Pharmacognostical And Phytochemical Evaluation of *Plumeria Obtusa*(Linn.) Leaves

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Abstract

The quality management of the crude medication and their formulations is of great importance in acceptance of their acceptableness in present system of drugs that's why the topic of herbal drug standardization is massively wide and deep. This can be achieved only if the herbal products are evaluated and analyzed using various techniques of standardization. Plumeria Obtusa which is a herbal medicinal plant and is used in treatment of hyper proliferative tissue with gastroprotective activity, Anti-mutagenic activity and Anti-bacterial activity. This study reports on the standardization of Plumeria obtusa based on organoleptic characters, physical and physio-chemical properties. The crude drug has been evaluated on the basis of the following parameter that includes the morphology, qualitative as well as quantitative microscopy and physio-chemical characteristic studies of the plant (leaves). As a very limited research has been carried out on the plant, under the present study assumes singular significance and it is supposed to contribute a great deal to the existing literature. These observations would even be of huge price within the ayurvedic and herbal identification and standardization of the drug in crude type conjointly this study would facilitate differentiation of the drug from its other species.

Keywords: *Gastro protective activity, Anti-mutagenic activity, Anti-bacterial activity, physio-chemical*

1. Introduction

Medicinal herbs which we have been using since ancient times, many of these, are used as herbal remedies. The use of herbal medicine has been encouraged due to toxicity and side effects of allopathic medicines.

Acoording to WHO herbal medicines as medicinal product that contain active ingredients, aerial or underground parts of the plant. In the last few years there we have been noticing a tremendous growth in the field of herbal medicine. It has become popular in developing as well as in developed countries owing to its natural origin and lesser side effect [1].

As per the WHO the process of the physicochemical evaluation of crude drug includes the aspects, as selection and procurement of crude material, safety, and stability checking of final product, and maintaining the records for the safety purpose as well as to provide the product information to consumer and product promotion. The Pharmacognostical

and Phytochemical screening of any herbal plant provide method for the evaluation of crude drug. In this paper, we are focusing on the Pharmacognostical evaluation of the leaves plant *Plumeria obtusa*, belonging to the family Apocyanceae[2]. The Plumeria Plant is commonly known as Frangipani or white Champa. Plumeria is a genus of lacticferous trees and shrubs and there are about 8 species have been reported in India. Plumeria obtusa is a, native to Greater Antilles,northern Central America & southern Mexico. It is a large shrub or small tree growing to a height of 8 metres (26 ft.)[3]. Also it is found in West Bengal, Kolkata & various other places in India[4].

Chemical constituents The areal parts of P.obtusa contain pentacyclic triterpenoids namely kaneroside, oleandrin, α-amyrin, neriucoumaric acid, isoneriucoumaric acid, alphitolic acid, oleanonic acid, methyl p-E-coumarate and scopoletin[5]. iridoids, 6-O-acetyl plumieride[6], [7], plumieride p-Z-coumarate [8],(3β)-3,27-Dihydroxylup-29-ene, Obtusilinin,Oleandrin, Obtusol[9].Obstusin ,Obstusilic acid, β-Hydroxy-27-[(Z)-p-coumaroyloxy]-urs-12-en-28- oic acid, Obtusinin, Obtusilin (¼ 3b-hydroxy-11-oxours-12-en-28-oic acid), Obtusinidin, Obtusidin, 27-[p-(E)-Coumaroyloxy] ursolic acid[15] (20Z)-Dammara-,20(22)-dien(3 β,20Z)-dammara-12,20(22)-dien-3-ol, (3β)-Olean-12-ene-3, 27-diol, 27-Hydroxyolean-12-en-3,Urs-12-en-3-one,(3β)-27-[(Z)-Feruloyloxy]-3-hydroxyurs-12-en-28-oic acid[10]. Uvaol (¼(3b)-3,28-dihydroxyurs-12-ene)[11]. The plant is mainly grown for its ornamental and fragrant flowers. *Plumeria obtusa* Linn (Apocynaceae) is used in the treatment of ulcers used for Anti-mutagenic activity[12].,Anti-bacterial activity[16],[17],[18],and treatment of hyper proliferative tissue[13]. and the methanolic extract of *Plumeria obtusa* from the stem barkwas evaluated for gastroprotective activity by pylorus ligationann indomethacin models. The extract showed activity due to reduction of gastric acid secretion, gastric cytoprotection and proton pump inhibition mechanism [19].

2. Materials and Methods

2.1. Collection of Plant

The leaves of *Plumeria obtusa* were collected from N.I.E.T. campus and surrounding areas during February 2018. A proper herbarium sheet containing stem,leaf,flower was prepared and plant was given authentication by Dr.K.C BHATT, NBPGR, PUSA. They were cleaned (washed) with water to remove solid particles, dust material and other debris. Then they were dried under sunlight for few days. The dried leaves were then grounded to a coarse powder in a grinder and stored in a well closed air –tight container.

2.2. Macroscopy

The following macroscopic characters for the fresh leaves were noted: Size and Shape, Colour, Odour, Taste, Lamina, Surface, Apex, Margin and Base.

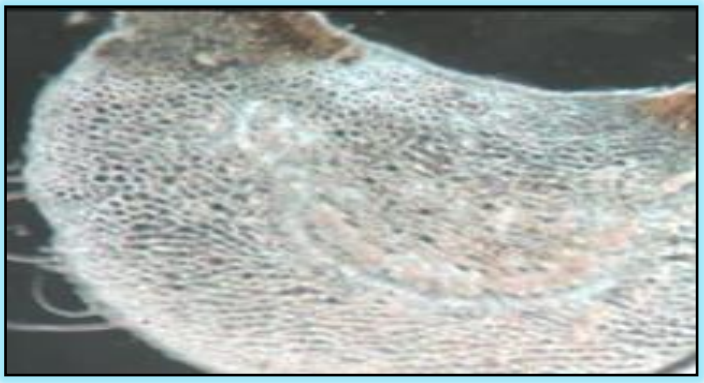

Figure 1. T.S of *Pobtusa*

2.3. Microscopy

2.3.1. Qualitative microscopy

In the solution of chloral hydrate epidermal membrane layer was cleared and warm for half an hour and dyed with Phloroglucinol and conc. HCL and mounted with glycerine and observed beneath the compound microscope. The following was observed. Epidermal cell, Stomata (on upperand lower surfaces) and trichomes .The transverse sections of the fresh leaves through the lamina and midrib as well as small quantity of powdered leaves were found to be cleared mounted and observed[14].

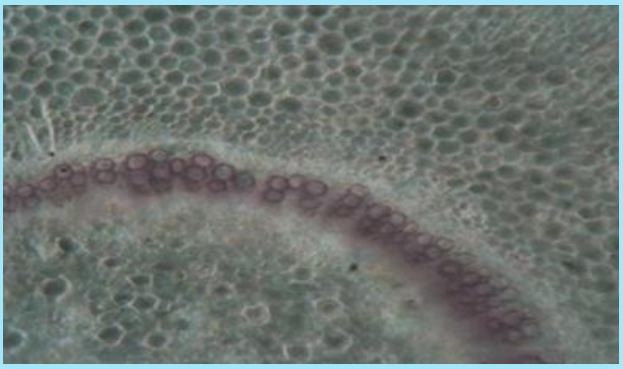

Figure 2. T.S of *P.obtusa* vascular bundles

2.3.2. Quantitative microscopy

Quantitative leaf microscopy to determine Palisade ratio, Stomatal index, Vein-islet number and Vein Termination number were carried out on epidermal strips.

Stomatal number & Stomatal index

1) The piece of leaf (middle part) was cleaned by boiling with chloral hydrate solution. The upper and lower epidermis was peeled out by means of forceps.

2) A camera Lucida and drawing board was arranged for making the drawing to scale.
3) A square of 1mm was drawn by means of stage micrometer.
4) The slide with cleared leaf was placed on the stage.
5) The number of stomata and epidermal cells was counted.
6) The stomatal number and stomatal index was calculated.

Figure 3. Lower leaf stomata

FIG 4. Upper leaf stomata

Vein –islet number & Vein termination number:

1) A piece of the leaf was cleaned by boiling in chloral hydrate solution for about thirty minutes.
2) A camera Lucida & drawing board was arranged for making drawings to scale.
3) Stage micrometer was placed on the microscope & a line of 1mm was drawn & a square was constructed on this line.
4) Moved the paper so that the squire is seen in the eye piece in the centre of the field.
5) The slide with the cleaned leaf was placed.
6) The vein islet number &vein termination number was counted.

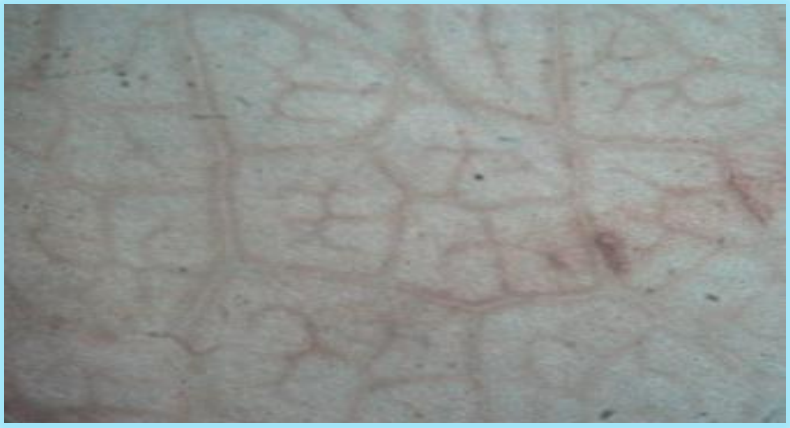

FIG 5. Vein Islet No

FIG 6. Vein Termination No

Palisade ratio

1) A piece of the leaf was cleaned by boiling in chloral hydrate solution for about thirty minutes.
2) A camera Lucida & drawing board was arranged for making drawings to scale.
3) The outline of four cells of the epidermis was traced off by using the 4 mm objective.
4) Then palisade layer was focused down &sufficient cells were traced off to cover the tracing of epidermal cells.
5) The palisade cells under the four epidermal cells were counted.

The average number of cells beneath single epidermal cells was calculated

3. Results

3.1. Macroscopy

The following macrocopic characteristics of fresh leaves were noted:

Table1. Macrocopic characteristics of fresh leaves of *P.obtusa*

Parameter	**Size**	**Shape**	**Colour**	**Margin**	**Odour**
Result	12-14cm, 4-6 cm wide	Lanceolate	Dark green	Entire	Slight
Parameter	**Taste**	**Base**	**Venation**	**Apex**	**Texture**
Results	Sweet-soul	Asymmetrical	Reticulate	Acute	Smooth

3.2. Microscopic Characters

3.2.1. Qualitative Microscopy

Midrib

The leaf has very thick midrib and thin lamina arising from the adaxial – lateral portion of the midrib. The ground tissue is differentiated into outer zone of smaller collenchyma cells and remaining portion being parenchymatous. The collenchyma zone is more in the upper part and less wide in the lower part. Narrow thick walled, circular or lobed laticifers are seen randomly dispersed in the ground tissue. The vascular system consists of a main, wide, bowl shapedthin strand and two small, less prominent accessory adaxial lateral strands. It consists of short, radial fibers of 3-5 angular xylem elements and a thin layer of phloem along the outer metaxylem side. Within the concavity of the vascular arcs these are numerous small nests of phloem elements which are known as inner phloem. The xylem elements are wide.

Lamina

The lamina is trichomatous on the outer side and smooth and glabrous on the inner side. The major lateral veins project prominently into conical outer part. The lateral veins and veinlets have a small cluster of xylem elements and a thin arc of phloem. The vascular bundles are subtended by thick mass of parenchyma. The cells are mostly rectangular. The outer epidermis is thin and the cells are narrow and cylindrical. The spongy mesophyll consists of several lobed parenchyma cells which are interlinked with each other around the air chambers.

Epidermal Trichomes

The trichomes are non-glandular or covering type and arise from a group of dilated epidermal ells. The trichomes are multicultural, uniseriate and unbranched. They are narrow and thick walled with smooth surface

Leaf margin

The marginal part becomes slightly thin with rounded bent down edge. The epidermal layer of the leaf margin is thin, the cuticle is very thick and smooth. The palisade – spongy parenchyma ifferentiation is absent in the marginal portion. It consists of 4 or 5 layers of small, thick walled compact parenchyma cells. The marginal part of the lamina is 150μm thick.

Epidermal cells and Stomata

The epidermal cells are rectangular to polyhedral in shape. The cells have straight or slightly wavy walls. The walls are moderately thick. The stomata are paracytic type; there are two subsidiary cells, lying on the lateral sides of the guard cells. The subsidiary cells may be equal or slightly unequal in size. The guard cells are elliptical with wide stomatal aperture.

3.2.2. Quantitative Microscopy

Table2. Quantitative microcopical characteristics of fresh leaves of *P.obtusa*

Parameter	Palisade ratio		Stomatal number		Stomatal index
	Lower	Upper	Lower	Upper	Lower
Result	6	4	8-9	6-7	23
Parameter	Veins-islet number		Veins-termination number		Stomatal index
	Lower	Upper	Lower	Upper	Upper
Result	10	11	23	27	28

3.2. Phytochemical investigation

Table3. Chemical constituents present in *P.obtusa* leaves

S. No.	**Secondary metabolites**	**Result**	**S. No.**	**Secondary metabolites**	**Result**
1	Alkaloides	+ve	6	Carbohydrates	+ve
2	Sapanin Glycosides	-ve	7	Proteins	-ve
3	Tannins	+ve	8	Oxalic Acid	+ve
4	Anthraquinone glycoside	-ve	9	Melic Acid	-ve
5	Cardiac glycoside	-ve	10	Tartaric Acid	+ve

3.3. Physical Parameter

Table 4. Some physical parameters calculated after doing analysis of *P.obtusa* leaves

S. No.	Parameter	Initial weight (gm)	Final weight (gm)	Result (%)
1	Ash value	2.00	0.19	9.5
2	Moisture content	1.50	1.41	7.3
3	Acid-insoluble content	2.00	0.01	0.5
4	Extractive Value(Ethanol)	2.50	0.25	20
5	Extractive Value(Water)	2.5	0.31	24.8

4. Discussion

P.obtusa is currently being used in the treatment of various disease condition .The standardization of a crude drug is an integral part of establishing its correct identity, before any crude drug can be included in a herbal pharmacopoeia standards and parameters must be established as per who guidelines.

P.obtusa is a plant that has been confused with other species due to their relative similarities The results of these investigation could therefore serve as basis for proper identification collection and investigation of the leaf described distinguishes it from other members of the genera.

Chemomicrocsopy and quantitative leaf microscopy are parameters that are unique to the plant and required in its standardization.

5. Conclusion

The purpose of this research work on standardization of herbal plant is to study the potential usefulness including evaluation, safety, and efficacy of *Plumeria obtusa* which is used to cure and possess gastroprotective properties and Anti-mutagenic activity, Anti-bacterial activity and used in treatment of hyper proliferative tissue. This study reports on the standardization of *Plumeria obtusa* based on organoleptic characters, physical and physico-chemical properties.

The macroscopic investigations revealed that the leaf shape was lanceolate, colour dark green and venation-reticulate.

Microscopic investigations revealed that the stomatal number (upper surface) was 6-7 and lower surface was 8-9.Vein islet number was 10 and vein termination number was 19.

Phytochemical screening in the methanolic extract showed the presence of alkaloid and tanins. Aqueous extract showed the physical investigations were done to ascertain the ash value, acid insoluble ash value, moisture content.

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