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Pharmacognostic and phytochemical studies on *Plumeria obtusa* L

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ABSTRACT

From last few decades there is an upsurge in the use of herbal medicines all over the world and these herbal drugs should be studied scientifically to develop their monographs to assure their quality. The present research work was an attempt to establish parameters for identification of *Plumeria obtusa* L. (Fam. Apocynaceae) according to the guidelines of WHO, by studying its morphological and organoleptic characters, detailed microscopic evaluation, histochemical studies, fluorescence analysis, loss on drying, extractive values, swelling index, foaming index and preliminary phytochemical screening of the leaves and flowers. Although conventional, but it is simple, easy and quick way for identification and standardization of herbal substances affordable even in developing countries. This is the first report on pharmacognostic and physicochemical studies on *P. obtusa* which can be helpful in establishing pharmacopoeial monograph of this plant.

Keywords: Phytochemical studies, Pharmacognostic studies, *Plumeria obtuse*.

INTRODUCTION

The genus *Plumeria* (family Apocynaceae) is a group of shrubs and trees consisting of eight species growing in the tropical areas of the world. In Pakistan, it is represented by two species i.e. *Plumeria obtusa* and *Plumeria rubra*, being grown as ornamental plants [1]. *P. obtusa*, commonly known as Singapore graveyard flower is an evergreen tree, grown as ornamental plant for the attractive and fragrant flowers. On commercial scale, essential oil extracted from its flowers is used in perfume industry [2, 3]. In traditional medicines, *P. obtusa* is used to treat itching, asthma, headache, bronchitis, ulcers, cough, fever, blood disorders, tumors and as purgative [4, 5]. In experimental models, *P. obtusa* has shown gastroprotective, antibacterial, antifungal, antioxidant and anti-inflammatory activities [6-10]. Iridoids and triterpenoids are the main phytoconstituents found in the leaves and flowers of this plant [11-17]. Traditional uses, phytoconstituents and biological studies reported in literature make this plant a suitable candidate to be studied further to unveil more useful biological activities and phytoconstituents. For all these studies to be done correctly, authenticity of the plant material is the crucial step. In this research work, we tried to set quality parameters to check the authenticity of *P. obtusa*.

MATERIALS AND METHODS

All the solvents, chemicals and reagents used were of analytical grade. Solutions and reagents were prepared according to United States Pharmacopoeia and British Pharmacopoeia.

Plant collection

Fresh leaves and flowers of *P. obtusa* were collected from vicinity of Bahauddin Zakariya University Multan. Specimens were identified by Dr. Zafarullah, Institute of pure and applied Biology, Bahauddin Zakariya University Multan and a specimen voucher number Stewart 565 was allotted. Plant material was used as fresh or shade dried depending on the nature of the experiments performed.

Macroscopic characters and preliminary tests

Macroscopic characters of fresh and shade dried leaves and flowers were evaluated. Small quantity of powder was pressed between the layers of filter paper to check oily stain and small quantity was mixed with water and shaken vigorously to check persistent froth indicative of saponins. Powdered plant material was also allowed to stand in water to observe gummy substances [18-20].

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Microscopic and histochemical studies

Microscopic and histochemical studies were performed according to the methods of WHO, Johansen and Khandelwal [21, 22].

Fluorescence analysis

Fluorescence analysis was performed by treating the powders with different reagents [23, 24].

Physico-chemical parameters

Physicochemical parameters were studied according to the guidelines of WHO [19].

Phytochemical studies

Preliminary phytochemical screening was done according to the prescribed procedures [25].

RESULTS

Morphological studies, organoleptic characters and some preliminary tests

Flowers

The flowers are produced on the cluster of stalks (compound cymose inflorescence) (Fig. 1). Each flower has green synsepalous sepals and white colored petals. Petals are fused at the base and form a funnel shaped tube (Fig. 2). Ovary is superior. Each of the sepals, petals and anthers are five in number. Length and width of petal is up to 4.1 and 2 centimeters respectively. Color of shade dried petals was light down, with the fragrance similar to the original fresh flower and the petiole was hard (Fig. 2). The powder of shade dried petals was light brown in color possessing the characteristic fragrance like fresh flowers and slightly sweet taste. No spot of oil was seen on the filter paper after pressing the powder between the layers of filter paper. Test performed for the gummy material was also negative.

Leaves

Leaves are simple, bright green in color, arranged alternatively with adaxial side shinier than abaxial side (Fig. 3). Leaves measure up to 26 cm in length and up to 10 cm in width. The pedicel of mature leaf is up to 5 cm (Fig. 4). Leaves contain soft hairs on both sides and have pinnately netted venation. Leaves present near the start of the branch are small in size and have cuneate leaf bases. They have coarse texture and are not flexible. The both sides of shade dried leaf blade were greenish black in color, odorless, hairy and crumpled. Powder of shade dried leaves was greenish back colored. Odor was almost similar to that of fresh plant with slightly bitter taste. No spot of oil was seen on the filter paper after pressing the powder between the layers of filter paper. Test performed for the gummy material was also negative.

Microscopic evaluation

Flowers

Surface tissues

On the surface of the petals, irregular epidermal cells and non-glandular trichomes were found. Stomata were absent (Fig. 5).

Powder

In powder of petals, fragmented irregular epidermal cells and non-glandular trichomes similar to those of fresh plant were observed.

Leaves

Surface view

Anomocytic type stomata were found on the abaxial side of the fresh leaves. Epidermal cells of both sides are of irregular shape, stomata occupying a wide area of the leaf surface. Trichomes were also observed on the abaxial side of the leaf (Fig. 6).

Transverse section of leaf through mid rib

The transverse section of the leaf through the mid rib shows the collenchyma cells making the outer smaller zone and parenchyma occupying the remaining part (ground tissues). This collenchyma zone is greater in size in upper region than in lower. Thick walled lactifers (circular shaped or lobed) are also dispersed in an irregular manner in these ground tissues. The vascular system of the leaf comprises of two regions. First is a major thin strand which is wide and resembles to a bowl in shape, while the other one consists of the accessory two lateral strands present on the upper surface and are less prominent. It comprises of angular xylem which is short and has three to five radial fibers while the phloem is thin layered and present along the outer metaxylem. There is also inner phloem in the form of small nests and present across the vascular bundle (Fig. 7).

Transverse section of leaf through lamina

Abaxial side of lamina is trichomatous while it is smooth on the adaxial side. The major lateral veins project from the conical abaxial part. A cluster of xylem elements along with a very of thin phloem arc are present in the vein lets. This vasculature is flooded by heavy masses of parenchyma cells which go up to the adaxial area in the upper epidermis. The adaxial surface has square shaped enlarged cells which are characterized by the presence of thick cuticle. In contrast the abaxial side has thin and narrow cylindrical shaped cells. There are many lobed parenchyma cells present in spongy mesophyll (Fig. 8).

Powder

The leaf powder has irregular shaped epidermal cells, with anomocytic stomata. Lactifers are observed quite abundantly in the leaf powder.



Figure 1: Cluster of the stalks at which flowers are produced



Figure 2: Flowers of *P. obtusa* A. Fresh; B. Shade dried

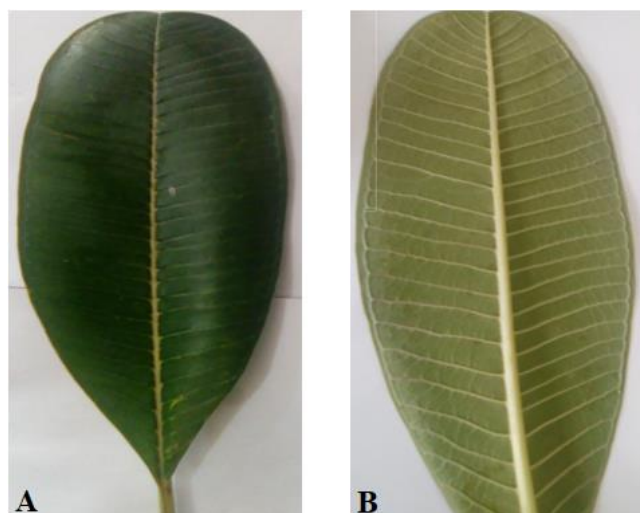


Figure 3: Leaves of *P. obtusa* A. Adaxial side; B. Abaxial side



Figure 4: Leaves of *P. obtusa* A. Length of leaf; B. Width of leaf; C. Length of pedicel

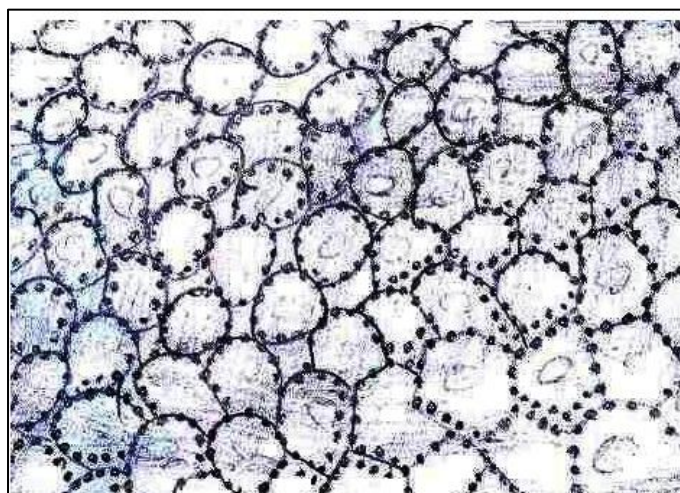


Figure 5: Surface of petals with irregular shaped cells and no stomata

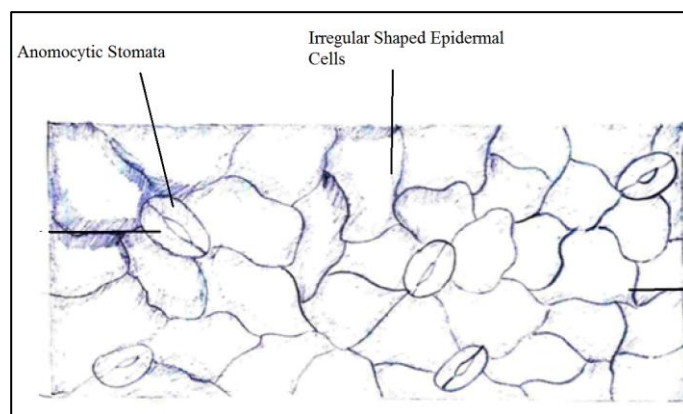


Figure 6: Abaxial surface of leaf showing anomocytic stomata

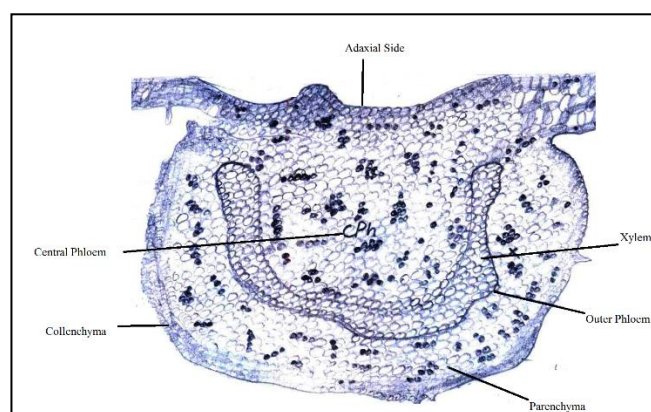


Figure 7: Transverse section of leaf through mid rib

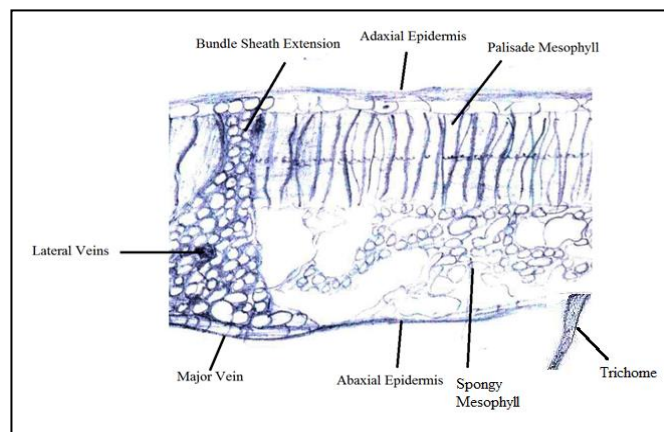


Figure 8: Transverse section of leaf through lamina

Histochemical studies

Table 1: Results of histochemical studies of flowers of *P. obtusa*

Phytoconstituents	Reagents used	Histochemical zone	Observation	Results
Lignin	Safranin solution	Vascular bundles	Pink color	Present
Lignin	Phloroglucinol solution	Trichomes	Cherry red color	Present
Lignin	Safranin solution	Trichomes	Pink color	Present
Lignin	Phloroglucinol solution	Vascular bundles	Cherry red color	Present
Starch granules	Iodine solution	Dried powder	Dark blue color	Present
Tannins	FeCl ₃ solution	Dried powder	Greenish black color	Present
Volatile oils and fats	Sudan red solution	Dried powder	No color	Absent

Table 2: Results of histochemical studies of leaves of *P. obtusa*

Phytoconstituents	Reagents used	Histochemical zone	Observation	Results
Lignin	Safranin solution	Xylem	Pink color	Present
Lignin	Phloroglucinol solution	Xylem	Cherry red color	Present
Lignin	Safranin solution	Trichomes	Pink color	Present
Lignin	Phloroglucinol solution	Trichomes	Cherry red color	Present
Starch granules	Iodine solution	Dried powder	Dark blue color	Present
Tannins	FeCl ₃ solution	Dried powder	Greenish black color	Present
Volatile oils and fats	Sudan red solution	Dried powder	No color	Absent

Fluorescence analysis

Table 3: Results of fluorescence analysis of flowers of *P. obtusa*

Treatment	Visible (Day light)	Ultra violet (Short wavelength)	Ultra violet (Long wavelength)
Powder as such	Blackish green	Blackish green	Blackish green
Powder in distilled water	Brown	Brown	Brown
Powder in ethanol	Black	Black	Dark black
Powder in conc. HCl	Yellowish brown	Green	Green
Powder in 10% H ₂ SO ₄	Dark black	Black	Black
Powder in iodine solution	Dark green	Green	Greenish back

Table 4: Results of fluorescence analysis of leaves of *P. obtusa*

Treatment	Visible (Day light)	Ultra violet (Short wavelength)	Ultra violet (Long wavelength)
Powder as such	Brown	Brown	Light brown
Powder in distilled water	Brownish green	Dark green	Brown
Powder in ethanol	Blackish brown	Black	Dark black
Powder in conc. HCl	Yellow`	Dark green	Blue
Powder in 10% H ₂ SO ₄	Dark brown	Black	Black
Powder in iodine solution	Dark green	Green	Blue

Physico-chemical parameters

Table 5: Physico-chemical parameters of flowers of *P. obtusa*

Physico-chemical parameter	Value
Loss on drying	1.1% w/w
Swelling index	9 ml
Foaming index	Less than 100
Alcohol soluble extractive value (Hot)	3% w/w
Water soluble extractive values (Cold)	9.52% w/w

Table 6: Physico-chemical parameters of leaves of *P. obtusa*

Physico-chemical parameter	Value
Loss on drying	19.64% w/w
Swelling index	5 ml
Foaming index	Less than 100
Alcohol soluble extractive value (Hot)	2.75% w/w
Water soluble extractive values (Cold)	7.85% w/w

Preliminary phyto-chemical analysis

Table 7: Preliminary phytochemical analysis of flowers and leaves of *P. obtusa*

Phyto-constituents	Flowers	Leaves
Alkaloids	Absent	Absent
Cardiac glycosides	Present	Present
Free anthraquinones	Absent	Absent
Anthraquinone glycosides	Absent	Absent
Tannins	Present	Present
Saponins	Absent	Absent

DISCUSSION

The aim of this research work was to identify and report some important pharmacognostic characters of the medicinally important plant *P. obtusa*, which will be helpful to develop a monograph of the plant in the official books.

This research work was confined to the leaves and the flowers of *P. obtusa*. When these parts of the plant are viewed in fresh state, they are very easy to identify and no issue arises in their use for medicinal purpose. But if the crude drug of these parts either dried or powdered form is used, many complications including identification and authentication arise. This is also accompanied by adulteration to varying limits. This research works offers a solution to such problems by establishing the standards for different parameters for this plant.

During this study plant material was tested for various pharmacognostical parameters. Not only the fresh parts of the plant under consideration were studied for their morphological and organoleptic characters but also their surface and trans-sectional microscopic views were also described. For example, presence of anomocytic stomata in the abaxial surface of leaf, presence of irregular epidermal cells in the leaves, absence of stomata in the petals and presence of irregular shaped epidermal cells. While leaf having pinnately netted venation with cuneate base and obtuse end represent some of the organoleptic characters of the plant. While in transaction of mid rib presence of characteristic central phloem, outer phloem, xylem and other accessory structures in a bowl shape provides a strong base for setting identification parameters for this plant. Along with this, dried and powdered form of these parts were subjected to fluorescence analysis, preliminary phyto-chemical analysis, physico chemical analysis and results were noted which represent the specific behavior of this plant, helpful in its authentication and identification.

There were few starch grains present in the leaf and petal powder. They produced the characteristic bluish black color. Different other color was also produced when the powders were treated with other reagents and also when observed under the day light and ultraviolet light of short and long wavelengths. All of these count for specific characteristics features of the plant material. High extractive values of petals and leaves with water as compared to with alcohol suggest that there is more water soluble phyto-constituents in these parts than in alcohol. This plant not only contain cardiac glycosides, which are in higher concentration in leaves than in petals but also contain tannins. Thus, future evaluation in this area can be helpful for discovery of new drug. Lignin is another phyto-constituent which is abundantly present in this plant. The powder of this plant does not produce the spot on the filter paper and test for gummy and mucilaginous material is also negative.

CONCLUSION

There are still a lot of possibilities to explore different hidden aspects of this plant by using latest developed techniques for example authentication based on DNA studies, microscopic evaluation for quantitative studies and elaboration of medicinally important phyto-constituents responsible for therapeutic effects.

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Conflict of interest

We declare that we have no conflict of interest.

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