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Development of Standardization Parameters of *Plumeria pudica* Linn: A wild species of Central India

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Abstract

Plumeria pudica Linn. commonly known as Nag champa is a fast growing, medium size plant belongs to family Apocynaceae. The plant is grown wildly or planted in garden and used traditionally for the treatment of fungal infections, inflammations and other vulnerable diseases. The fresh leaves and dried powder plant material were studied macro-morphologically and anatomically. Preliminary phytochemical investigation of plant material and WHO recommended parameters for the standardization were also performed. The present paper deals with study of various standardization parameters of *P. pudica* leaves to establish the quality control parameters of the species.

Key-words: *Plumeria pudica*, Standardization, Leaves

Introduction

India is endowed with a wealth of medicinal plants. It has a valuable heritage of herbal remedies like most developing countries the ethnic and rural population of India still relies on indigenous system of medicine to a great extent. Moreover, India exports large quantities of crude plants to the international markets at very cheap prices due to lack of economic profits to these currently almost worthless but abundant product of India is to screen these plants for the various biological and pharmacological activities. A sincere step is need to establish the standardization parameters of these medicinal plants to set their in-order to prove the safety, efficacy and genuity of these herbs so that manufactures can utilize them for identification and selection of the raw material for drug production.[1]

Plumeria pudica Linn. is a flowering plant of the family Apocynaceae. Different species of Plumeria are used for the cure of rheumatism, diarrhoea, blennorhea, venereal disease, leprosy, psychosis and diuresis etc.

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Plumeria species have also been investigated for isolation of irridoids and triterpenoids, which exhibited algicidal, antibacterial and cytotoxic activities. Iridoid glycosides were the first medicinally active compounds isolated from the species of *Plumeria.* Subsequently the latex and oil of some of these species were found to have other medicinally active constituents like sterols, carbohydrates, tannins, triterpenoids and alkaloids[2-3]. So, far no any systematic standards were developed for the selected species of *Plumeria* i.e., *P. pudica* Linn. therefore, the present work was conceived to reveal and set quality standards parameters.

Material and Methods

Collection and authentication of Plant Material

The leaves of *P. pudica* Linn. was collected in the month of Jan-Feb 2017 from the Malwa Region, Indore, (M.P.) and identified & authenticated by Dr. S.N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College,A.P.S. university, Rewa, M.P. and was deposited in our Laboratory,Voucher specimen No. PCog/PIL/001.

Standardization Parameters

Morphological studies

The morphology of leaves of the plant such as color, odor, size, shape, taste, surface characters and fractures was carried out as per standard protocol.[4]

Anatomical studies

The specimens of the proposed study were collected, care was taken to select healthy part and for normal organs. Then required samples of organ were fixed in FAA (formalin-5ml+ Acetic acid 5ml+ 70% Ethyl alcohol-90ml). Free hand transverse sections of fresh parts were taken, cleaned in chloral hydrate solution with gentle warming, stained with phloroglucinol and concentrated hydrochloric acid. They were mounted on slide in glycerine and studied under microscope. Microphotographs of sections were documented using microscope with camera, Nikon (14 mp). Descriptive terms of the anatomical features were revealed as given in the standard anatomy book .[5-6]

Quantitative microscopy

The fresh leaves of *Plumeria pudica* Linn. was subjected to standard procedure for the determination of various leaf constants (viz., Stomatal number, Stomatal index, Vein islet no, Vein termination number, Pallisade ratio).[11]

Physicochemical Evaluation

The dried parts of *PlumeriaPudica* Linn. were subjected to standard procedure for the determination of various physicochemical parameters .[7-10]

Successive Extraction of leaves

The shade dried coarsely powdered leaves of *Plumeria pudica* Linn. (250 g) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in a dessicator and percentage yield was determined.[9]

Preliminary Phytochemical Screening of Extract

Extract obtained after extraction were subjected for phytochemical screening to determine the presence of various active phytochemical present in the extracts. The standard procedure was adopted to perform the study[10-11].

Results and Discussion

The present work carries the results of 'Development of Standardization Parameters of *Plumeria pudica* Linn: A wild species of Central India.' It indicates the quality standards and utilization of selected plants for the treatment of several ailments among the inhabitants as mentioned in folk-lore and to validated scientifically.

Morphological studies

Leaves were simple, 10-20 cm long and 3-4 cm wide in size. The leaf margin morphology varies from pointed to smooth and leaf colour varies from light green to dark green, the leaf surface is smooth and have faint odor. (Figure 1, 2 & 3)

Table 1. Morphological features of leaves of *P. pudica* Linn.

S/No.	Features	Comments
1.	Color	UP=Green; LP=Light green
2.	Odor	Characteristics
3.	Taste	Pleasant
4.	Shape	Oblong
5	Size	L=14.6; W=2.3
6.	Fracture	Absent
7	Margin	Wavy& Entire

Figure 1. Leaf of *Plumeria pudica* Linn.

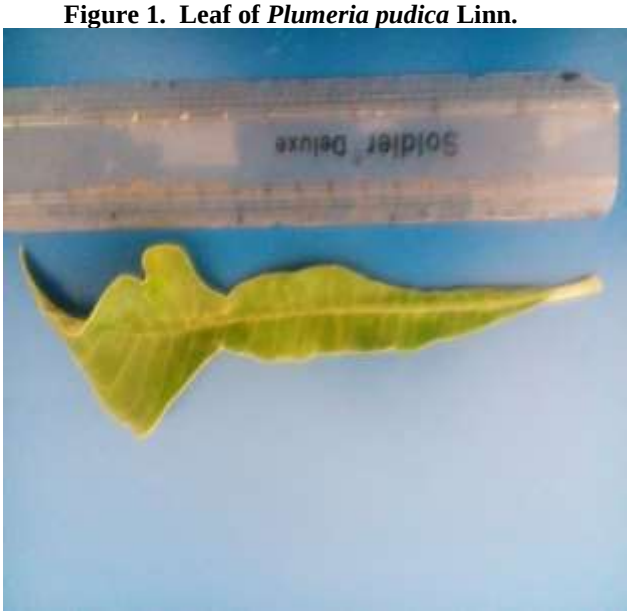


Figure 2. Measurement of leaf length of *Plumeria pudica* Linn.

Figure 3. Measurement of leaf width of *Plumeria Pudica* Linn.

Anatomical studies

The leaves *Plumeria pudica* Linn. is a typical dorsi-ventral leaf revealed the following characters. (Fig. 4) Epidermis is in two layers, one on each surface of the leaf, both the layers are composed of compactly arranged oval shaped cells. Intercellular spaces are absent. Lamina of transverse section shows an upper and lower epidermis covered by thin cuticle. Trichome are present on upper epidermis. Midrib consists of well developed collenchyma beneath the epidermis. It is the ground tissue that occurs between two epidermis region, composed of collenchymas cells. The mesophyll is characterized differentiated in to two region i.e., upper palisade parenchyma and lower spongy parenchyma. Pallisade parenchyma: It is composed of two to three layers of elongated, compactly arranged collenchymas cells. Intercellular spaces are absent. The cells contain very large number of chloroplast. Spongy parenchyma: It is composed of few layers of loosely arranged spherical or oval cholenchyma cells with prominent intercellular spaces. These cells contain few chloroplast cells. A vein represents the vascular bundles. They are found irregularly scattered in mesophyll due to reticulate venation. Each vein has bundle sheath composed of single layer of barrel shaped parenchyma cells. These bundle sheath included xylem and phloem. Xylem is found towards the upper epidermis and phloem towards lower epidermis. The vascular bundles are conjoint collateral with endarch xylem.

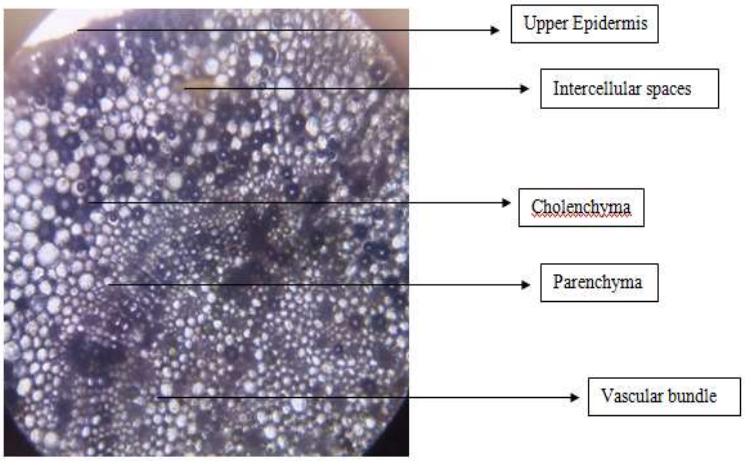


Figure 4. Anatomical Studies of leaf of *Plumeria Pudica* Linn.

Quantitative microscopy

The leaves constant such as stomatal numbers, stomatal index, palisade ratio and vein-islet numbers were determined and are presented (table 2).

Table 2. Leaf constant of *Plumeria pudica* linn.

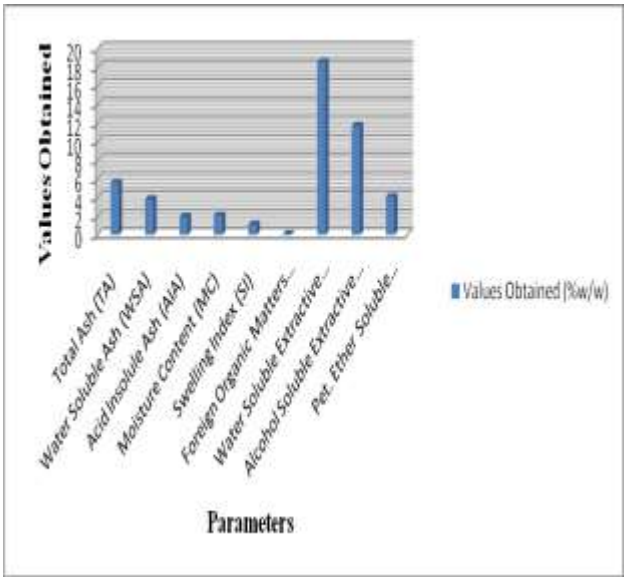
S/No.	Parameters	Values (1 mm^2)
1.	Stomatal numbers	
	Upper epidermis	3-7
	Lower epidermis	3-5
2.	Stomatal numbers	
	Upper epidermis	04.35-7.17
	Lower epidermis	19.32-28.32
3.	Pallisade ratio	
	Base:middle:apex	3.20:4.00:4.10
4.	Vein-islet number	15

Physiochemical Evaluation

The physicochemical evaluation of leaves of *Plumeria pudica* Linn. was carried out. Air dried material was used for quantitative determination of physiochemical values In this study ash values (total ash, acid insoluble ash and water soluble ash), moisture content, swelling index and foreign organic matters were determined (Table 3). Petroleum ether, alcohol and water soluble extractives were determined and were recorded. Alcohol and water extractive was determined as per WHO recommendations while petroleum ethersoluble extractive was determined due to the medicinal attributes of the extract. Water soluble extractive was found to be very high when compared to other extractable matter in the drug. (Graph 1)

Table 4. Physico-chemical analysis of Powdered Plant Material (*P. pudica* Leaves)

S/No.	Parameters	Values Obtained (%w/w)
1.	Total Ash (TA)	5.6183
2.	Water Soluble Ash (WSA)	3.8027
3.	Acid Insolule Ash (AIA)	1.9505
4.	Moisture Content (MC)	2.0832
5.	Swelling Index (SI)	1.1039
6.	Foreign Organic Matters (FOM)	0.0801
7.	Water Soluble Extractive value	18.5719
8.	Alcohol Soluble Extractive value	11.7325
9.	Pet. Ether Soluble Extractive value	4.0985



Graph 1. Physio-chemical analysis of *Plumeria pudica* Linn. leaves

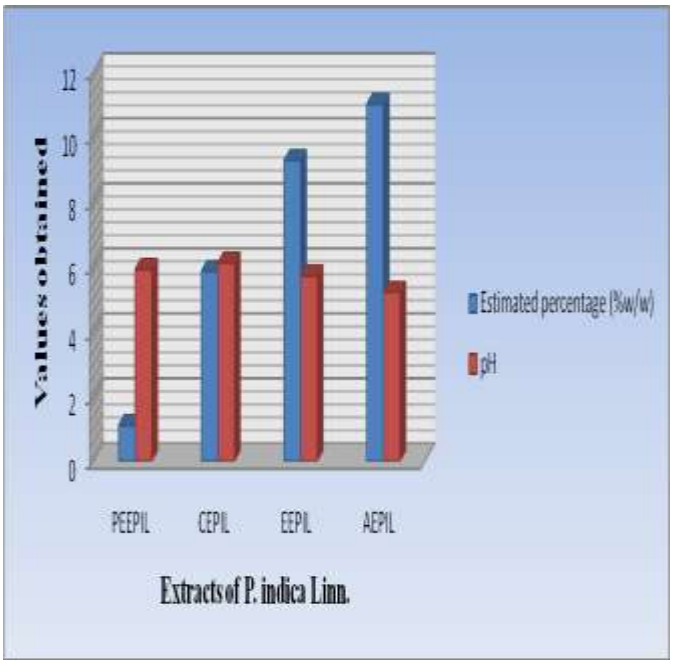
Extraction

The shade dried coarsely powder of leaves of *Plumeria pudica*Linn. was successively extracted with petroleum ether, chloroform, ethanol and water in a soxhlet apparatus. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator. The percentage yields of various extract along with their color, nature and pH were presented in Table 5 & Graph 2.

Table 5. Percentage yield of various extracts of *P. pudica* Linn. Leaves

Extract	Estimated percentage (%w/w)	Color of extract	Nature of extract	pH
PEEPIL	1.10	Light Brown	Solid Powder	5.9
CEPIL	5.84	Blackish Brown	Solid Powder	6.1
EEPIL	9.25	Blackish Green	Solid Powder	5.7
AEPIL	10.98	BlackishBrown	Semi Solid	5.2

Abbr. : PEEPIL: Pet. Ether extract of *P. pudica* Leaves, CEPIL: Chloroform extract of *P. pudica* Leaves, EEPIL: Ethanolic extract of *P. pudica* Leaves, AEPIL: Aqueous Extract of *P. pudica* Leaves, All values are Mean , n =3



Graph 2. % Yield and pH of *Plumeria pudica* Linn. leaves extracts

Preliminary Phytochemical Screening of Extract

S/No.	Constituents	Extract			
		PEE	CE	EE	AE
1.	Carbohydrates	-	-	+	+
2.	Glycosides	-	-	+	+
3.	Alkaloids	-	-	-	-
4.	Protein & Amino acid	+	+	+	+
5.	Tannins & Phenolic Compounds	-	-	-	-
6.	Flavonoids	+	+	+	+
7.	Fixed oil and Fats	-	-	-	-
8.	Steriods&Triterpenoids	+	+	+	+
9.	Waxes	-	-	-	-
10.	Mucilage & Gums	-	-	+	+

The extract obtained after extraction of plant material were subject to phytochemical screening which revealed the present of various active phytoconstituents**.** The results were presented in table 6.

Table 6. Preliminary phytochemical screening of PEE, CE, EE & AE of *P. pudica* leaves
Abbr.: - = Absent; + =Present; PEE=Pet. Ether extract; CE=Chloroform extract; EE=Ethanolic extract; AE=Aqueous extract

Conclusion

The standardization parameters of Indian medicinal plants *P. pudica* Linn. was studied which include the morphological, anatomical and physicochemical studies. The study of standardization parameters of species will be beneficial for the validation and assessment of quality control parameters of *P. pudica* Linn. to find out the presence of adulterant if any in order to establish the quality, safety and efficacy.

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