

Antihypertensive and Cardioprotective Property of *Mimosa pudica* Using Zebra Fish

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ABSTRACT

Background: Increase in blood pressure is generally termed as hypertension which is one of the common causes of cardiovascular diseases. Despite the several treatment methods, hypertension stands to be the major concern in developing countries. Angiotensin converting enzyme is one of the key contributors in regulating blood pressure where improper activation of angiotensin leads to hypertension. *Mimosa pudica* plant has been reported to exhibit several therapeutic properties and thus the current study was focused on evaluating antihypertensive property of phytochemicals found in the *Mimosa pudica* extract. **Materials and Methods:** Methanol extract of *Mimosa pudica* was examined for presence of potential phytochemicals by GC-MS and molecular docking studies were carried out using Autodock 4.2. **Results:** 07 phytochemicals were identified in the methanol extract of *Mimosa pudica*. Further molecular docking studies against angiotensin converting enzyme (ACE) was carried out and among the list of test compounds 9,12-octadecadienoic acid showed least binding energy with -6.6 kJ. **Conclusion:** Compounds selected as phytochemicals of *Mimosa pudica* from methanol extract of *Mimosa pudica*, specially 9,12-octadecadienoic acid considered for further evaluation of antihypertensive and cardioprotective activity.

Keywords: Angiotensin converting enzyme, *Mimosa pudica*, Methanol extract, Anti-hypertensive activity, Antihypertensive and cardioprotective activity.

INTRODUCTION

Hypertension is a growing concern in developing countries, because of unhealthy life style, and food habits. As reported by WHO, hypertension is recognized as one of the key risk factors for morbidity and mortality worldwide causing about one million deaths annually in all over the world (WHO, 2013). Clinically hypertension is categorized as High blood pressure (BP). Blood pressure is the force exerted due to blood flow against the walls of blood vessels, the intensity of force relies on cardiac output and resistance of blood vessels.^[1] Increase in blood pressure is associated with several risk factors mainly involving cardiovascular diseases which contribute in increased mortality rates.^[2] Among several conditions that impact blood pressure, angiotensin converting enzyme (ACE) system is reported to be the major contributor. Inappropriate activation of angiotensin in stenosis is therefore reported to be major cause of hypertension and cardiovascular morbidity.^[3] Individuals suffering with hypertension are reported with lesser signs of RAS activation; thus indicating that dysregulation of RAS contributes in elevated blood pressure. Therefore angiotensin catalyzing inhibitors and angiotensin receptor blockers are effectively capable of reducing blood pressure.^[4] Many of such Angiotensin converting enzyme (ACE) inhibitors are

currently used for treating primary hypertension. ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II, while renin blocks enzymatic action of renin, the conversion of angiotensinogen to angiotensin I.^[5]

Mimosa pudica L. is a perennial herb that belongs to *Mimosaceae* family. Phytochemical compounds isolated from *M. pudica* that are reported include terpenoids, tannins, alkaloids, sterols, and fatty acids.^[6-7] Medicinal properties of *Mimosa pudica* reported so far mainly include antidepressant, anticonvulsant, hyperglycaemic and anti-implantation activities.^[8-9]

Zebra fish (*Danio rerio*) is considered to be a potential model organism to study vertebrate biology both suited for genetic and developmental analysis.^[10] Therefore, Zebra fish was used in analyzing ACE inhibition that might be caused by phytochemicals of *Mimosa pudica* plant extract. Zebrafish are small, healthy and less expensive to maintain. An interruption of daylight triggers mating in Zebrafish. Zebrafish produces hundreds of offspring at weekly intervals providing scientists with an ample supply of embryos for the study. The approximate generation time for *Danio rerio* is three months. A male must be present for ovulation

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and spawning to occur. Females are able to spawn at intervals of two to three days, laying hundreds of eggs in each clutch. Fertilized eggs almost immediately become transparent, a characteristic that makes *D. rerio* a convenient research model.^[11] Thus in the current investigation, *M. pudica* plant extract was screened for phytochemical profile by GC-MS analysis, followed by evaluation of anti-angiotensin activity in *D. rerio* was carried out to prove the cardioprotective property of *M. pudica* extract by *in-vitro* approach.

MATERIALS AND METHODS

Plant Material Collection

Plant material was collected from vicinity of Chamarajpet, India. Plant leaflets were cleaned thoroughly with flowing water and air dried and later dried in oven for 24 hr. Plant material was coarsely powdered using mechanical blender.

Soxhlet Extraction

The homogenized plant material was subjected to soxhlet extraction.^[12] Plant material was placed inside the thimble, and the thimble was loaded on to soxhlet extractor placed on heating element and reflux condenser is placed at the top of the flask. As solvent heats up in distillation flask, the refluxed vapour travels to distillation arm and floods to thimble. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound was concentrated in the receiver flask. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the solid remains in the thimble and discarded. The extract was collected in the round bottomed flask was dried and used for GC-MS analysis, *in vitro* and *in vivo* studies.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of leaf methanol extract of *Mimosa pudica* was performed to identify different phytochemicals present in the extract. Oven temperature was maintained at 60°C and injection temperature was 260°C with split mode of injection and liner velocity flow control. 57.4 kpa pressure was adjusted for GC that provides the column flow of 1.0 ml/min and linear velocity of 36.5 cm/sec, with a distinct flow of 3.0 ml/min. and split ratio was 10.0.

Identification of Compounds

Phytochemical compounds present in plant extract of *Mimosa pudica* was identified by matching peaks obtained in the chromatogram with Wiley MS Libraries and confirmed by matching mass spectra of peaks with available online literature.^[13]

Experimental Animals

Adult zebra fish purchased from local aquarium, and were maintained in glass tanks, with proper aeration at 28°C. Zebra fish were subjected to light and dark photoperiod and fed until sanitation. Embryos were collected and reared separately in sterile 50ml petri dishes and kept in incubator at 28°C. Dead embryos were removed and water was changed at regular intervals of time.^[13]

In-vitro Isolation of ACE

Adult Zebra fish eyes were dissected and homogenized with 10 volumes of 10 mM Tris-HCl buffer at 4°C (pH 8.2) and centrifuged at 5000 rpm for 7 min at 4°C. To the supernatant 2.0 volumes of 10 mM buffer was added and centrifuged at 5000 rpm for 10 min. The supernatant was dialyzed and stored at 4°C for further use.

ACE inhibition assay

Methanol extract of *Mimosa pudica* was checked for its potential to inhibit ACE activity. Experiment was carried out according to the method by^[13] with further modifications, where 0.5 ml reaction mixture containing 300 µmol NaCl, 3 mM HCl in 40µmol and phosphate buffer at pH 8.3. The mixture was incubated for 30 min at room temperature and later kept in boiling water bath for 10 min. To the same reaction mixture, 3 ml of 0.2 M phosphate buffer (pH 8.3) was added. The mixture was stirred vigorously until turbidity becomes transparent and centrifuged at 1000 rpm for 10min. To the enzyme reaction mixture, *Mimosa pudica* leaf methanol extract was (20, 40 and 60 mg/ml) added and incubated for 10 min. Later, to the same enzyme reaction mixture substrate molecule Hippuryl-L-histidyl-L-leucine^[16] was added and incubated for another 10 min. Enzyme activity was measured by taking absorbance at 380 nm using spectrophotometer.

In vivo Screening of ACE Inhibition

In-vivo screening of ACE inhibition was carried out using Zebra fish embryos. Treatment group embryos were incubated in 50ml petri dishes with 30ml methanol extract of *Mimosa pudica* at three different concentrations viz., 20, 40 and 60 mg/ml and control group embryos were maintained untreated. Embryos were allowed to incubate for 72hr and were observed under microscope to measure heart beat at an interval of one minute up to 30 min.

In-silico Screening of ACE Inhibitors

In-silico molecular docking studies was carried out using Autodock 4.0^[17] on ACE enzyme and phytochemical compounds identified from methanol extract of *Mimosa pudica*.

Ligand Preparation

Phytochemical compounds identified through GC-MS analysis of *Mimosa pudica* methanol extract were used for molecular docking studies. Sdf files of identified compounds were downloaded from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>)^[12] of National Center for Biotechnology Information. Compounds were further designed using MarvinSketch (www.chemaxon.com/)^[18] and further converted to pdb.file format using online tool Open Babel.

Protein Preparation

The pdb structure of angiotensin converting enzyme of human was downloaded from Protein Data Bank, all hetero atoms removed from the pdb flatfile. Further the protein structure with hetero atoms was added with C-terminal oxygen using SPDBViewer.

RESULTS

GC-MS Analysis of Extracted Compounds

GC-MS is one of the widely used techniques for identifying compounds in plant extracts. Gas chromatography and mass spectroscopy analysis carried out on methanol extract of *Mimosa pudica* revealed with seven compounds. The compounds identified were Alanyl glycine, Alanyl valine, Alanin ethyl ester, Alanin ethyl ester, 1 Octamine N methyl, 1 Octamine N methyl, Phytol, 9,12 Octadecadienoic acid, and 13 Eicosdienoic acid methyl ester. Highest peak area of 65.10% was shown by 9,12 Octadecadienoic acid and lowest peak area was reported by 13 Eicosdienoic acid methyl ester with 2.88%. The total ion chromatogram along with the peak areas are shown in Figure 1. And detailed tabulation of GC-MS analysis is represented in Table 1. Peak area obtained by GC-MS analysis determines concentration of unknown sample.

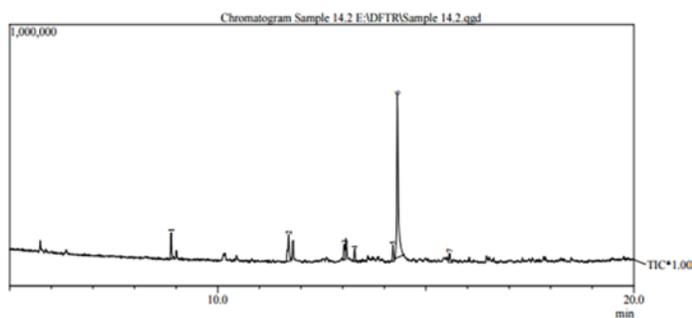


Figure 1: Chromatogram of methanol extract of *Mimosa pudica*.

Table 1: Phytochemical profile of *M. pudica* methanol extract as identified from GC-MS analysis.

Peak	Retention Time	I. Time	F. Time	Peak Area	Area%	Compound
1	8.882	8.858	8.925	160364	6.47	Alanyl glycine
2	11.707	11.642	11.767	277541	11.20	Alanyl valine
3	13.047	13.000	13.067	162435	6.56	Alanin ethyl ester
4	13.290	13.258	13.325	82868	3.35	N-methyloctylamine
5	14.210	14.167	14.242	110018	4.44	Phytol
6	14.319	14.242	14.458	1612785	65.10	9,12-Octadecadienoic acid
7	15.571	15.542	15.625	71344	2.88	13-Eicosdienoic acid methyl ester

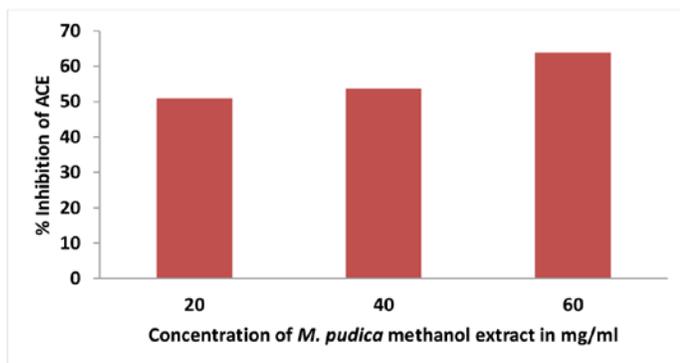


Figure 2: Graph showing the percentage inhibition of ACE induced by *M. pudica* methanol extract.

In-vitro ACE inhibition assay

Activity of ACE enzyme isolated from zebra fish eyes was tested for its inhibition using *M. pudica* methanol extract with a concentration of 20, 40 and 60 mg/ml, which showed the percentage inhibition of 50.92%, 53.70% and 63.88% respectively (Figure2).

In vivo screening of ACE inhibition

ACE inhibition study was evaluated by measuring heart rate by stopwatch counting method in zebra fish embryos of 52 hr post –fertilization. Embryos were incubated in water without plant extract (control group) showed the heart rate of 129.33±1.63 (mean ± SD) beats per minute; whereas embryos of treatment group treated with plant extract of *Mimosa pudica* at the concentration of 20, 40 and 60 mg/ml showed 122.5± 3.271, 121.33± 3.77 and 106.83 ± 5.67 beats per minute respectively. The results

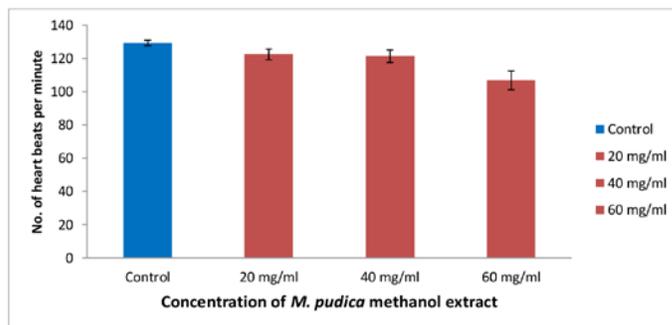


Figure 3: Graph showing the heart rate regulation induced by *M. pudica* methanol extract.



Figure 4: Zebra fish embryo of 52 hr post –fertilization.

clearly indicated that treatment group showed controlled heart rate, than compared to control group (Figure 3).

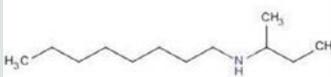
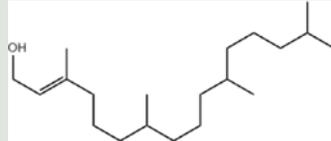
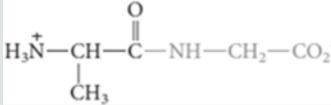
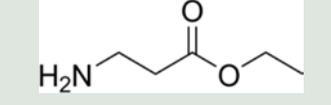
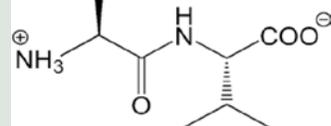
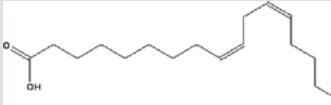
These results can be potential in supporting the data that phytochemicals isolated from methanol extract of *M. pudica* might have potential in reducing blood pressure, where further investigation can help in treating hypertension. Zebra fish has emerged in recent years for studying cardiovascular diseases because of cellular and molecular mechanisms of heart development and are highly conserved between zebra fish and human.^[19] Microscopic view of zebra fish embryo 52hr post fertilization is shown in Figure 4.

In-silico Screening of ACE Inhibitors

Compounds identified through GC-MS analysis were docked with human angiotensin converting enzyme and checked for best interaction to understand ability of compounds present in *Mimosa pudica* to inhibit ACE enzyme. List of compounds along with the binding energy are listed in the below Table 2.

Among 7 different compounds docked with ACE, the molecule 9,12 octadecadienoic acid showed highest inhibition with binding energy -6.6 and therefore the compound can be further evaluated to check antihypertensive effect.

Table 2: Structures of phytochemicals used for molecular docking studies.

	Compound	Mode	Affinity (kcal/mol)	Structures
1	N-methyloctylamine	1	-3.8	
2	Eicosdienoic acid methyl ester	1	-4.8	
3	Phytol	1	-4.8	
4	Alanyl glycine	1	-4.8	
5	Alanine ethyl ester	L	-4.8	
6	Alanyl valine	1	-5.8	
7	9,12 Octadecadienoic acid	1	-6.6	

DISCUSSION

GC-MS Analysis

GC-MS analysis studies reported previously has also shown similar compounds that are known to be availing several pharmaceutical properties. Phytochemical compounds isolated were analyzed^[13] through GC-MS and FTIR showed presence of 13 compounds. Pharmaceutical applications of *Mimosa pudica* is reported by several investigations due to the presence of a wide variety of phytochemical compounds, for example *in-vitro* antitumor activity of *Mimosa pudica* was reported due to the presence of phenolic and other 11 compounds screened through GC-MS analysis. Anti-diabetic property of methanol extracts of *Mimosa pudica* was reported with the presence of potential phytochemicals mainly including organic acids, quinolones, quinone, phenolic compounds and dodecaborane as major constituents.^[21] Therefore presence of potential phytochemicals in plant extract of *Mimosa pudica* reported in the current study could be the reason for inhibition of ACE enzyme and thus helping in regulating hypertension.

ACE Inhibition

Results reported by ACE inhibition can be potential in supporting the data that phytochemicals isolated from methanol extract of *M. pudica* might have potential in reducing blood pressure, where further investigation can help in treating hypertension. Zebra fish has emerged in recent years for studying cardiovascular diseases because

of cellular and molecular mechanisms of heart development and are highly conserved between zebrafish and human.^[21] Methanol extract of *M. pudica* has been reported with several pharmacological potentials, one of them also include anti-diabetic property.^[17]

In-silico ACE inhibition

Several *in-silico* molecular docking studies with several plant extracts are reported to inhibit ACE. *Trigonella foenum* containing 9, 12 Octadecadienoic acid has been reported to show good interaction with ACE, indicating its ability to cause ACE inhibition.^[18] Further other reports indicate that LC-MS analysis of methanol extracts from *Cucumis melo var. cantalupensis* with the presence of 9, 12 Octadecadienoic acid have showed good binding affinity with ACE. *In-silico* characterization of ACE enzyme from pigeon peas is also reported with a novel octapeptide that is capable of inhibiting ACE enzyme, which was confirmed through molecular docking and simulation studies.^[19] These investigations strongly support the results of the current investigation and thus indicate the antihypertensive activity of *M. pudica*.^[22]

CONCLUSION

Angiotensin converting enzyme is a key target associated with hypertension and therefore compounds capable of inhibiting ACE can become potential molecules in managing blood pressure. ACE inhibitors competitively bind to the enzyme and stop its conversion to angiotensin which in turn regulates blood pressure. In the current

study, phytochemical compounds studied from methanol extract of *Mimosa pudica* revealed 07 phytochemical compounds. GC-MS results showed highest concentration of 9, 12 Octadecadienoic acid in the plant extract. *In-vivo* ACE inhibition study, showed much lesser heart rate than compared to control group. Further *in-silico* evaluation through molecular docking revealed 9, 12 Octadecadienoic acid showed highest inhibition of ACE with lowest binding energy. Methanol extract treated zebra fish embryos showed regulated heart rate in *in-vitro* conditions. Therefore 9, 12-octadecadienoic acid can be considered for further studies to check its ability to act as antihypertensive agent.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

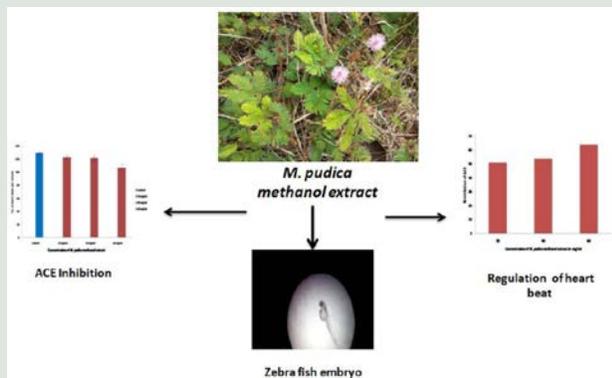
ABBREVIATIONS

GC-MS: Gas chromatography and mass spectrometry; **ACE:** Angiotensin resestsin enzyme; **FTIR:** Fourier transform infrared spectrometry; **HPLC:** High performance liquid chromatography.

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GRAPHICAL ABSTRACT



SUMMARY

M. pudica popularly known as touch me not plant is commonly found in cold and moist areas. Several studies support potential pharmacological activities of different extracts from *M. pudica*. In current study methanol extract of the plant was studied for cardioprotective and antihypertensive activity. An important enzyme related to antihypertensive activity is ACE, *in-vitro* ACE inhibition activity was reported by the plant extract. GC-MS analysis revealed presence of potential phytochemicals in the extract and 9, 12 Octadecadienoic acid was found to show best interaction with ACE, also considering to be inhibiting the enzyme. Therefore the compound can be further evaluated using *in-vivo* studies for understanding cardioprotective and antihypertensive activity.

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