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Pharmacognostic and Phytochemical Evaluation of Root of *Plumeria pudica* Jacq.

Sujata Mukherjee[1], Dr. Jignesh Kevalia*[2]

[1]Institute of Teaching and Research in Ayurveda, Jamnagar, Department of Pharmacognosy, India.
[2]*M.Sc (Ayu), Ph.D., Senior Lecturer & Head, Dept. of Pharmacognosy, India.

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ABSTRACT:

Plumeria pudica Jacq. was typically termed as bridal bouquet that is an ornamental flowering plant belonging to the Apocynaceae family. It is mainly cultivated as an ornamental plant for its showy white flower. The plant possesses the medicinal properties like antidiarrheal, anti-inflammatory, anti-bacterial, anti-tumour, as purgative, skin problems, cough asthma, bronchitis, piles, fever, as well as used in blood disorder, dysentery, nociceptive and many more. The botanical and pharmacognostical studies of root of *P. pudica* were performed to know about the taxonomical order, cell structure of the plant parts. In addition to that the important phytoconstituents were also examined for detailed analysis. The analysis includes its physico-chemical parameters along with the elucidation of compounds like alkaloid, glycosides, tannin, and lipid contents. Thin Layer Chromatographic profile was also performed to find out the nature of polar and non-polar substances present in the plant P. pudica by using various solvent systems.

Keyword: Bridal bouquet, *Plumeria pudica*, laticeferous cells, extra-pharmacopoeial, *anukta dravya*

INTRODUCTION:

Approximately 354,696 plant species are available in the world whereby; the Indian flora is rich with thousands of flowering plant species. According to botanical survey of India 44,500 species of plants are identified and classified, amongst them around 18 species of the genus Plumeria, *P. pudica* is reported and distributed throughout India which is classified taxonomically as below:

Plumeria pudica Jacq.[1]

Kingdom: Plantae; Division: Spermatophyta; Subdivision: Angiosperm; Class: Dicotyledons; Subclass: Gamopetalae; Series: Bicarpellatae; Order: Gentianales; Family: Apocynaceae; Genus: Plumeria; Species: *P. pudica*; Syn. *P. caracasana*; *P.cochleate*[2]

Their Common/vernacular names are "Wild Plumeria, Bridal bouquet, White frangipani, Fiddle leaf plumeria," [2]

Habit: *Plumeria pudica* is a medium sized shrub with tap root system. It has woody, erect stem; young stem is light green in colour with gland & mature stem is greyish green in colour. Leaf simple, arranged in whorled, sometimes almost lobed when immature, margin - entire, apex- unique fiddle-shaped or spoon- shaped (spathulate), dark green in colour. Inflorescence is cymose (reduced polychasial cyme), secondary peduncles usually developed, terminally with many flowers, the primary peduncle is about 7 cm long. Flower is complete, pedicellate, 8-12 cm long, bracteate, bracteolate, actinomorphic, hypogynous, bisexual, pentamerous. **(Fig no.- 1.1)**

Global habitat:

This plant is local to Columbia, Venezuela and Panama, *P. Pudica* has striking flowers that are white along with an extended blooming time period which happen to have made it a favourite along with scape grow in south Florida as well as the Caribbean. It also grows in Central, tropical & South America.

Distribution in India:

In India, it is found to be present in regions like Kerala: Idukki district; Sikkim: Sikkim; Gujarat because of its showy flower also cultivated more or less throughout the nation.

Flowering: August-March [3]

General use: It is mainly utilized as purgative, in the treatment of diarrhoea, itch cure, cough asthma, bronchitis, piles, fever, blood disorder dysentery, and tumours. It is also prescribed as antibacterial, antidiarrheal, anti-inflammatory, nociceptive, in Alzheimer's diseases, bowel problems, possesses anti-helminthic properties, gynaecological disorders, etc.

Albeit, pharmacological activities were already carried out on the leaves and latex of *Plumeria pudica*. But here focuses were mainly given on the work which has not reported yet on the root part of the plant especially the studies of pharmacognostical and phytochemical aspects. Hence, its detailed morphology, anatomy, physico-chemical parameters, qualitative tests, and thin layer chromatographic work has carried out to authenticate the plant *Plumeria pudica*.

MATERIALS AND METHOD:

Collection & Authentication of drug:

Selected plant was collected from its natural habitat and identification as well as authentication was done by pharmacognosist in pharmacognosy department of ITRA-pharmacy, Jamnagar, Gujarat, India.

Preparation of powder drug:

After the collection of plant, the root was separated and was thoroughly cleaned in order to make them free from dust or other filthy materials or foreign materials, then shade dried to maintain its properties and natural appearance. For proper drying it was then cut into small pieces and were powdered separately in a clean mechanical grinder or mixture. The powder was then sieved through mesh 80 (♯) and stored in air tight containers so that it can be used as and when required for further studies to observe specific microscopical characters or for its utilization in phytochemical investigations.

Organoleptic evaluation: Organoleptic characters of samples by means of the organ of sense that includes color, odor, and taste, texture, etc. were also carried out and reported. [4,5,6]

Microscopical evaluation: Free hand sections of root was taken and cleared with chloral hydrate. First observed in diluted glycerine and then stained with phloroglucinol and Conc. HCL to examine its diagnostic microscopical characters, arrangement of tissue, contents present in the cells, presence and absence of cell wall lignin, etc.

Microphotographs were taken by using Carl Zeiss Trinocular microscope attached with camera.[4,5,6]

Physicochemical evaluation:

Physicochemical study of the samples were carried out by using various physiochemical parameters as mentioned in Ayurvedic Pharmacopoeia of India. All determinations were performed in triplicate and the results are presented as mean value.[7]

Analytical study:

Loss on drying:

Loss on drying of the sample is determined by taking 2g accurately weighed sample in dried Petri dish and dried in an oven at 1100c till constant weight. The weight was noted and loss on drying was calculated and expressed as % w/w.

$$\text{Percentage of L.O.D} = \frac{\text{Loss on drying}}{\text{weight of sample}} \times 100 = \%\text{w/w}$$

Determination of total ash:

About 2gm of the sample was accurately weighed and taken in a previously dried and weighed with silica crucible. It was then incinerated in a muffle furnace at a temperature not exceeding 450^0C until free from carbon. Then sample was kept for self-cooling and weighed, from the weight of the residue, ash value was obtained, it was calculated in percentage on the basis of air-dried sample.

$$\text{Percentage of ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100 = \%\text{w/w}$$

Determination of Acid-Insoluble Ash:

Treatment of ash with hydrochloric acid leaving virtually only silica. Hence it is done to detect the silica in the drug. The ash of the drug was boiled with 25 ml of 6N HCl for 5 minutes. The insoluble matter was collected on ash less filter paper (Whatman filter paper No. 40). It was washed with hot water until free from chloride, dried in oven, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the sample.

$$\text{Percentage of A.I.A} = \frac{\text{Weight of A.I.A}}{\text{Weight of sample}} \times 100 = \%\text{w/w}$$

Determination of water-soluble extractive:

5g accurately weighed sample was taken and macerated with 100 ml distilled water in a closed flask for 24 hours. The flask was shaken intermittently during first six hours and allowed to stand for 18 hours. Next day it was filtered. 20 ml of filtrate was taken in a previously dried and weighed, porcelain-evaporating dish and evaporated on a hot water bath. It was dried till constant weight in an oven and weighed. From the weight of the residue the water-soluble extractive was calculated on the basis of air-dried sample.

$$\text{Percentage of W.S.E} = \frac{\text{Wt.of extract} \times \text{Vol.of Water}}{\text{Vol.of Filtrate} \times \text{Wt.of sample}} \times 100\ \%\text{w/w}$$

Determination of Acid soluble Extractive:

A methanol soluble extractive of the sample was determined in the similar way like water-soluble extractive but by using alcohol (95%) instead of water. The percentage of methanol soluble extractive was calculated on the basis of air-dried sample and expressed in % w/w.

$$\text{Percentage of A.S.E} = \frac{\text{Wt.of extract} \times \text{Vol.of Alcohol}}{\text{Vol.of Filtrate} \times \text{Wt.of sample}} \times 100\ \%\text{w/w}$$

Determination of P^H:

5g of sample was weighed and transferred to a clean conical flask. Add 50ml distilled water. Shake it continuously with the help of clean and dry glass rod for about 45min.

Filter through cotton so as to remove the insoluble portion. The pH value is found out from pH meter by calibrating it previously with standard buffer solution of pH 4 and 7. Thereby on dipping the electrode in the sample solution pH of the sample can be read from the pH meter.

Determination of TLC:

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminum oxide, cellulose, or silica gel.

The factors affecting retardation factor are the solvent system, amount of material spotted, adsorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent.

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RESULTS & DISCUSSION:

a) Macroscopy of root (Table no.1.1) (Fig no.-1.2)

Colour	Outer brown; inner creamish yellow
Odour	Characteristic
Taste	Slight bitter
Texture	Coarse
Size	Approx 30 cm X 2 cm
Shape	Irregular; cylindrical

Fig No.1.1: Whole plant

Fig No.1.2: Morphology of root

b) Microscopy of Root:

Diagrammatic T.S. of the root is circular in outline, and shows a narrow region of cork and cortex followed by wide zone of vascular bundle occupying almost major area of the section.

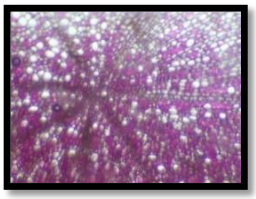
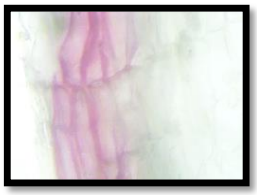
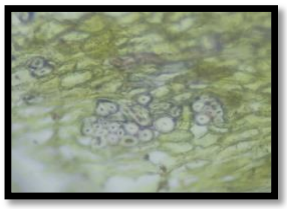
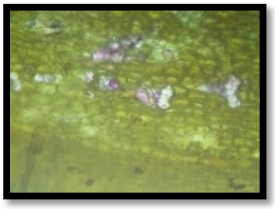
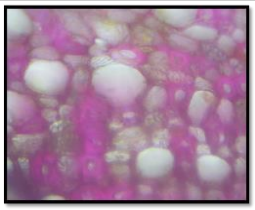
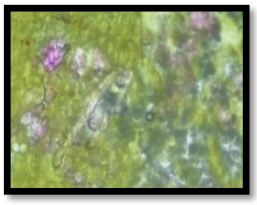
Detailed T.S. shows compactly and tangentially arranged 3 – 5 layered, lignified cork cells followed by 1 – 2 layered cork cambium occasionally filled with brown content.

Cortex is parenchymatous, thin walled, compactly arranged, 10 – 15 layered, at places groups of lignified sclerenchymatous fibres and horizontally arranged groups of laticeferous cells are embedded throughout the parenchymatous zone.

Steler region consists of vascular bundles with narrow parenchymatous phloem, occasionally embedded with phloem fibres followed by wider lignified xylem elements consisting vessels, xylem parenchyma and xylem fibres that distinctly separated by bi to tri-seriate medullary rays arranged vertically and extended towards the central zone of stele. Xylem shows isolated or in groups of 2 to 3 vessels occasionally arranged in radial rows. Abundant simple and compound starch grains and few rhomboidal prismatic crystals of calcium oxalates are found throughout the parenchymatous cells of the section. **(Fig no. 2.1-2.8)**

Micrometric measurement of T.S of root (Table no.1.2):

Sr. No.	Character	Measurement
1	**Prismatic crystal**	**4×4µm**
2	**Starch grain (4x)**	**0.4µm^2**

 Fig. 2.1: Diagrammatic section of root	 Fig 2.2: Diagrammatic view of lignified region	 Fig 2.3: Lignified central region	 Fig 2.4: Lignified cork layers
 Fig 2.5: Starch grains	 Fig 2.6: Lignified pericyclic fiber	 Fig 2.7: Lignified xylem vessels	 Fig 2.8: Phloem region with laticiferous cells

c) **Powder microscopy of root:**

Organoleptic character of root powder (Table No.1.3):

Sr. No.	Character	Observation
1	Colour	Light brown
2	Taste	Slight bitter
3	Odour	Characteristics
4	Texture	Slightly coarse

Microscopic evaluation of root powder:

Diagnostic characters of root powder show characters like cork in surface view, simple fibre, cells with brown contents, simple and compound with mostly 2-celled, starch grains few with distinct centric hilum; silica deposition, prismatic crystals of calcium oxalate, fragments of lignified cork cell, fragments of isolated or in groups of lignified fibres & fragments of vessels with annular thickening **(Fig no.3.1-3.8)**. The micrometric study is mentioned below in **Table no.1.4**.

Root powder micrometry (Table No.1.4):

Sr. No.	**Powder Character (40x X 10x)**	**Measurements**
1	Starch grain	0.1 μm^2
2	Prismatic crystal	4×4µm
3	Oil globules	0.1 μm^2

Fig 3.1: Root powder

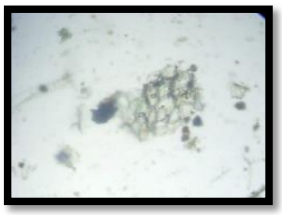


Fig 3.2: Cork in surface view

Fig 3.3: Simple fiber

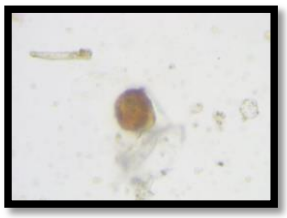


Fig 3.4: Brown content

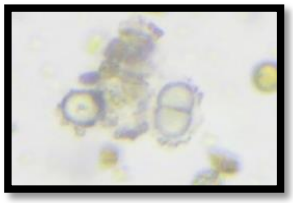


Fig 3.5: Simple & compound starch grains

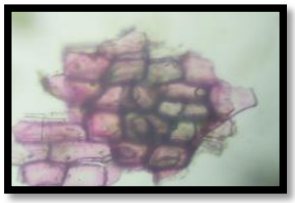


Fig 3.6: Lignified cork cell

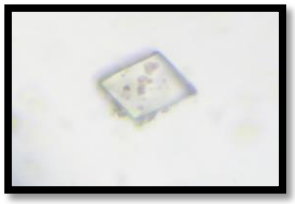


Fig 3.7: Prismatic crystal

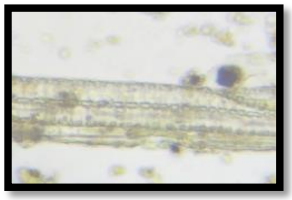


Fig 3.8: Fragment of annular vessels

Histochemical evaluation:

T.S. of root and their powder shows presence of starch grains, calcium oxalate crystals and lignified cells by histochemical tests which are enlisted below in the table no.1.5.

Sr. No	Reagent	Observation	Characteristics	Results
1	Phloroglucinol + Conc. HCl	Red	Lignified cells	++
2	Iodine	Blue	Starch grains	++
3	Phloroglucinol + Conc. HCl	Dissolved	Ca Ox - crystals	++

PHYSICO-CHEMICAL AND PHYTOCHEMICAL EVALUATION:

Aalytical values of *P. pudica* Jacq. root powder including its physicochemical parameters was observed by performing tests like loss on drying, total ash, acid insoluble, etc. the ash value was around 20.48% w/w; the acid insoluble ash value was 2.01% indicate the presence of the mineral composition. Likewise, all other parameters were carried out based on the pharmaceutical standards. They are mentioned in the below **table no.1.6.**

Chemical parameters of root powder: Table no.1.6

Parameters (w/w)	Root
Loss on drying	50%
Total ash value	20.48%
Acid insoluble ash	2.01%
Water soluble extractive value	1.73%
Alcohol soluble extractive value	3.32%
P^H (aqueous)	6

Phytochemical screening in primary stages was done by performing qualitative tests of root powdered to confirm the presence of different chemical constituents of secondary metabolites using solvents like methanol and water-soluble extracts of the sample.

Alkaloid was present in alcoholic extract from the sample while, saponin glycoside and carbohydrate were present only in water extract, amount of lipid particles was reported also in root using alcoholic extracts. The data mentioned in the **table no.1.7.**

Phytochemical test for root powder: Table no.1.7

Test	Root
Test for alkaloid	+
Test for phenolic compound	-
Test for glycoside	-
Test for coumerine glycoside	-
Test for carbohydrate	+
Test for fats and oils	+
Test for saponin	+

TLC: Methanolic extract of root powder of root showed 3 spots at 254 nm UV and 2 spots respectively at 366 nm UV using Chloroform & Methanol (7:3) v/v solvent system. A desirable R_f value lies between 0.03 and 0.9625, since it is likely that other compounds present in the mixture will be visible on plate. R_f value is used to compare and help identical compounds and to compare known and unknown substance to determine if they are same.

TLC of root (Table No.1.8):

Sample	Detection condition	No. of spot	R_f value
ROOT	254 nm UV	3	0.0375, 0.3375, 0.7
	366 nm UV	2	0.6, 0.7
	Common spot	1	0.7

DISCUSSION:

Plumeria pudica Jacq is an extra-pharmacopeial plant which is called "*ANUKTA DRAVYA"* in *Ayurveda*. This plant is native to Columbia, Venezuela & Panama. It is mainly utilized as purgative, in diarrhoea, itch cure, cough asthma, bronchitis, piles, fever, blood disorders dysentery, and tumours, it also possesses antibacterial, anti-inflammatory, analgesic[8] etc activities. It is hugely ethnomedicinally used by some tribals in Brazils, Venezuela etc. In India besides of its medicinal properties, this plant is also cultivated as an ornamental plant in garden for its showy flowers that used for religiotic purpose for worshiping god.

However, it has a great scope to use in treating various diseases in Ayurveda and could achieve good market attention even in allopathy. The presence of phytoconstituents especially compound like alkaloids shows great opportunities to find out its usages in humans to cure deadly and challenging diseases as alkaloids are highly potent to humans even in small dosages.

Conclusion:

Plumeria pudica Jacq. is hugely cultivated as ornamental plant in India. But it has great medicinal value. In future this plant will be used in both allopathic and ayurvedic formulation. This paper will be helpful for further study in research purpose.

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