



# Antioxidant And Antidiabetic Efficacy Of *Mimosa pudica*: A Phytochemical Perspective

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## Abstract

The medicinal properties of *Mimosa pudica*, a widely used plant in traditional medicine, were investigated through phytochemical screening, antioxidant, and antidiabetic assays. Antioxidant potential was evaluated using ABTS and hydrogen peroxide ( $H_2O_2$ ) scavenging assays. The methanol extract exhibited potent radical-scavenging activity with a low  $IC_{50}$  value, suggesting its capacity to neutralize reactive oxygen species (ROS), which are implicated in oxidative stress and related diseases such as cancer, cardiovascular diseases, and diabetes. Furthermore, the in vitro antidiabetic activity of *Mimosa pudica* was assessed by measuring the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. The methanol extract showed strong inhibition of both enzymes, indicating its potential in managing postprandial blood glucose levels, a key factor in diabetes management.

The findings highlight *Mimosa pudica* as a promising natural source of antioxidants and antidiabetic agents. Its rich phytochemical profile, particularly the high flavonoid and phenol content, underpins its therapeutic potential. Statistical analysis confirmed that methanol extracts were more bioactive compared to hexane extracts. 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. Further research is needed to isolate and characterize the active compounds responsible for these bioactivities and to explore their mechanisms of action.

**Keywords:** *Bioactive compounds,  $\alpha$ -Amylase inhibition assay, Phenolic compounds, Antidiabetic activity.*

## Introduction

*Mimosa pudica*, commonly known as the sensitive plant, sleepy plant, action plant, touch-me-not, or shame plant, is a creeping plant from the Fabaceae (pea or legume) family. It is well-known for its unique response to touch: the compound leaves fold inward and droop when disturbed, only to reopen a few minutes later, which makes it popular for its curiosity value. [1] In its early stages, *M. pudica* grows with an erect stem, but as it matures, it tends to sprawl, often becoming floppy and adopting a creeping or trailing form. The slender stems are branched and may vary from sparsely to densely prickly, growing up to 1.5 meters (5 feet) in length, while its typical upright height is around 30 cm (1 foot). [2]

The plant's leaves are bipinnate, each with 1–2 pairs of pinnae and 10–26 leaflets per pinna. Like the stems, the petioles have prickles. In mid-summer, *M. pudica* produces pale pink or purple flower heads from the leaf axils, with blooms becoming more abundant as the plant ages. Each flower lasts less than a day, typically wilting by the next. [3] The delicate, spherical flower heads measure about 8–10 mm (0.3–0.4 in) in diameter, not including the stamens. [4] The florets are red at the top, and the filaments range in color from pink to lavender, while the pollen grains are round and approximately 8 microns in diameter.

*M. pudica* belongs to the Mimosoideae subfamily, which includes around 400 species of shrubs and herbs mainly found in Africa, North America, and Asia. It also holds a prominent place in Greco-Arab, Ayurvedic, and Chinese medicine for its wide range of therapeutic applications. [5]

The medicinal benefits of plants often stem from the diverse array of secondary metabolites they produce. Each plant species or taxonomic group has a unique combination of these compounds, contributing to its specific therapeutic properties. [6] In recent decades, there has been a growing interest in developing antioxidant-based treatments to prevent and manage complex diseases, including atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer. This has led to an increased focus on natural sources of antioxidants, found in foods such as berries, tea, herbs, oilseeds, beans, fruits, and vegetables.

Numerous herbs and spices—such as rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several traditional Chinese medicinal plants—have demonstrated antioxidant activity. The primary bioactive compounds in these plants are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. Alongside these, vitamins C and E and beta-carotene are also recognized for their antioxidant properties. [7]

The role of reactive oxygen species (ROS) in health and disease has received increasing attention in recent years. Reactive oxygen species include both free radicals, such as superoxide anions ( $O_2^-$ ) and hydroxyl radicals ( $OH^-$ ), and non-radical species like hydrogen peroxide ( $H_2O_2$ ) and singlet oxygen ( $O_2^*$ ). [8] Many studies now highlight plants as a potent source of natural antioxidants, offering promising solutions for combating oxidative stress and related diseases.

## MATERIALS AND METHODS

### 1.1 Collection of Plant Materials

The fresh plant sample of *Mimosa pudica* were collected randomly from the yercaud, Salem, Tamil Nadu. Sample materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

### 1.2 PREPARATION OF EXTRACT

Crude sample extract was prepared by Soxhlet extraction method. About 20 gm of powdered material was uniformly packed into a thimble and extracted with 250 ml of hexane and methanol extract separately. The process of extraction must be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that, the extract was taken in a beaker and kept on hot plate and heated at 30–40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C till future use.

### 1.3 PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis was carried out for all the methanol and hexane extracts of *Mimosa pudica* as per standard methods [9]

#### 1.3.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

**Mayer's test:** Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

#### 1.3.2 Detection of Flavonoids

**H<sub>2</sub>SO<sub>4</sub> test:** Extracts were treated with few drops of H<sub>2</sub>SO<sub>4</sub>. Formation of orange colour indicates the presence of flavonoids.

#### 1.3.3 Detection of Steroids

**Liebermann- Burchard test:** 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H<sub>2</sub>SO<sub>4</sub>. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

#### 1.3.4 Detection of Terpenoids

**Salkowski's test:** 0.2g of the extract of the whole sample was mixed with 2ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> (3ml) was carefully added to form a layer. A reddish- brown coloration of the inner face was indicating the presence of terpenoids.

#### 1.3.5 Detection of Anthroquinones

**Borntrager's test:** About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl<sub>3</sub> was added to the filtrate. Few drops of 10% NH<sub>3</sub> were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

#### 1.3.6 Detection of Phenols

**Ferric chloride test:** Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

#### 1.3.7 Detection of Saponins

**Froth test:** About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

#### 1.3.8 Detection of Tannins

**Ferric chloride test:** A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered, and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

### 1.3.9 Detection of Carbohydrates

**Fehling's test:** 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

### 1.3.10 Detection of Oils and Resins

**Spot test:** Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

## 1.1 Quantitative Phytochemical Analysis

### 1.1.1 Estimation of Flavonoids

5ml of *Mimosa pudica* Methanolic extract sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid content was calculated by difference in weight [10]

### 1.1.2 Determination of Total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The *Mimosa pudica* methanolic extract samples were made up to mark and left to react for 30 min for color development. This was measured at 505nm [11]

## 1.2 In Vitro Antioxidant activity

### 1.2.1 ABTS radical scavenging activity

ABTS radical-scavenging activity of the extract was determined according to [12]. The ABTS +cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ( $K_2S_2O_8$ ) solution, stored in the dark at room temperature for 16 hrs. Before use, this solution was diluted with ethanol to get an absorbance of  $0.700 \pm 0.020$  at 734 nm. The plant extract at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950  $\mu$ l of ABTS.+ solution and 50  $\mu$ l of BHT. As for the antiradical activity, ABTS scavenging ability was expressed as  $IC_{50}$  ( $\mu$ g/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where  $A_0$  is the absorbance of the control, and  $A_1$  is the absorbance of the sample.

### 1.2.2 Hydrogen peroxide scavenging activity

The scavenging activity of extract towards Hydrogen peroxide radicals was determined by the method described by [13]. Solution of hydrogen peroxide (40mM) was prepared in phosphate buffer pH 7.4. Concentration of hydrogen peroxide was determined by measuring the absorption at 230nm using a spectrophotometer. Extract 0.1mg/ml in distilled water were added to hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula.

$\% IC_{50} = [(Absorbance\ of\ Sample - Absorbance\ of\ control) / Absorbance\ of\ control] \times 100$

Where, Abs control was the absorbance of the control (without extract) at 560 nm; Abs sample was the absorbance in the presence of the extract at 560 nm. The experiment was repeated in triplicate.

### 1.3 *In Vitro* Anti-Diabetic Activity

#### 1.3.1 Determination of $\alpha$ -Amylase Inhibitory Activity Reagents

1. Acetate buffer (0.1 M) - 820.3mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water.
2. Iodine-iodide indicator - 635mg Iodine and 1gm potassium iodide in 250ml distilled water.

Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer.

#### Procedure

In alpha amylase inhibition method 1ml substrate (potato starch (1% w/v)), 1ml of drug solution different concentrations were added to (such as 250, 500, 750 and 1000 $\mu$ g/ml) 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) were added. The above mixture was incubated for 1 hr. Then 0.1ml Iodine-iodide indicator was added in the mixture. Absorbance was taken at 565nm in UV-Visible spectroscopy [14]

$$\text{Inhibition of alpha- Amylase (\%)} = (Abs_{\text{sample}} - Abs_{\text{control}} * 100) / Abs_{\text{sample}}$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

#### Calculation of 50% Inhibitory Concentration ( $IC_{50}$ )

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions.

Percentage inhibition (I%) was calculated using the formula,  $I\% = (As - Ac) / Ac * 100$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

#### 1.3.2 *In Vitro* $\alpha$ -Glucosidase Inhibition Assay

The *in vitro*  $\alpha$ -glucosidase inhibitory effect of the crude extract was evaluated by using p-nitrophenyl-a-D glucopyranoside (p-NPG) substrate solution (using 0.1 M potassium phosphate buffer, pH 6.8). Similarly, 0.1 unit/ml of alpha-glucosidase was dissolved using potassium phosphate buffer. All the samples were dissolved in dimethyl sulfoxide, and 20 ml of each sample was mixed with the same volume of enzyme solution. Subsequently, a volume of 40 ml substrate solution was added for initiation of the reaction and incubated at 37°C for 40 minutes. Then, 80 ml of 0.2 M sodium carbonate in phosphate buffer, pH 6.8 was added to terminate the reaction. Finally, the amount of released p- nitrophenol (pNP) was measured at 405 nm. The  $\alpha$ -glucosidase inhibitory activity was expressed as percent inhibition and determined as follows. The  $IC_{50}$  values of the crude extract, solvent fractions, and acarbose were calculated from the dose-response curve through interpolation from the linear regression analysis.

**Statistical Analysis-** All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Turkey's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at  $p \leq 0.05$ .

## Results and Discussion

### 5.1 Yield Obtained

Yields from *Mimosa pudica* were extracted using methanol and hexane as solvents. Methanol extraction produced a 40% higher yield compared to hexane extraction. Due to the higher yield, methanol extract was chosen for further analyses. (Table 1). The yield percentage is calculated by comparing the mass of the extract obtained to the mass of the original plant material.

$$Yield(\%) = \frac{Mass\ of\ Extract}{Mass\ of\ Plant\ Material} \times 100$$

| Extract  | Yield (%) |
|----------|-----------|
| Methanol | 23.54     |
| Hexane   | 11.23     |

**Table 1:** Comparison of yield obtained in methanol and hexane extracts of *Mimosa pudica*.

### 5.2 Phytochemical Analysis

Phytochemical screening was conducted qualitatively on both methanol and hexane extracts of *Mimosa pudica*. The methanol extract exhibited a broader range of bioactive compounds, including alkaloids, flavonoids, steroids, terpenoids, phenols, saponins, tannins, and carbohydrates. In contrast, hexane extract displayed fewer phytochemicals, specifically flavonoids, steroids, terpenoids, carbohydrates, and oils/resins.

The presence of these phytochemicals has therapeutic implications, as they are known to contribute to various medicinal effects such as antioxidant, antidiabetic, and anticancer properties. The presence of flavonoids and phenols, explained in table 2, in particular, is noteworthy, as these are known for their strong antioxidant activity, which plays a key role in mitigating oxidative stress and preventing cellular damage caused by reactive oxygen species (ROS). Other secondary metabolites such as alkaloids and saponins are linked to antimicrobial, anti-inflammatory, and analgesic effects, suggesting that *Mimosa pudica* may possess a broad spectrum of therapeutic potential. [15]

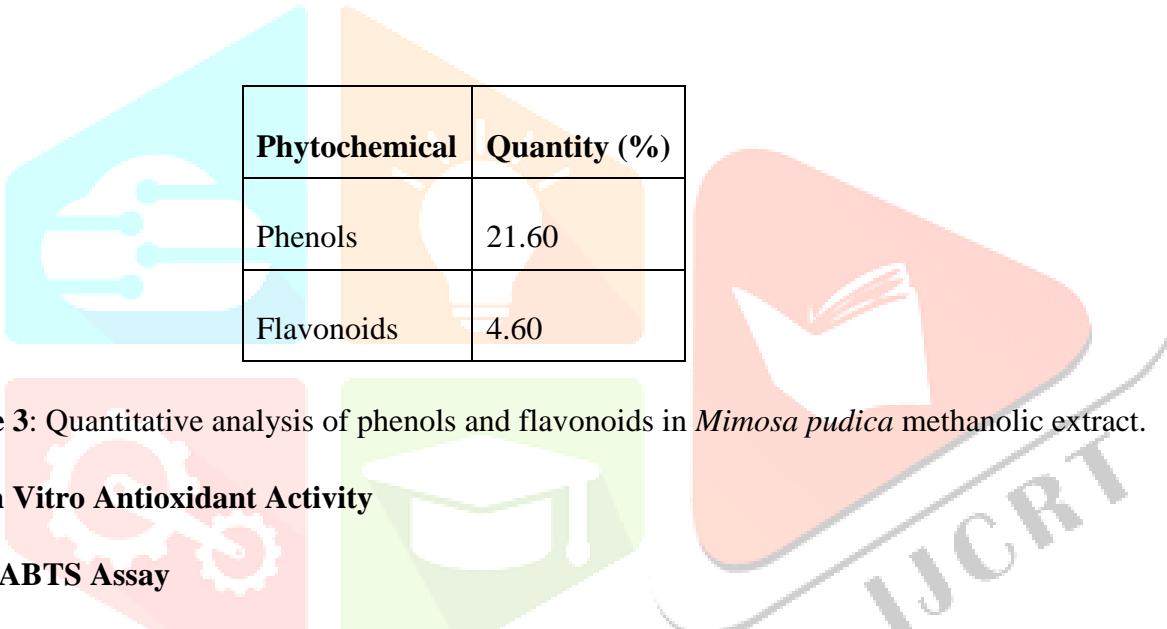
| Phytochemical | Test                                | Observation                      | Methanol Extract | Hexane Extract |
|---------------|-------------------------------------|----------------------------------|------------------|----------------|
| Alkaloids     | Mayer's test                        | Cream color                      | +                | -              |
| Flavonoids    | H <sub>2</sub> SO <sub>4</sub> test | Reddish brown/orange precipitate | +                | +              |
| Steroids      | Liebermann-Burchard                 | Violet/blue/green color          | +                | +              |
| Terpenoids    | Salkowski                           | Reddish-brown precipitate        | +                | +              |
| Phenols       | Ferric chloride                     | Deep blue/black color            | +                | -              |
| Saponin       | Stable persistent                   | +                                | -                |                |

|               |                           |     |   |   |
|---------------|---------------------------|-----|---|---|
| Tannin        | Brownish green/blue black | +   | - |   |
| Carbohydrates | Color variation           | +   | + |   |
| Oils & Resins | Filter paper method       | N/A | - | + |

**Tables 2:** Phytochemical analysis for methanol and hexane extracts of *Mimosa pudica*.

### 5.3 Quantitative Phytochemical Analysis

Quantitative analysis of the methanol extract showed a significantly higher content of flavonoids and phenolic compounds compared to the hexane extract. The flavonoid content was determined by extraction with aqueous methanol, and the phenolic content was assessed using a colorimetric method, illustrated in Table 3. Quantitative assessment of the methanol extract revealed the percentage composition of key phytochemicals.[16]



| Phytochemical | Quantity (%) |
|---------------|--------------|
| Phenols       | 21.60        |
| Flavonoids    | 4.60         |

**Table 3:** Quantitative analysis of phenols and flavonoids in *Mimosa pudica* methanolic extract.

### 5.4 In Vitro Antioxidant Activity

#### 5.4.1 ABTS Assay

The antioxidant activity of both hexane and methanol extracts was evaluated using the ABTS radical scavenging assay and hydrogen peroxide scavenging assay. In the ABTS assay, the methanol extract exhibited a marked scavenging ability, with an  $IC_{50}$  value lower than the hexane extract, indicating stronger radical-neutralizing properties. The  $H_2O_2$  scavenging activity showed similar trends, with the methanol extract demonstrating a more significant reduction in hydrogen peroxide levels. [12]. These results are indicative of the plant's strong ability to counteract oxidative stress, which is implicated in various chronic diseases such as cardiovascular diseases, neurodegenerative diseases, and cancer. The potent antioxidant capacity of *Mimosa pudica* suggests its potential as a natural source of antioxidants for therapeutic and preventive applications in oxidative stress-related diseases.[17] . The  $IC_{50}$  value obtained was 642.92  $\mu\text{g}/\text{ml}$ .

#### 5.4.2 Hydrogen Peroxide Assay

Hydrogen peroxide scavenging activity was measured, yielding an  $IC_{50}$  value of 621.30  $\mu\text{g}/\text{ml}$ .Increased concentration corresponded with greater scavenging ability, indicating the methanol extract's potential to protect against oxidative stress[3]

| Concentration (μl) | ABTS assay Inhibition (%) | Hydrogen peroxide Inhibition (%) |
|--------------------|---------------------------|----------------------------------|
| 50                 | 34.87                     | 32.62                            |
| 250                | 40.79                     | 41.13                            |
| 500                | 45.39                     | 47.52                            |
| 750                | 50.66                     | 53.90                            |
| 1000               | 54.61                     | 59.57                            |

**Table 4:** ABTS assay and Hydrogen peroxide scavenging assay results for *Mimosa pudica* methanol extract.

### 5.5 In Vitro Antidiabetic Activity

The antidiabetic potential of *Mimosa pudica* was assessed by examining its inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase, two key enzymes involved in carbohydrate digestion and glucose metabolism. Both extracts exhibited significant enzyme inhibition. The methanol extract showed a higher degree of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition compared to the hexane extract. This suggests that the methanol extract may be more effective in managing blood sugar levels by slowing down carbohydrate digestion and glucose absorption. [18] This is particularly important for controlling postprandial hyperglycemia in individuals with diabetes. The observed inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase point to the potential antidiabetic properties of *Mimosa pudica*, which could support its traditional use in managing diabetes and blood sugar regulation.[19]

#### 5.5.1 Alpha-Amylase Activity and Alpha-Glycosidase Activity

Alpha amylase is an enzyme that hydrolyses alpha bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. As a result, methanol extract of *Mimosa pudica* shows significant activity as compared to acarbose standard drug. At different concentrations, the % inhibition of alpha amylase activity revealed that even if the inhibition is almost similar in both acarbose standard and methanol extract of *Mimosa pudica* shows better activity. Because medicinal plants are used to treat numerous causes at the same time, they can regulate disease progression through multiple pathways. The *in vitro* antidiabetic activity is carried out for two parameters one is Alpha Amylase Inhibition Method and Alpha Glycosidase Inhibition Method. The IC<sub>50</sub> for Alpha Amylase Inhibition Method (610.53  $\mu$ g/ml) and Alpha Glycosidase Inhibition Method (688.10  $\mu$ g/ml) illustrated in table 5. Methanol extract showed a high inhibitory effect in both assays, comparable to the antidiabetic drug acarbose, but with the advantage of potentially fewer side effects. These findings suggest that *Mimosa pudica* methanol extract could serve as a complementary antidiabetic agent.

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**Table 5** Alpha amylase and alpha glycosidase activity of *Mimosa pudica* Methanolic extract and Hexane extract

| Sl. No | Concentration (μl) | alpha amylase activity   |  | Alpha Glycosidase activity  |  |
|--------|--------------------|--|--|---|--|
|        |                    | <i>Mimosa pudica</i> Methanolic extract - (alpha amylase activity) | <i>Mimosa pudica</i> Hexane extract - (alpha amylase activity) | <i>Mimosa pudica</i> Methane Extract - (Alpha Glycosidase activity) | <i>Mimosa pudica</i> Hexane extract - (Alpha Glycosidase activity) |
| 1      | 50                 | 34.87  | 32.62  | 35.97   | 33.5   |
| 2      | 250                | 40.79  | 41.13  | 40.29   | 42.2   |
| 3      | 500                | 45.39  | 47.52  | 45.32   | 47.3   |
| 4      | 750                | 50.66  | 53.9   | 51.8  | 55.2   |
| 5      | 1000               | 54.61  | 59.57  | 56.83   | 58.4   |

**Graph 1:** Alpha amylase and alpha glycosidase activity of *Mimosa pudica* Methanolic extract and Hexane extract

