ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytochemical Screening And Biological Activities Of *Mimosa pudica* L.

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ABSTRACT

The objective of this study is to investigate phytochemical constituents, antioxidant, antimicrobial, antiplatelet aggregation activities and enzyme inhibitory studies from medicinal plant *Mimosa PudicaL*. The qualitative and quantitative phytochemical screening was performed for the two extracts and it showed the presence of carbohydrates, phenols, alkaloids, flavonoids, and glycosides. The antioxidant property was evaluated by DPPH activity, reducing power assay and total antioxidant activity, and was shown that methanol extract of *Mimosa Pudica L*. showed more antioxidant property which has IC_{50} value 240.96µg/ml compared to the petroleum ether extract of *Mimosa Pudica L*. Which has the IC_{50} value of 487.5µg/ml. The antimicrobial activity was performed using the Macro-dilution method to identify the MIC and MBC of the extract against *E. coli* and *Aspergillus niger.* Antiplatelet aggregation activity, anti-inflammatory activity and Enzyme inhibition was performed spectrophotometrically. *Lipoxygenase* enzyme was extracted from Soybean seeds using n-hexane. Enzyme inhibition was performed. The methanol extract showed the highest enzyme inhibition activity among all the other extracts.

Keywords: *Mimosa Pudica*, Antioxidant, Antiplatelet aggregation, lipoxygenase inhibition.

https://doi.org/10.33887/rjpbcs/2025.16.2.6

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ISSN: 0975-8585

INTRODUCTION

Ever since ancient times, in search of rescue for disease, people have looked for drugs in nature. Medicinal plants continue to be an important therapeutic aid for the alleviating ailments of humankind, which are rich sources of different types of medicines and produce various bioactive molecules. They are rich in secondary metabolites which are chemically and taxonomically extremely diverse compounds with obscure function. Many phytochemicals are widely used in human therapy, agriculture, veterinary and scientific research. *Mimosa PudicaL*. is one such medicinal plant having numerous medicinal properties such as anti-oxidant, antimicrobial, anti-inflammatory etc. *Mimosa pudica L.* is a creeping annual or perennial herb. It has been identified as lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. *M. pudica* is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. *M. Pudica* also have anti-inflammatory properties by which they can block both the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism.

MATERIALS AND METHODS

Extraction of the plant material

The plant material was crushed using a blender and fine powder was collected. The fine powder was extracted using Soxhlet Apparatus using methanol and petroleum ether as solvents [1].

Phytochemical Screening

Qualitative Analysis

Tests for proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids were performed as per the standard protocol.

Quantitative Analysis

Estimation of total phenolic contents

The amount of total phenol for aqueous, methanol, ethanol and chloroform extract were determined by the Folin-Ciocalteu reagent method. 2.5 ml of 10% Folin- Ciocalteu reagent and 2 ml of 2% Na_2CO_3 were added to 0.5 ml of plant extract. The mixture was then incubated at room temperature for 30 minutes. gallic acid was used as standard (1mg/ml). The absorbance of the sample was measured at 765nm [2][3]. All the tests were done in triplicates and the results were determined from standard curve and were expressed as gallic acid (trihydroxy benzoic acid) equivalent (mg/g of extracted compound).

Estimation of carbohydrates using Anthrone method

The amount of carbohydrates was estimated using the Anthrone method using glucose as standard. 5ml of anthrone reagent was added to the sample and it was incubated at 90ºC for 5 minutes. The absorbance was read at 620nm [4]. Standard glucose was used with the concentration of 0.2mg/ml.

Estimation of alkaloids by Harborne method

5 g of the sample was taken and 200 ml of 10% acetic acid in ethanol was added to the sample and allowed to stand for 4 hours. Then the solution was filtered and the extract was concentrated on water bath conc. ammonium hydroxide was added drop wise and the whole solution was allowed to settle and the precipitate was then washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed and this was the amount of alkaloid present in the plant material [5].

Estimation of Flavonoids using Aluminium Chloride method

Extract was separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the

absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of rutin (200-1000µg) plotted by using the same procedure and total flavonoids were expressed Rutin equivalents (RE) in mg/gm. sample.[6]

Antioxidant Activity

DPPH Radical Scavenging Activity

Different concentrations of sample(100-500µg/ml) were taken and the volume was made up to 1ml using methanol. To these aliquots 1ml of 0.1mM DPPH was added. The tubes were kept for incubation for 30mins in the dark. The absorbance was read at 517nm after 30minutes. gallic acid was used as the standard curve (1mg/ml).[7] From the absorbance the inhibition percentage was calculated. The formula used was:

$$A_0 - A_S / A_0 \times 100,$$

where A_0 is control and A_S is absorbance of sample.

Reducing Power Assay

Different concentration of samples(100-500µg/ml) were taken in the test tube. To these tubes 1ml of phosphate buffer was added and 1ml of potassium ferricyanide was added. This was then kept for incubation at 50ºC for 20 minutes. After 20 mins, 1ml of trichloro acetic acid was added to all the tubes. To these tubes 0.5ml of distilled water and 0.5ml of ferric chloride was added. The absorbance was measured at 700nm. gallic acid was used as the standard to measure the activity.

$$A_0 - A_S / A_0 \times 100,$$

where A_0 is control and A_S is absorbance of sample.

Total Antioxidant Activity

Firstly, phosphomolybdenum reagent was prepared by mixing 28mM sodium phosphate, 4mM ammonium phosphate and 0.6M sulphuric acid. Aliquots of samples were taken in the test tube. To all the tubes 1ml of the reaction mixture was added. The tubes were closed and it was incubated at 95ºC for 90minutes. The absorbance was read at 695nm.

Antimicrobial Activity

Macro-dilution method

Minimum Inhibition Concentration

Primary inoculum was prepared by using laboratory strain of *E. coli* and *Aspergillus niger*that was collected from the Department of Microbiology, The Oxford College of Science. The inoculum was cultivated using Muller Hilton broth. The culture was kept in a rotary shaker incubator. After 24 hours of incubation, the inoculum was taken and it was poured in different tubes. To this different concentration of sample(200-800µg/ml) was added and kept in the incubator. After 24 hours of incubation the turbidity was checked for the *E. coli* strain and after 48 hours of incubation the turbidity was checked for the *Aspergillus* strain. Based on the turbidity the minimum inhibition concentration is studied [8].

Minimum Bactericidal Concentration

The Primary inoculum is taken after 24 hours incubation and it is poured in different tubes. To these tubes various concentrations of the samples(200-800µg/ml)were added. Muller Hilton agar plates are prepared. The tubes are then inoculated in the plates using an inoculum loop. The plates are sealed and kept in the incubator. After 24 hours and 48 hours of incubation, growth colonies were checked to identify the MBC. The standard used was hydrogen peroxide.

Antiplatelet Aggregation Activity

Blood was collected from the healthy donors in the tubes containing the 3% tri-sodium citrate. Blood was centrifuged at 3000 rpm for 10min to obtain the platelet rich plasma (PRP). This platelet rich plasma was again centrifuged at 5000rpm for 15min to obtain the platelet poor plasma (PPP). The PRP and PPP are separated, where PPP is used to adjust the cell count. The PRP is then tested with different aliquots(100,300,500µg/ml)of our plant sample, where the 0.1% $CaCl_2$ is used as an aggregating agent. The tubes were incubated for 2-3min at 37^0C, and the absorbance is read in a colorimeter at 600nm [9]. The Antiplatelet aggregation activity was calculated by using the formula

$$\text{Antiplatelet activity (\%)} = A_c\text{-}A_t / A_c \text{ x}100$$

Where A_C % is aggregation of PRP control, A_T% is aggregation of PRP treated with extract.

The assay was performed using quadruplets, which was compared with the standard heparin.

Extraction of Enzyme

Lipoxygenase enzyme was extracted from Soybean seeds. The soybean seeds were grinded into fine powder. The powder was mixed with 3 times more volume of n-hexane. It was centrifuged at 10000 rpm for 30 minutes. The supernatant was collected and it was centrifuged again for 10000 rpm for 30mins. The cycle was repeated for one more time. The final supernatant was precipitated using polyethylene glycol. The upper layer was collected and was stored in cold condition. This was used as crude extract of enzyme.[10]

Inhibition of Lipoxygenase Enzyme

Firstly, the Enzyme activity was performed by using linoleic acid as substrate in presence of a borate buffer. The reaction mixture was kept for incubation at room temperature for 30 mins. The product (lipoxygenase hydroperoxides) is then taken in various volumesin-order to estimate its amount. The absorbance is read at 235nm using a spectrophotometer. In the reaction mixture, various concentrations of sample (100-500µg/ml)were added in-order to identify the inhibition percentage. The concentration of the lipoxygenase hydroperoxides were calculated using beer-lambert law. The absorbance was taken using Spectrophotometer.[11]

$$A_0 - A_S / A_0 \times 100,$$

where A_0 is control and A_S is absorbance of the sample.

RESULT AND DISCUSSION

Qualitative Analysis of Phytochemicals

The phytochemical screening was performed, in the methanol extract the presence of carbohydrates, phenols, flavonoids, glycosides, steroids and alkaloids was seen and it showed the absence of proteins, tannins, saponins, terpenoids. In the petroleum ether extract the presence of carbohydrates, phenols, tannins, flavonoids, glycosides, alkaloids were observed, it showed the absence of proteins, saponins, terpenoids.[12] The obtained results are shown in table 1.

Quantitative Analysis of Phytochemicals

Estimation of total Phenol Content

After the Qualitative Analysis, the extracts were subjected for quantitative analysis. For total phenolic content, the folin-colteau method was used.gallic acid was used as a standard.

Among the two extracts, the phenol content was highest in the methanol extract of *Mimosa Pudica L* with 242µg/ml. Whereas the petroleum ether extract showed 144µg/ml. [13]The results obtained for total phenolic contents are shown in table2.

Estimation of Carbohydrate using Anthrone Method

Carbohydrate estimation was performed using theanthrone method. D-Glucose was used as a standard to estimate the content of carbohydrates. Among the two extracts, it revealed that the methanol extract containedmore quantity of carbohydrates66µg/ml when compared to the petroleum ether extract 22µg/ml. The results for carbohydrate content are shown in table 3.

Estimation of Alkaloids using Harborne method

The extracts were subjected to the Harborne method, which is a dry weight method to identify the alkaloid percentage.

The total alkaloids present in the methanol extract is found to be 37% whereas in the petroleum ether extract it was found to be 24%. It revealed that the methanol extract has more alkaloid content when compared to the petroleum ether extract.

Estimation of Flavonoids using Aluminum Chloride method

The crude extracts were then subjected to quantify the amount of flavonoids. We carriedout the aluminum chloride method to quantify the flavonoids. The standard that we used was Rutin.[14]Among the two extracts, it revealed that the methanol extract containedmore quantity of flavonoids86µg/ml when compared to the petroleum ether extract66µg/ml.The obtained results are shown in table 4.

Antioxidant Activity

The Extracts were taken to investigate the antioxidant property, by using various methods. Firstly, DPPH scavenging activity was done to check the percentage of inhibition. All the extracts were taken in different concentrations(100-500µg/ml)to perform this activity. The standard used was gallic acid.

DPPH Free radical Scavenging Activity

The extracts were taken for DPPH free radical scavenging assay, the standard taken was gallic acid.

From the linear equation of the graph, The IC_{50}values were calculated and found to be:355.1µg/ml for standard gallic acid, 240.96µg/ml for methanol extract and 487.5µg/ml for petroleum ether extract. This showed that methanol extract has the more antioxidant property when compared to the standard gallic acid and petroleum ether extract.[15] That is the methanol extract of *Mimosa pudica L*. has more ability of reducing the DPPH, in which the methanol extract contains more antioxidants.The results are shown in the table 5 and graph (Figure 1).

Reducing Power Assay

The extracts were further taken for reducing power assay using ferric chloride method. The standard that was taken was gallic acid.

From the linear equation of the graph, the IC_{50} values were calculated and found to be 570µg/ml for standard gallic acidand 512µg/mlpetroleum ether extract, 204.57µg/ml for methanol extract. From the obtained results it is revealed that the methanol extract has more reduction potential compared to the standard gallic acid and petroleum ether extract.The results are shown in the table 6and graph (Figure 2).

Total Antioxidant Property

The total antioxidant activity is the measure of the amount of free radicals scavenged by the test solution, being used to evaluate the antioxidant capacity of the biological sample. The total antioxidant activity of the methanol extract was found to be 84.4µg/ml and petroleum ether extract was found to be 54.4µg/ml when compared with standard ascorbic acid. Hence it shows that the methanol extract has more antioxidant activity compared to the petroleum ether extract.

Anti-microbial Activity

The extracts were then subjected to anti-microbial activity. The Minimum Inhibitory Concentration and Minimum Bactericidal concentration both were performed using the test organism *E. coli* and *Aspergillus niger*[17].

Minimum Inhibitory Concentration

The Minimum Inhibitory concentration against the test organism *E. coli* was found to be 300µg/ml for methanol extract and 500µg/ml for petroleum ether extract. The Minimum inhibitory concentration against the test organism *Aspergillus Niger* was found to be 500µg/ml for methanol extract and 300µg/ml for petroleum ether extract. When compared with standard, the minimum inhibitory concentration was seen to be 200(µg/ml) which was more efficient than the *M. Pudica* extracts.[18]The results are seen in Table 7.

Minimum Bactericidal Concentration

The tubes that were incubated for MIC were inoculated onto the Muller Hinton Agar plates. It was observed that 600µg/ml of methanol sample and 800 µg/ml of petroleum ether sample showed the complete inhibition of E. coli growth.

It was observed that 800 µg/ml of both methanol and petroleum ether samples showed the complete inhibition of *Aspergillus niger* growth.

Antiplatelet aggregation activity:

The methanol extract of*Mimosa Pudica L*. was tested for the aggregation activity, which was compared with the standard drug heparin. The test was carried out in quadruplets.

The samples were taken in quadruplets and were compared with the standard heparin for the antiplatelet aggregation, in which our plant sample showed almost the similar activity compared to the standard drug. This showed that our plant sample has the property to inhibit platelet aggregation [19].The results obtained are displayed in the graph (Fig 3) and Table 8.

Enzyme Inhibition:

Enzyme inhibition was performed for methanolextract of the plant sample. Enzyme activity was performed by using lipoxygenase along with linoleic acid as substrate, in the presence of a borate buffer. The absorbance was read at 234nmusing a spectrophotometer. To the enzyme reaction mixture, various concentrations of the sample were added and then the inhibition percentage was calculated.The results are seen in Table 9 and graph (Fig4).

The IC_{50} value for the methanol extract was found to be 150µg/ml and for the standard drug sodium diclofenac was found to be 50µg/ml. Our plant sample showed very less inhibition when compared to the standard drug. From the above IC_{50} values it is seen that methanolic extract has very less inhibition activity compared to the diclofenac, where more of our plant sample is needed to inhibit the enzyme Lipoxygenase.

ISSN: 0975-8585

Sl.no	Bioactive constituents	Methanol extract	Petroleum ether extract
1	Proteins	-	-
2	Carbohydrates	+	+
3	Phenol	+	+
4	Tannins	-	+
5	Flavonoids	+	+
6	Saponins	-	-
7	Glycosides	+	+
8	Steroid	+	-
9	Terpenoids	-	-
10	Alkaloids	+	+

Table 1: Phytochemical screening of *Mimosa Pudica L.*

Estimation of total phenol content

Sl. no	Extracts	Absorbance at 725nm	Concentration (µg/ml)
1	Methanol	0.72	242
2	Petroleum ether	0.44	144

Table 2: Total Phenolic content of *Mimosa Pudica L.*

Estimation of carbohydrate using anthrone method

Sl.no	Extracts	Absorbance at 620nm	Concentration (µg/ml)
1	Methanol	0.32	66
2	Petroleum ether	0.11	22

Table 3: Carbohydrate content of *Mimosa Pudica L*

Estimation of Flavonoids using aluminum chloride method

Sl.no	Extracts	Absorbance at 620nm	Concentration (µg/ml)
1	Methanol	0.070	86
2	Petroleum ether	0.060	66

Table 4: Total Flavonoid content of *Mimosa Pudica L*

Antioxidant Activity

Sl no.	Concentration (µg)	Methanol extract % inhibition	Petroleum ether % inhibition	Standard
1	100	42.45	38.75	66.7
2	200	48.5	40	76.6
3	300	53.75	43.45	81.4
4	400	58.25	46.5	84.8
5	500	63.75	51.75	86.2

Table 5: DPPH free radical scavenging assay for *Mimosa pudica L*

ISSN: 0975-8585

Sl.no	Concentration(µg)	Methanol extract % inhibition	Petroleum ether % inhibition	Standard % inhibition
1	100	57.3	76	97.22
2	200	50	74.6	94.4
3	300	44	73.33	90.27
4	400	36	70	84.72
5	500	29.3	68	83.33

Table 6: FRAP free radical scavenging assay for *Mimosa pudica L*

Minimum Inhibitory Concentration

Concentration (µg/ml)	200	400	600	800	Positive control	Negative control
E. coli	+	-	-	-	+	-
	+	+	-	-	+	-
Aspergillus niger	+	+	-	-	+	-
	+	-	-	-	+	-

Table 7: Minimum Inhibition Concentration of *Mimosa Pudica L.*

Antiplatelet aggregation activity

Sl.no	Concentration (µg)	Standard heparin (%)	Sample A (%)	Sample B (%)	Sample C (%)	Sample D (%)
1	100	84.05	76.81	75.35	73.91	75.35
2	300	86.95	84.05	82.60	85.50	89.05
3	500	92.75	91.30	88.40	89.85	91.30

Table 8: Percentage of Antiplatelet activity

Enzyme Inhibition

Sl.no	Concentration of sample (µg)	Absorbance At 480 nm	methanol enzyme inhibition %	Standard enzyme inhibition %
1	100	0.42	48.14	89
2	200	0.39	51.85	89.5
3	300	0.36	55.55	91
4	400	0.33	59.25	92
5	500	0.30	62.96	94

Table 9: Percentage of enzyme inhibition

DPPH radical scavenging activity

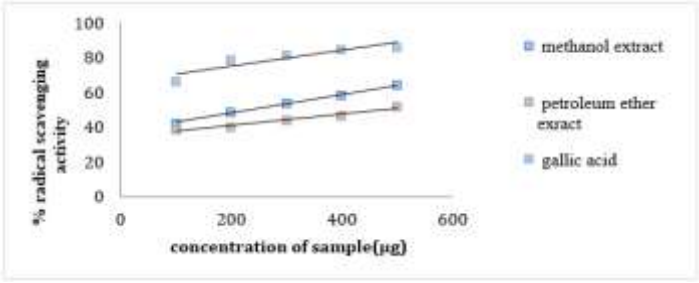

Figure 1: DPPH activity of *Mimosa Pudica L.*

ISSN: 0975-8585

Reducing Power Assay:

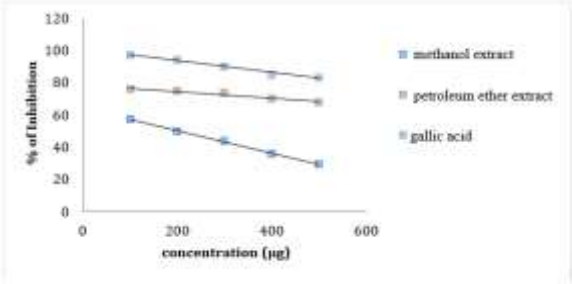

Figure 2: FRAP activity of *Mimosa Pudica L.*

Antiplatelet Aggregation Activity

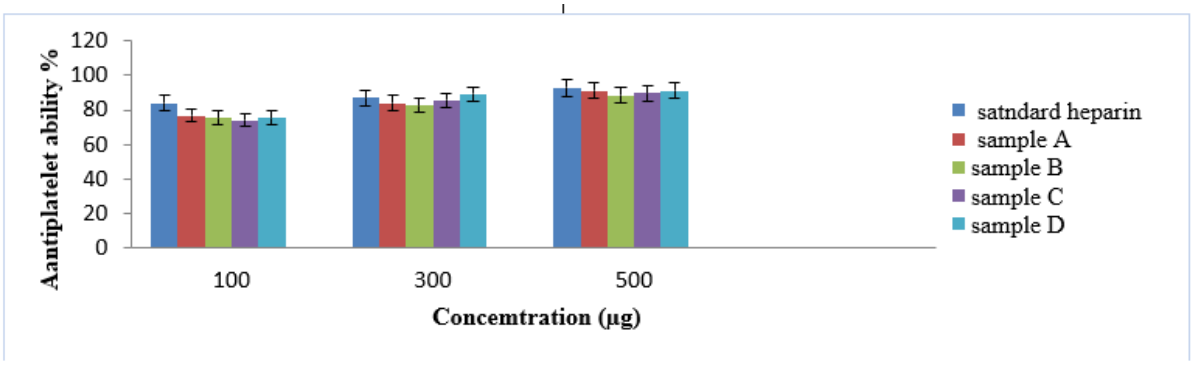

Fig 3: Percentage Inhibition of Antiplatelet Aggregation

Inhibition of Lipoxygenase Enzyme

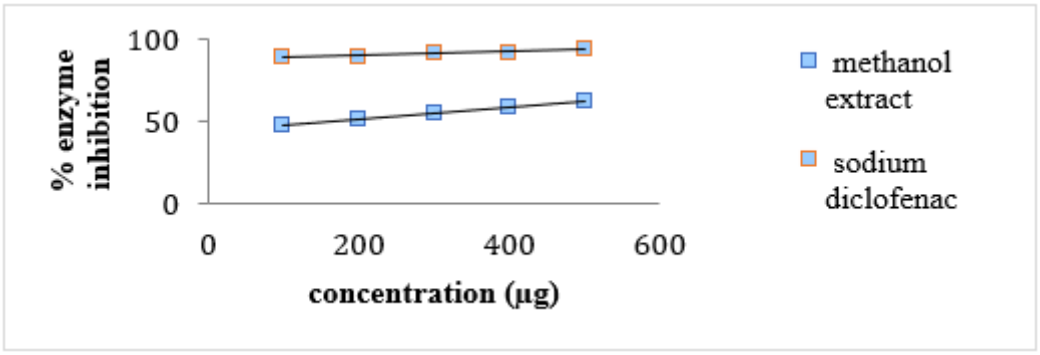

Figure 4: Inhibition of Lipoxygenase enzyme by *Mimosa Pudica L*

CONCLUSION

The results revealed the presence of medicinally important constituents in the plant studied. Many evidence gathered in the earlier studies which confirmed the identified Phytochemicals to be bioactive. Several studies confirmed the presence of these Phytochemicals contributing medicinal as well as physiological properties to the plant studied in the treatment of different ailments. Therefore, extracts of the dry powder of *Mimosa pudica L.* plant could be seen as a good source for useful drugs. This study was designed to investigate the phytochemical characterization and evaluate the in vitro antioxidant activity of *M Pudica L.* which was used as anti-ulcer, anti-inflammatory in Ayurveda remedies. To conclude, the above experimental studies clearly indicate that different extract of powder of *M Pudica L.* showed effective free radical scavenging activity which can be attributed to the presence of flavonoids

and phenolics along with other compounds. phenolic compounds, flavonoids and other secondary metabolites were detected in two different extracts. Methanolic extract showed the powerful result of containing the phenolics, flavonoids, DPPH free radical scavenging assay, antiplatelet aggregation activity and enzyme inhibition of Lipoxygenase.

The extracts showed the effective antimicrobial activity in Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC).This plant does have an antiplatelet aggregation activity, the methanol extract of this plant showed similar activity to the standard Heparin. The enzyme inhibition study of lipoxygenase was done, the methanol extract showed better inhibition. This confirmed that the plant sample can be used as an anti-inflammatory agent.

The traditional medicine practice is recommended strongly for this plant as well as suggested that further work should be carried out to isolate, purify and characterize the active constituents that are responsible for the pharmacological activities in this plant. Also, additional work is encouraged to elucidate the possible mechanism of action of these extracts. Further investigations on the different extracts of the *M Pudica L.* on the antiplatelet aggregation activity will be performed.

ACKNOWLEDGMENT

The Authors of this study are thankful for The Oxford College of Science for providing the laboratory facilities in order to complete the research study.

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