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Photochemical screening and pharmacognostic studies on *Mimosa pudica* L (Sensitive plant)

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Abstract
Mimosa pudica L is a medicinal herb. It is also called sensitive plant shame plant prayer plant etc. The aim of study was to investigate the pharmacognostic and phytochemical studies on important medicinal plant viz., *Mimosa pudica L.* The physico-chemical parameters like Total ash, extractive values and various drugs were determined. The extractive values can be used for detecting Adulteration. The preliminary phytochemical compounds and fluorescence study was screened by Alkaloids, Flavanoids, Steroids, Tannins, Glycoside, Amino acids etc. other major and minor constituents were also present.

Keywords: *Mimosa pudica*, Phytochemical, Flurescence analysis, Medicinal uses, Anatomical study.

1. Introduction
Mimosa pudica L. is a creeping perennial herbs often grown for its curiosity value the compound leaves fold inward and droop when touched and reopen within minutes. It belongs to the family Mimosaceae. It is otherwise known as sensitive plant, humble plant, shame plant, touch me not, (Germplasm Resource Information Network, 2008) sleeping grass, Tropical Biological association) Prayer plant etc. The species epithet ‘’pudica’’ is a Latin equivalent for ‘’shrinking’’ or ‘’Bashful’’ because of its curious nature and easy procreation. There are several reports on the antimicrobial activity of Mimosa *pudica L.* has been extensively used in Siddha, Ayurvedic, Unani and Homeopathi medicine and become modern medicine. It is also used in Jaundice, Asthma, Conjunctivitis, Cut wound and glandular swelling liver is considered metabolism detoxification, secretary function, in the body and its disorders are numerous with the no side effective (Aarthi and Murugan, 2011) [1]
The plant has been well documented for its use as Antiseptic, Antimicrobial, Antimalarial, Immunostimulating and Diuretic activity and is used as remedy for Flu, Cough, Rabies, and Tuberculosis etc, (Mukesh Chandra *et al*., 2010) [19]. It is known to possess sedative, emetic, and tonic properties and has been used traditionally in the treatment of various ailments including Alopecia, Diarrhoea, Dysentery, Insomnia, Tumour, and urinogential infections, etc (Dr Duke’s phytochemical and Ethnobotanical database, 2007) [4]

2. Classification
Class	:	Dicotyledons
Subclass	:	Polypetalae
Series	:	Calciflorae
Order	:	Rosales
Family	:	Leguminasae
Subfamily	:	Mimosaceae
Binomial	:	*Mimosa pudica L*

Fig 1: A flowering of habit on mimosa pudica

2.1 Synonyms
Sanskrit-Samanga, varakranta, Namaskari, Assamese-Laubilata, Adamalati, Bengali-Lajaka, Lajjavanti, English-Touch me not, Gujrati-Risamani, Lajavanti, Lajamani, Hindi-Chhuimui, Lajauni Kannada- Muttidasenui, Machikegida, Lajjavati,, Malayalam-Thottavati, Marathi-Lajalu,, Oriya-Lajakuri,, Punjabi-Lajan, Tamil- Tottavadi, Tottalchurungi Telugu-Mudugudamara Urdu-Chhimui.

Table. 1. Morphological description of M. pudica

Plant parts	Description
Branches	Short prickly branches with glandular hairs
Leaves	Bipinnate, sensitive to touch
Flower	Axillary globose head, lilac pink in colour
Stem	Erect, slender and well branched
Calyx	Companulate
Petals	Petals crenate towards base
Pods	1.5 to 2.5 cm long, closely prickly on the sutures and falcate

Mimosa is usually a short prickly plant with its branches growing close to ground. It grows up to a height of about 0.5 m and spread up to 0.3 m. The stem of Mimosa is erect, slender, prickly and well branched, leaves are bipinnate, fern like and pale green in colour with a tendency of closing when disturbed. These are quadri- pinnate, often reddish, leaflets 15 to 25 pairs, acute, bristly, usually 9 to 12mm long and 1.5 mm wide. Flowers of this plant are axillary in position and lilac pink in colour usually occurring in globose heads. Calyx companulate, and petals are crenate towards the base. Flowering occurs from August to October in Indian conditions. Fruits of Mimosa are pods 1.5 to 2.5 cm long falcate and closely prickly on sutures.

3. Materials and Methods
1. Sample Collection
Mimosa pudica L. plant leaves and root were collected various from Thiruvarur, and Thanjavur Dt. During the March 2016.

2. Processing of the plant leaves
The fresh leaves of the plant were washed with double distilled water and shade dried and then milled into coarse powder using a mechanical grinder Dried powder was stored in an airtight container and was used for extraction.

3. Preparation of extract for biological and phytochemical screening
The powder of plant material 150 gm was mixed with different solvent like Ethanol, Methanol, Petroleum ether, and Acetone, The leaf and root powder was mixed with water boiled and filtered with a muslin cloth and it was condensed in hot air oven at 50'c coarsely powdered plant material was soaked in ethanol for 3 days filtered and allowed to condense at 50'c. The different types of solvent extracts were stored in a container and refrigerated for further use (Jonathan, 2009) [14].

3.1. Screening procedure
1) Test for Alkaloids
2) Dragendroff's test
One ml of the extract was added with 1ml of Dragendroff's reagent (potassium bismuth iodine solution). An orange-red precipitate indicates the presence of alkaloids.

3) Mayer's test
One ml of the extract was added with 1ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow of cream coloured precipitate indicates the presence of alkaloids.

4) Test for Steroids
Two ml of acetic anhydride was added with 0.5 ml of extract of each sample with 2ml of H_2So_4. The colour changed from violet to blue or green in some samples indicates the presence of steroids.

3.2. Test for Terpenoids
Salkowski test
0.5 ml of the extract was added with 2ml of chloroform was added and 3ml of concentrated H_2SO_4 was also carefully added to form layer. A reddish brown coloured of the interface indicates the presence of terpenoids. This type of reaction was observed and recorded.

3.3. Test for Flavonoids
1) Alkaline reagent test
Few drops of dilute ammonia were added to a portion of the leaf extract and concentrated Hcl was also added. A yellow colouration indicated the presence of flavonoids. The reaction was observed and recorded.

2) Zinc Hydrochloric test
Few drops of extract were added with zinc dust and concentrated Hcl. The presence of red colouration indicates the presence of flavonoids.

3) Aluminium test
Few drops of the extract was added with 1% aluminium solution was added. Formation of yellow coloured indicated the presence of flavonoids. The type of reaction was observed and recorded.

3.4. Test for Saponins
Two gram of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously until the formation of a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously, and observed for the formation of emulsion.

3.5. Test for Tannins
1) Lead acetate test: A little quantity of the test solution was mixed with basic lead acetate solution Formation of white precipitate indicates the presence of tannins.
2) One ml of the extract was added with ferric chloride solution. Formation of a blue black or brownish green colour product shows the presence of tannins.
3) A little quantity of the extract was tested with treated with aqueous ammonia is solution. A deep green colour indicates the presence of tannins and the type of reaction was observed and recorded

3.6. Test for phlobatannins
The aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate indicates the presence of phlobatannins.

3.7. Test for cardiac glycosides

Keller-killiani test

0.5ml g of extract was diluted with 5ml water and 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1ml of concentrated sulphuric acid. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar, characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer.

3.8. Test for Proteins

Xanthoprotein test -One ml of the extract was added with 1ml of concentrated nitric acid. A white precipitate is formed and it is boiled and cooled. To that 20% of sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

3.9. Test for Carbohydrates

Two ml of the extract was added with 1ml of Barfoed's reagent and boiled. Reddish brown precipitates indicate the presence of carbohytrates.

4. Test for Aminoacids

Ninhydrin test

Three ml of test solution was added with 3 drops of 5% ninhydrin solution in a tube and heated in boiling water bath for 10 minutes. Formation of purple or bluish colour indicates the presence of amino acids

5. Test for reducing sugars

1) Fehling's test

One ml of the extract was added with equal quantities of Fehlings solution A and B and upon heating, formation of red precipitate indicates the presence of sugars.

2) Benedict's test

Five ml of Benedict's reagent was added with 1ml of extract solution and boiled for 2 minutes and cooled. Formation of red precipitate was showed the presence of sugars.

A few ml of extract was treated with saffranine solution. Pink colour formation indicates the presence of lignin.

6. Test for inulin

A few ml of the extract was added with 1ml solution of naphthol and 0.5 ml sulphuric acid. formation of brownish red colour indicates the presence of inulin.

7. Fluorescence Analysis

Fluorescence characteristics of the powdered drug with different chemicals were observed in day light and ultraviolet light at 254 nm. The powdered leaf was treated with various solvents like hexane, alcohol, chloroform, benzene, acetone and ethyl acetate and acids like 1N hydrochloric acid, 50% sulphuric acids and alkaline solutions like aqueous and alcoholic NaOH. Observations of fluorescence analysis were recorded at 0.24 and 48 hours. Horborne JB *et al.*, 1998.

8. Anatomical studies of the plant

The required samples of root and stem were cut and removed from the plant and fixed in FAA(Formalin in 5ml + Acetic acid 5ml+ 70% Ethyl alcohol 90ml).After 24 hrs of fixing the schedule given by (Sass., 1940) was followed. Infilteration of the specimens was carried by gradual addition. The specimens were cast into paraffin blocks.

9. Sectioning

The paraffin embedded specimens were sectioned with the help of the sections were 10-12 m in size. Dewaxing of the sections was performed by customary procedure (Johansan1940. The sections were stained with Toludine blue as according to the method prescribed by O' Brien *et al.*, 1964 [20]. Wherever necessary, the sections were also stained with saffranin and Fast-green.

10. Photomicrographs

Microscopic description of tissues are micrographs necessary photographs of different magnifications were taken with Nikon Lab photo 2 microscopic unit. For normal observation bright field was used (Easu 1964) [7].

11. Results and Discussion

The preliminary "Phytochemical" screening of ethanol, methanol, acetone, and petroleum ether, extract of the leaves of Mimosa pudica L. showed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, terpenoids, phenols, amino acids, protein, inulin, steroids, and absence of glycoside, resin, fat and oil, reducing sugar, phlobatanins, phytosterol, (Table 2 and 3).

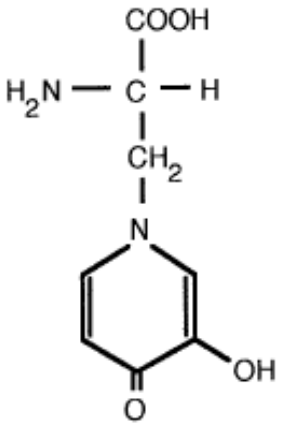

Fig. 2: Structure of Mimosine

Table 2: Phytochemical screening of different solvent of Mimosa pudica L.

Chemical constituent	Test	Ethanol	Methanol	Petroleum ether	Acetone
Alkaloid	Mayers test	+	+	+	+
	Dragendroff' test	+	+	+	+
	S.Wagners test	+	+	+	+
Carbohydrates	Molisch'test	+	+	+	+
	Benedicts test	+	+	+	+
	Fehling'test	+	+	+	+
Glycosides	Modified, Borntragers	–	–	–	–
	Legal test	–	–	–	–
Saponins	Foam test	+	+	+	+
	Froth test	+	+	+	+
Phytosterols	Salkowski test	–	–	–	–
	Libermann Burchard	–	–	–	–
	Tschugaiew test	–	–	–	–
Fat and oil	Staintes	–	–	–	–
Resins	Acetone water test	–	–	–	–
Phenols	Ferric chloride test	+	+	+	+
Tannins	Alkaline reagent	+	+	+	+
Flavanoids	Gelatin test	+	+	+	+
	Lead acetate test	+	+	+	+
Steroids	Steroids test	+	+	+	+
Inulin	Inulin test	+	+	+	+
Phlobatannins	Phlobatannins test	–	–	–	–
Terpenoids	Salkowski test	+	+	+	+
Protein	Xanthoprotein test	+	+	+	+
Amino acids	Ninhydrin test	+	+	+	+
Reducing sugar	i)Fehling's test	–	–	–	–
	ii)Benedict's test				

Table 3. Phytochemical screening of different solvent in fluorescence characteristics of Mimosa pudica L.

S. No	Test	Day light	UV light	Day light	UV light	Day light	UV light
1	Chloroform	Red	Light yellow	Orange	Greenish yellow	Orange	Yellow
2	Hexane	Light brown	Light yellow	Light brown	Greenish yellow	Light brown	Greenish yellow
3	Benzene	Red	Yellow	Red	Brown	Red	Brown
4	Aqueous NaOH	Majenta	Brownish green	Dark red	Dark brown	Dark red	Greenish brown
5	Alcoholic NaOH	Dark red	Yellow	Dark red	Greenish yellow	Dark red	Greenish red
6	1N Hcl	Pale yellow	Yellow	Red	Yellowish red	Red	Yellowish red
7	Ethanol	Sandal	Whitish yellow	Sandal	Greenish yellow	Yellow	Greenish yellow
8	Ethyl acetate	Pale yellow	Pale yellow	pale yellow	Greenish Yellow	yellow	Greenish yellow
9	Acetone	Light brown	Yellow	Dark brown	Greenish yellow	Yellow	Greenish yellow
10	50% sulphuric acid	Pink	Pinkish brown	Pink	Brown	Black	Dark brown

13. Microscopic feature

Anatomical characteristics of Mimosa pudica L. in root

Epidermis

Epidermis is multilayerd, composed 3-4 row of closely set thin walled parenchyma tissue rectangular cells.

Cortex

The cortex is composing circular intercellular, parenchyma cells. Innercortex is composed by multiseriate cell layer and form a endodermis pericycle is the outermost uniseriate layer of stele.

Vascular bundles

Vascular bundles are surrounded by sclerenchyma, vascular bundle is polyarch and radial 4-5 in numbers, phloem strand alternate to xylem strand, exarch, two protoxylem are in outside and metaxylem are inside. Phloem lies towards periphery and metaphloem lies towards the centre,

Pith: small pith observed central in position. (Fig-3.a)

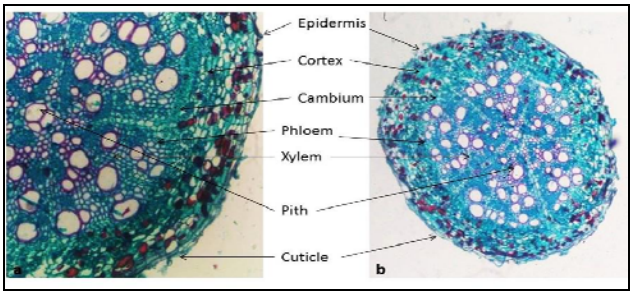

Fig 3: Transverse sections of the roots (a) and stem (b)

Anatomical characteristics of Mimosa pudica L. in stem.

Yong stem measuring 1.5 mm thick was studied. It shows initial stage of secondary growth. It is somewhat four-angled in cross sectioned view. The outline is smooth and even. The stem consists of a distinct continuous epidermis, cortex, vascular bundle, and pith.

Epidermis

Is thin and the epidermal cells are squarish or rectangular, coated with thick cuticle, stomata are frequently seen in the

epidermis.

Cortex
Is 150 mm wide. It consists of chlorenchyma and parenchyma cells which are compact and homogenous. (Fig-3.b)

Vascular bundle
Is in the form of the outside of the stem. It consists of about 25 discrete vascular bundles, separated from each other by narrow medullary rays. The rays are 3-5 cells wide, as they reach the periphery, the cells dilate tangentially into rectangular cells (Fig-3.b). The vascular bundles are open and collateral remain scattered in parenchymatous ground tissue. Cambium with 2-3 layers is located in between phloem and xylem, endarch radially elongated a thick semicircular mass of sclerenchymatous bundle-cap occurs on the cortex part of the phloem of each bundle. Primary xylem occurs secondary xylem consists of outer cluster of wide vessels and inner narrow, compact band of thick walled fibres and a few wide vessels.

Pith
It is wide, homogenous, and parenchyma cells are angular and thick walled they vary and cells of different sizes intermixed. (Fig-3.b)

14. Discussion
Hence the results of the pharmacognostic and phytochemical studies may be useful to supplement information in respect to its identification, authentication and standardization. These parameters are to be useful in the preparation of the herbal monograph for its proper identification in the near future. In the present era, medicinal herb resources are abundant, but these resources are dwindling fast due to the onward March of civilization (Vogel, 1991) [25] As it is already reported Phytochemical compounds such as Terpenoids are commonly implicated in the antiplasmodial activity of many plant (Philipson et al., 1991 Francois *et al.* 1996., Ghoshal *et al.*, 1996 Asase *et al.* 2010) [21] [12] Flavanoids showed significant antiparasitic activity Kim., 2004 [17], Monbrison et al. 2006 [18], Tasdemir et al., 2006 [23]. Although a significant number of studies have been used to obtain purified plant chemical, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veermuthu et al., 2006) [24] Earlier proofs on other plant groups have suggested that difference in the anatomical systems of stems and roots are vital sources of taxonomic inference in different groups of flowering plants (Edeoga and Okoli, 1997, 2001) [1, 8]

15. Conclusion
The study of preliminary phytochemical and pharmacognosy studies of Mimosa pudica L. used for different solvent was shown and determined the phytochemical compounds like Alkaloids, Flavanoids, Steroids, Tannins, Glycocides, Amino acids, major and minor compound was also identified. In the present pharmacognonostic study the identification standization of herbal drugs which are used herbal medicine and various traditional uses.

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