

# Journal of Pharma Research Available online through www.jprinfo.com

Research Article ISSN: 2319-5622

## PHARMACOGNOSTICAL, PHYSIOCHEMICAL & ANTIMICROBIAL ACTION OF MIMOSA PUDICA LEAVES

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Received on: 08-04-2016; Revised and Accepted on: 19-04-2016

## ABSTRACT

**M**imosa pudica Family Mimosae known as sensitive plant in English and lajvanti or chuimui in Hindi language. The plant is distributed throughout in India in moist locality. A diffuse prickly under shrub, is about 45-90 cm in height. Leaves bipinnately compound, pinnate 2-4 delicately arranged with 10-20 pairs of leaflets, rachis clothed with ascending bristles. Flowers pink, in globose heads, peduncles prickly, usually in auxiliary pairs all along the branches. Fruits bristly pods, flat, straw colored consisting of 3-5 one seeded segments. The roots and leaves are commonly used in treatment as bitter, astringent, acrid, cooling, vulnerary, alexipharmic, diuretic antispasmodic, emetic, constipating and febrifuge. The aim of the present study was to evaluate Pharmacognostical, Physiochemical and Antimicrobial Potential of Mimosa pudica Plant. Establishment of Pharmacognostic profile of the Plant will assist in standardization which can guarantee quality, purity and identification of samples. In the present study we describe Pharmacognostical feature, including macroscopy, microscopy, analytical profile and antimicrobial investigation from the whole plant of Mimosa pudica. These observations would be of immense value in the botanical identification and standardization of the drug in crude form. Mimosa pudica leaves extract were used for antimicrobial activity towards pathogens i.e. bacteria and fungi. The activity was tested against Hydro alcoholic extract of Mimosa pudica against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus in vitro at different concentrations of 25, 50 and 100 µl/ disc and the results have been illustrated.

Key Words: Pharmacognostical, Physiochemical, Antimicrobial, Mimosa pudica.

## INTRODUCTION

The plants provided food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances. Herbs had been used by all cultures throughout history. Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals <sup>[1]</sup>. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of chemistry in 16th century, plants had been the source of treatment and prophylaxis. Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again. The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake [2].

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: *Rhei rhisoma*, camphor, *Theae folium*, *Podophyllum*, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra <sup>[3, 4]</sup>. The Indian holy books Vedas

\*Corresponding author: Pankaj Pradhan Assistant Professor (Department of Pharmacognosy), Himachal Institute of Pharmaceutical Education & Research, Nadaun, Himachal Pradesh. Ph. +919929870878. \*E-Mail: pnkj.pradhan@gmail.com mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc <sup>[5]</sup>. The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 prescriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc <sup>[6, 7]</sup>.

According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense [8]. In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (Inula helenium L. Asteraceae) was named in honor of Elena, who was the centre of the Trojan War. As regards the plants from the genus Artemisia, which were believed to restore strength and protect health, their name was derived from the Greek word artemis, meaning "healthy" [9]. Herodotus (500 BC) referred to castor oil plant, Orpheus to the fragrant hellebore and garlic, and Pythagoras to the sea onion (Scilla maritima), mustard, and cabbage. The works of Hippocrates (459-370 BC) contain 300 medicinal plants classified by physiological action: Wormwood and common centaury (Centaurium umbellatum Gilib) were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; oak and pomegranate as astringents [10, 11]

Theophrastus (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time <sup>[12, 13]</sup>. Among others, he referred to cinnamon, iris rhizome, false hellebore, mint, pomegranate, cardamom, fragrant hellebore, monkshood, and so forth. In the description of the plant toxic action, Theophrastus under scored the important feature for humans to become accustomed to them by a gradual increase of the doses. Owing to his consideration of the said topics, he gained the epithet of "the father of botany," given that he has great merits for the classification and description of medicinal plants <sup>[14, 15]</sup>. In his work "De re medica" the renowned medical writer Celsus (25 BC–50 AD)

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quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc  $^{\rm [16]}.$ 

Mimosa pudica Linn known as sensitive plant in English and lajvanthi or chuimui in local Hindi language. The plant is distributed throughout in India in moist locality. A diffuse prickly under shrub, 45 - 90 cm in height. Leaves bipinnately compound, pinnate 2-4, digitately arranged with 10 -20 pairs of leaflets, rachis clothed with ascending bristles. Flowers pink, in globose heads, peduncles prickly, usually in auxiliary pairs all along the branches. Fruits bristly pods, flat, straw coloured, consisting of 3-5 one seeded segments. The roots and leaves are commonly used in treatment. The roots are bitter, astringent, acrid, cooling vulnerary, diuretic, alexipharmic, resolvent, antispasmodic, emetic, constipating, and febrifuge. They are useful in vitiated conditions of pitta, leucoderma, vaginopathy, metropathy, ulcers, dysentery, inflammations, burning sensation, hemorrhoids, jaundice, asthma, fistula, small pox, strangury, spasmodic, affections and fevers [17]. The leaves are bitter, sudorific and tonic, and are useful in hydrocele, hemorrhoids, fistula, scrofula, conjunctivitis, cuts and wounds and hemorrhages. The whole plant is used internally for vesicle calculi and externally for odema, rheumatism, myalgia and tumors of the uterus <sup>[18]</sup>. Literature available from all possible scientific sources revealed very little research work on this selected medicinal plant, whereas folklore claim that *Mimosa pudica*, were used in treatment of various diseases and aliments, and they claim for their promising activity but there is no inbuilt scientific proof in support of the utility of this plant or plant products against various types of microbes. So, the present study is investigated to exploring the details of Phytochemical, Pharmacognostical & Antimicrobial Potential of hydro alcoholic extract from Mimosa pudica.

#### **MATERIALS & METHODS**

#### **Plant Material:**

The plant *Mimosa pudica* belonging to family "Mimosaceae" are available in Himachal Pradesh. For Present work the plant *Mimosa pudica* was collected in the month of April 2015, from Nadaun. After collection of plant, it was authenticated by Mr. Vinod Kumar at Rajasthan University. After identification, a voucher specimen was kept at the Department of Botany in Rajasthan University, Jaipur.

#### Treatment: (Drying)

The whole plant was collected and washed with water. Kept in sunlight for thirty minutes and then dried under shade. Then it was powdered by means of a Grinder.

## **Physiochemical Parameters:**

### Foreign Matter:

The 50 gm sample was spread in a thin layer, and the pieces of foreign matter were sorted out by visual inspection. The powder of foreign matter was sifted through a 250 micron sieve. All portions of the foreign matter were pooled and weighed.

#### Loss on Drying:

 $10~{\rm gm}$  of the drug was weighted in a tarred evaporating dish. It was dried at  $105^{\circ}{\rm C}$  for 5 hours and weighed. The drying and weighing was continue at 1 hour interval until difference two successive weighing correspond not more than 0.25%.

#### Total Ash value:

About 3 gm accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding  $450^{\circ}$ C until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated.

#### Acid insoluble ash value:

To the crucible containing the total ash was added 25 ml of hydrochloric acid. The crucible was then covered with a watchglass and the mixture was boiled gently for 5 minutes. The watchglass was rinsed with 5 ml of hot water and this liquid was added in to the crucible. The insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper contain the insoluble matter was transferred to the original crucible, dried on a hotplate and ignite to constant weight. The residue was allowed to cool in a desiccator for 30 minutes and then weighed.

## Water soluble ash value:

Total ash obtained was boiled for 5 minute with 25 ml of water. Insoluble matter was collected in a crucible or an ashless filter paper. Washed with hot water and ignite for 15 minute at temperature not exceeding 450°C. Weight of insoluble matter was substracted from the weight of the ash, the difference in weight was representing the water soluble ash. Percentage of water soluble was calculated with reference to the air dried drug.

### Extractive Values:

### Alcohol soluble extractive value:

5 gm of the air dried coarsely powdered drug was macerate with 100 ml of alcohol of the specified strength in a closed flask for 24 hour. Shaking frequently during 6 hour and allowed to stand for 18 hour. Filter rapidly and evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at  $105^{\circ}$ C to constant weight and weigh.

#### Water soluble extractive value:

5 gm of the air dried coarsely powdered drug was macerate with 100 ml of distilled water in a closed flask for 24 hour. Shaking frequently during 6 hour and allowed to stand for 18 hour. Filter rapidly and evaporate 25 ml of the filtrate to dryness in tarred flat bottomed shallow dish and dry at  $105^{\circ}$ C to constant weight and weigh. <sup>[19]</sup>

#### **Pharmacognostical Parameters:**

Macroscopical studies:

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of leaves and various plant parts of *Mimosa pudica* were observed.

#### Microscopic studies:

Microscopic studies were carried out by preparing thin sections of leaf. The thin section was collected in watch glass and bleached with bleaching agent along with little boiling. Thin section was further washed with water, stained with Saffranin and mounted in glycerin for observation. Same time quantitative microscopy also carried out with help of plant leaves.

#### Extraction:

The powdered plant material (40g) was extracted with combination of ethanol and distilled water (50:50) in Soxhlet extractor for 48 hours. After this procedure, extracts was filtered through Whatman No.1 filter paper. This crude sample was then subjected to Antimicrobial action.<sup>[20]</sup>

#### Antimicrobial Activity:

#### Microorganism:

Four Reference Bacteria, Vic. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATTCC 27853 and *Bacillus cereus* ATCC 6633 were used during the present study and were obtained from Dr. B. Lal Institute, Jaipur, Rajasthan. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

### Antibacterial Assay:

Muller- Hinton Agar plates are prepared by pouring 10-15 ml of the medium in to each sterilized Petridish and are allowed to set at room temperature. The cell suspension is inoculated over the surface of agar medium using sterile cotton swab. The two cups are scooped in each plate using a sterile cork borer of 8 mm diameter. Then the solution of test compounds (25  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l and control 100  $\mu$ l) are added in cups by using micropipettes and these plates are incubated at 37°C for 48 hr. Standard drug ciprofloxacin (5 mcg) is used. The Zone of inhibition is measured in mm for each organism <sup>[21]</sup>.

## RESULTS

### **Physico-Chemical Parameter:**

The Physicochemical parameters were investigated and reported in table no. 1. The above studies enable the identification of the plant material for future investigation and form an important aspect of drug studies.

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### Table No. 1: Physiochemical Parameters of Mimosa pudica

S. No.	Parameters	Values Obtained
1	Foreign Matter	0.5 %
2	Moisture Content	2%
3	Total Ash	16%
4	Acid Insoluble Ash Value	8.95%
5	Water Soluble Ash Value	9.35%
6	Alcohol Soluble Extractive Value	10%
7	Water Soluble Extractive Value	9.5%

## Pharmacognostical Evaluation:

## Quantitative Investigation:

*Macroscopy:* Digitately compound with one or two pairs of sessile, hairy pinnae, alternate, petiolate, stipulate, linear lanceolate; leaflets 10-20 pairs, 0.6-1.2 cm long, 0.3-0.4 cm broad, sessile, obliquely narrow or linear oblong; obliquely rounded at base, acute, nearly glabrous; yellowish-green. Quantitative microscopy includes stomatal number, stomatal index, palisade ratio, vein-islet number and vein termination number. The value obtained for leaf constant is tabulated in Table 2.

### Table No. 2: Quantitative Investigation of Mimosa pudica

Sample	Type of Stomata	Stomatal Index		Vein Islet No. per mm <sup>2</sup>		Vein Termination No. per mm <sup>2</sup>	
Identity		Upper	Lower	Upper	Lower	Upper	Lower
Leaves	Diacytic	23.20	25.20	18.16	13.12	23.16	12.13

## Microscopy:

Petiole shows single layered epidermis with thick cuticle; cortex 4-7 layered of thin walled, parenchymatous cells; pericycle arranged in a ring; 4 central vascular bundles present with two smaller vascular bundles arranged laterally, one in each wing.

*Midrib* - shows single layered epidermis, covered with thin-cuticle; upper epidermis followed by a single layered palisade, spongy parenchyma single layered, pericycle same as in petiole; vascular bundle single.

*Lamina* - shows epidermis on both surfaces, palisade single layered; spongy parenchyma, 3-5 layers consisting of circular cells; rosette crystals and a few veins present in spongy parenchyma.

### Antimicrobial Activity:

Hydro alcoholic extract is test for antibacterial activity. The doses of 1000 mg /ml of extracts were made by dissolving appropriate quantity of extracts in DMSO. Standard drug Ciprofloxacin is used. The solution of test compounds (25  $\mu$ l, 50  $\mu$ l, 100  $\mu$ l and control 50  $\mu$ l) are added in cups by using micropipettes and these plates are incubated at 37°C for 48 hr. The zone of inhibition is measured in mm for each organism. Controls with DMSO did not show any activity. The crude extract shows positive antimicrobial activity against both gram positive and negative bacteria (table 3, fig. 1, 2, 3, 4.)

## Table No. 3: Showing Effect of Hydro alcoholic extract of Mimosa pudica on microbial growth

S. No.	Drug → Microorganism↓	Hydro-alcoholic Extract(25 µl)	Hydro-alcoholic Extract (50 µl)	Hydro-alcoholic Extract (100 µl)	Ciprofloxacin (50 µl)
1	Escherichia coli	15 mm	19 mm	23 mm	24 mm
2	Staphylococcus aureus	12 mm	15 mm	19 mm	27 mm
3	Pseudomonas aeruginosa	7 mm	11 mm	14 mm	26 mm
4	Bacillus cereus	5 mm	8 mm	10 mm	29 mm

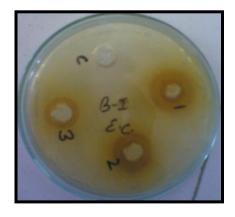


Fig. 1: Zone of Inhibition against Escherichia coli

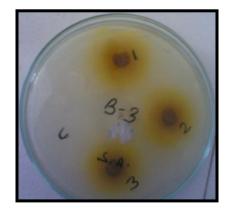


Fig. 2: Zone of Inhibition against Staphylococcus aureus

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Fig. 3: Zone of Inhibition against *Pseudomonas aeruginosa* 

## CONCLUSION

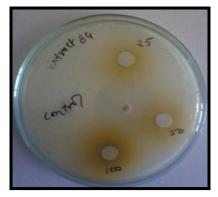
**M**any of the existing synthetic drugs cause various side effects. Hence, development of plant- based compounds is required to meet this demand for production of newer drugs with minimal side effects. The plant Mimosa pudica has been examined to gain an insight of its Pharmacognostical and Phytochemical behavior. In the present study, whole plant extract of Mimosa pudica was subjected to antimicrobial activity. The authenticated plant material was subjected to pharmacognostical & physiochemical standardization. All the Physicochemical parameters were investigated for Mimosa pudica. The above studies enable the identification of the plant material for future detailed investigation. From above studies, it is concluded that the susceptibility of various microbial agents to different concentrations of Mimosa pudica indicates that plant is the potential source for antimicrobial compound and is useful for rationalizing the use of this plant in primary health care. So further work on the profile in order to determine the nature of bioactive principles present in the plant and their mode of action. In the present study, Mimosa pudica leaf extracts possesses a strong antimicrobial activity against all tested microorganisms and the plant contains potential antimicrobial components for the therapy of infections. The present investigation clearly demonstrates the significant antibacterial activity of Hydro alcoholic extract of Mimosa pudica against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus in vitro. These results indicate the potential use of this plant in management of bacterial diseases caused by Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus. These local ethanomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

### ACKNOWLEDGEMENT

The authors are thankful to HIPER, Nadaun & Dr. B. Lal Lab, Jaipur for providing necessary facilities to carry out the research work. The authors are also thankful to the Mr. Ashok Sharma, Honorable Chairman, Himachal Institute of Pharmaceutical Education & Research, Nadaun & Dr. Parshuram Rai, Principal, HIPER, Nadaun for providing necessary facilities and financial support for this study.

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#### Fig. 4: Zone of Inhibition against Bacillus cereus

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## How to cite this article:

Pankaj Pradhan et al.,: PHARMACOGNOSTICAL, PHYSIOCHEMICAL & ANTIMICROBIAL ACTION OF MIMOSA PUDICA LEAVES, J. Pharm. Res., 2016; 5(4): 52-55.

Conflict of interest: The authors have declared that no conflict of interest exists. Source of support: Nil