ability of plant extracts to eliminate free radicals and reduce oxidative stress.

The present study was designed to evaluate the phytochemical constituents and investigate the in vitro anti-inflammatory, antidiabetic and antioxidant activities of ethanolic and hexane stem extracts of *Mimosa pudica* L.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The stem of *Mimosa pudica* L. was collected from Cuddalore-Saankarapuram Rd, Athiyur, Tamil Nadu, 605801, India which was authenticated by Dr.J. Suresh Kumar, M.sc., M. Phil, PhD., PGDCA. The collected plant material was washed thoroughly with water to remove adhering impurities, shade dried and powdered. The dried powder was stored in an airtight container until further extraction.

Preparation of Crude Extracts

The powdered stem material of *Mimosa pudica* was subjected to Soxhlet extraction using ethanol and hexane as solvents. The extraction process was continued until complete exhaustion of the plant material. The obtained extracts were concentrated by evaporation of solvents and stored for further pharmacological evaluation.

Preliminary Phytochemical Screening

The ethanolic and hexane extracts were subjected to preliminary phytochemical screening to identify the presence of various secondary metabolites. The extracts were tested for alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides and carbohydrates using standard qualitative procedures.

S. NO	TEST	OBSERVATION	INFERENCE
1	ALKALOIDS Hager's Test: Few ml of filtrate with 2ml Hager's reagent	Creamy white precipitate	Presence of alkaloids
2	FLAVANOIDS Lead acetate test: 1ml of extract with 10% lead acetate solution	Yellow precipitate is formed	Presence of flavonoids
3	CARBOHYDRATES Barford's test: 1ml of filtrate with 1ml of	Red precipitate is formed	Presence of carbohydrates

	reagent heat for 2mins		
4	PROTEINS Millions test: 2ml of filtrate with few drops of millions reagent	White precipitate is formed	Presence of proteins
5	PHENOLICS Iodine test: 1ml of extract with diluted iodine solution	Translucent red colour is formed	Presence of phenolics
6	STEROIDS Liebermann-Burchard test: A plant extract with dissolved anhydrous chloroform and add 1ml of acetic anhydride and add 1ml of conc.H ₂ SO ₄ solution	Green or Reddish blue colour is formed	Presence of Steroids
7	SAPONINS Foam test: 2ml of extract 15 sec shake it stands for 5mins	Foam is formed	Presence of saponins
8	TERPENOIDS Salkowski test: 2ml of extract with 1ml of chloroform then add conc. H ₂ SO ₄	Reddish brown colour	Presence of terpenoids
9	GLYLCOSIDES Keller- killiani test: A plant extract and add glacial acetic acid containing trace of ferric chloride and add Conc.H ₂ SO ₄	Brown ring at the junction	Presence of Glycosides
10	TANNINS Lead acetate test: Plant extract with lead acetate solution	Cream coloured or Gelatinous precipitate	Presence of Tannins

Invitro Anti-inflammatory Activity

Protein Denaturation Assay

The anti-inflammatory activity of *Mimosa pudica* stem extracts was evaluated by inhibition of protein denaturation method using egg albumin. Different concentrations of extracts (100–500 µg/mL) were prepared using suitable solvent. The reaction mixture contained egg albumin, phosphate buffer (pH 6.4) and different concentrations of extracts. Diclofenac sodium was used as the standard drug.

The mixtures were incubated at 37°C for 15 minutes followed by heating at 70°C for 5 minutes. After cooling, absorbance was measured at 660 nm. Percentage inhibition of protein denaturation was calculated using the formula.

$$\% \text{ Inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100}{100}$$

Invitro Antidiabetic Activity

Glucose Uptake Assay

The glucose uptake activity of *Mimosa pudica* stem extracts was evaluated by glucose uptake assay. Different concentrations of ethanolic and hexane extracts (100–500 µg/mL) were prepared. Metformin was used as the standard drug.

The reaction mixture containing glucose solution and extract was incubated for predetermined time intervals. The glucose concentration was estimated by measuring absorbance at 540 nm. The percentage glucose uptake was calculated using the formula:

$$\% \text{ Glucose uptake} = \frac{[\text{Initial glucose concentration} - \text{Final glucose concentration}]}{\text{Initial glucose concentration}} \times 100$$

Invitro Antioxidant Activity

Hydrogen Peroxide Scavenging Assay

The antioxidant activity of *Mimosa pudica* stem extracts was determined by hydrogen peroxide scavenging assay. Different concentrations of extracts (100–500 µg/mL) were prepared. Ascorbic acid was used as the standard antioxidant.

The reaction mixture contained phosphate buffer (pH 7.4), hydrogen peroxide solution (40 mM) and different concentrations of extracts. The absorbance was measured at 230 nm against a blank. The percentage scavenging activity was calculated using the formula:

$$\% \text{ H}_2\text{O}_2 \text{ scavenging activity} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}]}{\text{Absorbance of control}} \times 100$$

RESULTS

Percentage Yield of Extracts

The percentage yield of ethanolic and hexane extracts obtained from *Mimosa pudica* stem was calculated after Soxhlet extraction. The ethanolic extract showed higher yield compared to the hexane extract, indicating better extraction of polar phytoconstituents.

Table 1: Percentage yield of different extracts of *Mimosa pudica* stem.

S.No.	MIMOSA PUDICA EXTRACTS	INITIAL WEIGHT(g)	EXTRACT OBTAINED(g)	PERCENTAGE YEILD (%)
1	Ethanol	35	6	17.1
2	n-hexane	35	3	8.5

Preliminary Phytochemical Screening

The preliminary phytochemical screening revealed the presence of various secondary metabolites. Ethanolic extract showed the presence of phenolics, flavonoids, tannins, saponins, alkaloids and glycosides, whereas hexane extract showed limited phytoconstituents.

Table 2: Phytochemical profile of *Mimosa pudica* stem extracts.

S. No.	Phytochemical test	Ethanolic extract	n-Hexane extract
1	Alkaloids	+	—
2	Flavonoids	+	—
3	Saponins	+	—
4	Tannins	+	—
5	Phenolic compounds	+	—
6	Terpenoids	+	+
7	Steroids	+	+
8	Glycosides	+	+
9	Proteins	—	—
10	Carbohydrates	—	—

(+ Present, – Absent)

In Vitro Antioxidant Activity

The antioxidant activity of *Mimosa pudica* stem extracts was evaluated by [DPPH/H₂ O₂] radical scavenging assay using ascorbic acid as standard. The percentage inhibition increased with increasing concentration of extracts.

Table 3: Antioxidant activity of *Mimosa pudica* stem extracts.

S.No.	Concentration(μg/mL)	Ethanolic	Extract	n-Hexane	Extract
		Absorbance (nm)	% inhibition (%)	Absorbance (nm)	% inhibition (%)
1	100	0.449	35.1	0.536	22.5
2	200	0.355	48.7	0.444	35.8
3	300	0.264	61.8	0.358	48.3
4	400	0.175	74.7	0.271	60.8
5	500	0.093	86.6	0.198	71.4

CONTROL ABSORBANCE = 0.692

ASCORBIC ACID (STD) = 0.069

% INHIBITION OF STD = 90.02%

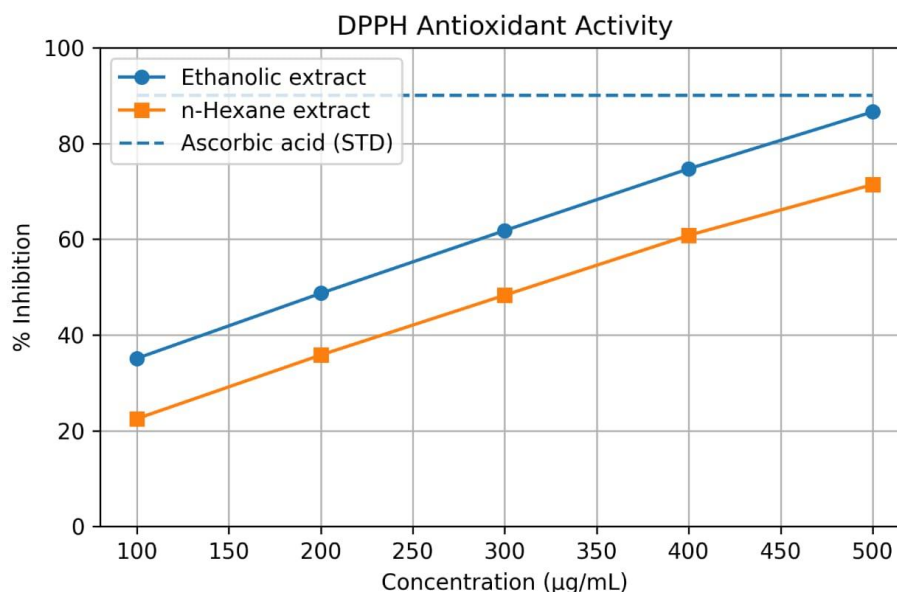


Figure 1: Concentration-dependent antioxidant activity of standard ascorbic acid and *Mimosa pudica* extracts.

***In Vitro* Anti-inflammatory Activity**

The anti-inflammatory activity was evaluated by egg albumin protein denaturation method using diclofenac sodium as standard drug.

The ethanolic extract exhibited significant inhibition of protein denaturation compared with hexane extract.

Table 5: Effect of *Mimosa pudica* stem extracts on protein denaturation assay.

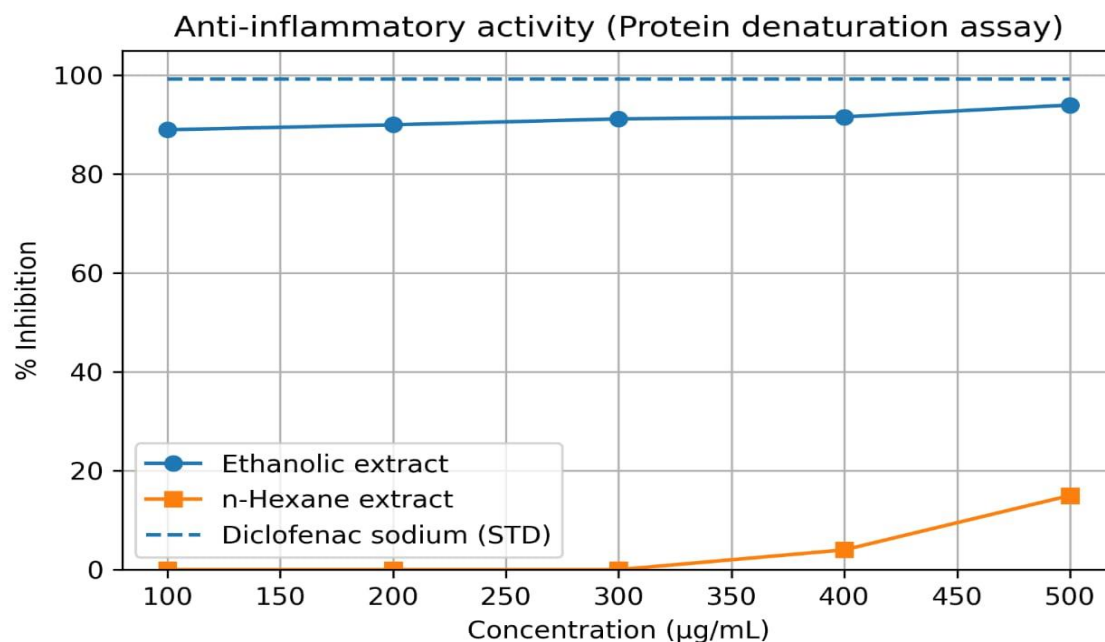
S. No.	Concentration(µg/mL)	Ethanolic Absorbance (nm)	Extract % inhibition (%)	n-Hexane Absorbance (nm)	Extract % inhibition (%)
1	100	0.026	89.0	0.499	0
2	200	0.025	90.0	0.487	0
3	300	0.022	91.2	0.368	0
4	400	0.021	91.6	0.240	4.0
5	500	0.015	94.0	0.120	15.0

CONTROL ABSORBANCE = 0.250

DICLOFENAC SODIUM (STD) = 0.0018

% INHIBITION OF STD = 99.28%

Figure 2: Concentration-dependent Anti-inflammatory activity of extracts compared with diclofenac sodium.



***In Vitro* Antidiabetic Activity**

The glucose uptake activity of *Mimosa pudica* stem extracts was evaluated using metformin as standard. Both extracts showed concentration-dependent glucose uptake activity.

Table 6: Glucose uptake activity of *Mimosa pudica* stem extracts.

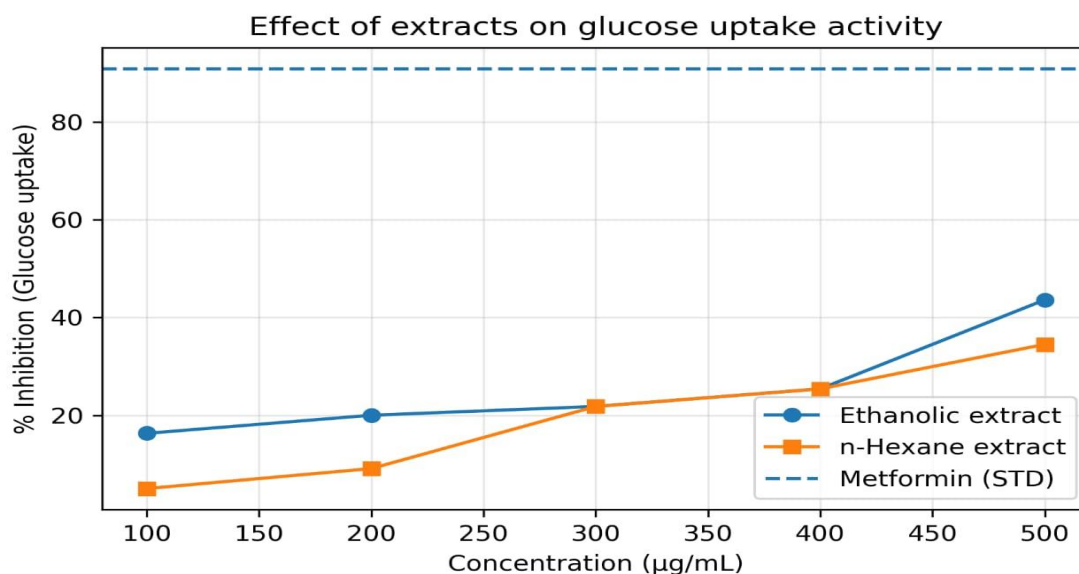
S. No.	Concentration(µg/mL)	Ethanolic Absorbance (nm)	Extract % inhibition (%)	N-Hexane Absorbance (nm)	Extract % inhibition (%)
1	100	0.046	16.3	0.052	5.0
2	200	0.044	20.0	0.050	9.09
3	300	0.043	21.8	0.043	21.8
4	400	0.041	25.4	0.041	25.4
5	500	0.031	43.6	0.036	34.5

CONTROL ABSORBANCE = 0.055

METFORMIN (STD) = 0.005

% INHIBITION OF STD = 90.90

Figure 3: Concentration-dependent Anti-diabetic activity of extracts compared with metformin.



DISCUSSION

The present study was carried out to evaluate the *in vitro* antioxidant, anti-inflammatory, and antidiabetic activities of *Mimosa pudica* stem extracts prepared using ethanol and hexane as extraction solvents. The phytochemical analysis revealed that the ethanolic extract contained alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, and glycosides, whereas the n-hexane extract contained only terpenoids, steroids, and glycosides. Proteins and carbohydrates were absent in both extracts. The higher diversity of bioactive phytoconstituents in the ethanolic extract suggests that ethanol is a more effective solvent for extracting polar secondary metabolites. Phenolic compounds, flavonoids, tannins, and saponins are well known for their antioxidant, anti-inflammatory, and antidiabetic activities, which likely contributed to the enhanced biological effects observed in the present study.

The DPPH assay showed a concentration-dependent increase in free radical scavenging activity for both extracts. The ethanolic extract exhibited significantly higher antioxidant activity, increasing from 35% inhibition at 100 µg/mL to 87% inhibition at 500 µg/mL, while the n-hexane extract increased from 23% to 72% inhibition. Although both extracts showed lower activity than the standard ascorbic acid (~90%), the ethanolic extract demonstrated strong antioxidant potential. This superior activity can be attributed to the presence of phenolic compounds and flavonoids, which effectively neutralize free radicals by donating hydrogen atoms or electrons.

The protein denaturation assay demonstrated excellent anti-inflammatory activity for the ethanolic extract, with inhibition values ranging from 89% to 94%, which were comparable to the standard diclofenac sodium ($\approx 99\%$). In contrast, the n-hexane extract exhibited very low inhibition, reaching only 15% at 500 $\mu\text{g/mL}$. The marked anti-inflammatory activity of the ethanolic extract may be due to its rich content of flavonoids, tannins, saponins, and phenolic compounds, which are known to inhibit protein denaturation and inflammatory mediators.

Both extracts enhanced glucose uptake in a concentration-dependent manner. The ethanolic extract showed greater activity, increasing from 16% at 100 $\mu\text{g/mL}$ to 44% at 500 $\mu\text{g/mL}$, whereas the n-hexane extract increased from 5% to 35%. Although both extracts showed lower activity than metformin ($\approx 90\%$), the ethanolic extract demonstrated a moderate glucose uptake effect. The improved glucose uptake may be associated with the synergistic action of flavonoids, alkaloids, and phenolic compounds, which have been reported to improve glucose metabolism and insulin sensitivity.

Overall, the phytochemical profile strongly supports the biological activities observed in the three *in vitro* assays, indicating that the presence of multiple bioactive constituents in the ethanolic extract is responsible for its superior pharmacological potential.

CONCLUSION

The present investigation demonstrated that the ethanolic extract of *Mimosa pudica* stem exhibited superior phytochemical composition and biological activities compared to the n-hexane extract. Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, and glycosides in the ethanolic extract, whereas the n-hexane extract contained only terpenoids, steroids, and glycosides.

The DPPH antioxidant assay revealed strong free radical scavenging activity for the ethanolic extract, approaching that of the standard ascorbic acid. The protein denaturation assay demonstrated potent anti-inflammatory activity, with inhibition values comparable to diclofenac sodium. In the glucose uptake assay, the ethanolic extract exhibited moderate antidiabetic activity and consistently outperformed the n-hexane extract, although both extracts were less effective than metformin.

These findings indicate that the superior biological activities of the ethanolic extract are closely associated with its higher content of phenolic compounds, flavonoids, tannins, and other bioactive phytochemicals. Therefore, the ethanolic stem extract of *Mimosa pudica* may serve as a promising natural source of *invitro* antioxidant, anti-inflammatory, and antidiabetic agents.

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