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Morphological and anatomical characterization of *Plumeria obtusa* L.: An Ayurvedic medicinal plant

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Abstract

The development of commercial natural products has significant obstacles due to the exploitation, adulteration and substitution of herbs. In order to standardize medicinal plant, the morphological microscopic inspection is the initial stage. The present investigation deals with morphological evaluation of leaf, seed pod, flowers and anatomical evaluation of *Plumeria obtusa* L. seed pod. *P. obtusa* (Apocynaceae) is an outdoor decorative plant. Size, colour, odour and taste of plant material were evaluated under morphological features. The traditional procedure of section cutting was used to analyze the seed pod's anatomy. Using a high-resolution microscope, examinations of microscopic structures were carried out. The plant's leaves are dark green colour, thick and leathery according to the morphological inspection. The winged seed pods are brown to dark brown at maturity stage having T shape double pod with course texture. Stomatal number (7-9 sq. mm), stomatal index (21.88-24.32%), vein islet number (12-15/mm^2) and vein termination number (16-20/mm^2) were observed in leaf constant evaluation. Cyclocytic and sunken stomata, lignified peripheral and xylem fibres, rhomboidal shape calcium oxalate crystals, vascular bundles, *etc*., were all discovered in detailed microscopical study. The morphology of leaves and microscopic findings of the plant *P. obtusa* can be used for standardization and authentication.

1. Introduction

Quality control evaluation of ethnomedicine helps in their regulation or authentication purpose (Kumar *et al.*, 2022). The WHO also stressed the need to investigate medicinal plants to understand better their properties, safety and efficacy (Warrier, 2021). In the process of scientific research and botanical quality control, the morphological, anatomical, and microscopic characterization of plants has immense importance (Grazina *et al.*, 2020).The characteristics are applied for authentication of the plants and also for detection of contaminations, adulteration and substitutions in plant products (Michetti *et al.*, 2019; Upton *et al.*, 2011). For microscopic standardization evaluation of structural, cellular and molecular aspects of herb or herbal parts is performed with various microscopic techniques (Tam *et al.*, 2006). As mentioned in many pharmacopoeias, microscopy has been used since long time to identify herbal products. Microscopic studies are preferred due to numerous advantages such as need of small amount of sample, rapidity, reliability, cost effectiveness and simplicity of the procedures (Au *et al.*, 2009).

P. obtusa plant is geographically distributed in northern central America and southern Mexico, Greater Antilles as well as in Florida (Herbal and Natural Medicine, 2021; Nabi and Lalit, 2017). *P. obtusa* is a member of the Apocynaceae family, with common names such as Champa (India), Singapore graveyard flower (Singapore), Melia (Hawaii), White Frangipani (Australia), Araliya (Sri Lanka) and Temple tree (United Kingdom) (Bihani *et al.*, 2021). The *P. obtusa* has many medicinal uses (Bihani *et al.*, 2021; Dogra, 2016; Shinde *et al.*, 2014). Therefore, it is necessary and an important step to standardize it through the suitable depiction of its quality standards.

The morphological and anatomical characters of *P. obtusa* were evaluated in this study with an aim to identify its imperative features so that they can be effectively applied for herbal and herbal products standardization. The studies will aid to justify safe and effective use of herb as medicine by ensuring quality of the herb used.

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2. Materials and Methods

2.1 Collection and authentication of plant material

P. obtusa plant was harvested from Akole (Mehenduri), Ahmednagar, Maharashtra, India. After that, the plant's leaves and seed pods were cleaned to eliminate dust and other contaminants. The herbarium of the plant submitted for authentication at Botanical Survey of India (BSI), Pune, Maharashtra, India. The seed pods were air dried under the shade before being electrically ground into powder, sieved through a 20-mesh sieve, and kept in an airtight container for future research.

2.2 Chemicals

The chemicals used for study were of analytical grade and includes phloroglucinol, glycerin, chloral hydrate, potassium hydroxide, *etc*.

2.3 Morphological characterization

The morphological characters are simpler to notice and more useful to employ. These characters have traditionally been used more frequently than anatomical and molecular characters. Despite being outdated, morphology continues to serve as the foundation for solving taxonomical challenges. Morphological evaluation of *P. obtusa* leaves, flowers and seed pods have been accomplished according to herbal medicine quality control techniques used by the WHO. We examined the leaves' surface, fracture, colour, odour, shape, and size. Through magnifying lens, internal and external structure of leaves and other parts was thoroughly observed (World Health Organization, 2020).

The colour and shape was confirmed by visual examination. The leaves and seed pods size were measured using scale. Some amount of crude drug was stored in a beaker to test the smell, and it was detected by repeatedly inhaling air over the substance. Prior to studying different odour sensations including aromatic, rancid, fruity, mouldy and musty, the odour strength was first determined whether, it is strong, distinct, none, or weak). Taste was also determined.

2.4 Anatomical evaluation

2.4.1 Determination of stomatal number and index

The average number of stomata per square millimeter (mm) of epidermis is defined as stomatal number and it was determined using the procedure given by Kumar *et al.* (2012). The leaf sample was boiled with chloral hydrate to get cleaned epidermis and with forceps lower and upper epidermis were pealed. The tissue is then placed on the slide using glycerin. A camera lucida was fixed onto the microscope under high power and a drawing sheet was placed onto the platform to trace the magnified microscopic images easily. A stage micrometer was used to draw a square having an area of 1mm^2. The mounted slide was then placed onto the stage of the microscope. The epidermal cells and the visible stomata were subsequently traced onto a drawing sheet and counted. The Stomatal index is calculated by making use of the formula;

$$I = S/E + S \times 100$$

Where I is stomatal index, S is number of stomata per unit area and E is number of epidermal cells in same unit area of cleared leaf epidermis (From margin and midrib).

2.4.2 Vein-islet number and vein termination number determination

The average number of vein-islets per square millimeter (mm) of leaf surface is counted as vein-islet number. The vein-islet and vein termination number were determined using the standard procedure (Shah, 2016). The slide with the clean epidermis was mounted on microscope's stage fixed with camera lucida, and the veins were traced within square, drawing outlines of islets which lay on two adjacent sides of square. The number of vein islets in the square was then counted. Wherever the islets overlapped the sides of square, only two adjacent sides of square were considered. The average number of vein islets from four such adjoining squares to obtain value for 1 mm^2.

Vein terminations are the vein endings observed on the leaf epidermis. The vein termination number is the average number of vein terminations per millimeter square of the leaf epidermis. Wherever the vein terminations intersected the sides of the square, only two adjacent sides were considered. Therefore, the mean of vein terminations from four adjoining squares was measured to obtain final value of 1 mm^2.

2.4.3 Qualitative microscopic evaluation of plant

The microscopic evaluation was studied as per standard procedures (Kokate *et al.*, 2008; Pandya *et al.*, 2010). To soften the seed pods, they were cut into small pieces and boiled in water for 2 min. Transverse section of softened seed pod was taken through midrib to observe epidermis and stomata. Clear, transparent sections were prepared by treating them with choral hydrate and warming gently over a micro-bunsen flame. Thinnest sections were stained with phloroglucinol solution, followed by few drops of HCl after drying for 5 min. Then the sections were covered with coverslip for observation under Olympus CX-21i trinocular Microscope.

3. Results

3.1 Authentication of plant

Fresh plant was collected and submitted to Botanical Survey of India, who has validated the same based on herbarium and the accessible database and provided the authenticity certificate vide no. BSI/WRC100-1/TECH/2019/62, dated 19 December, 2019.

3.2 Morphological characterization

P. obtusa is mostly an outdoor ornamental plant. A shrubby, angiosperm, terrestrial plant, growing either as a small shrub or tree between 0.9-6.1 meters high. The dry soil, moderate water, and full or semi-shade are the plant preferences for growth. The leaves, stem and its branches are yield a milky sap. The branches are succulent, fleshy, thick, widely spaced and pale green in colour. The fresh plant, leaf and seed pods are given in Figure 1.

3.2.1 Leaves

The leaves are thick and leathery, dark green, a little shiny, have tertiary veins, obovate with blunt ends and are seen in groups near tips of branches petiolate, obovate to oblong-obovate long (6-22 cm) and wide (2-7 cm). Leaves are arranged in spiral pattern. It is a simple leaf with reticulate venation. The apex is rounded. The apex of leaf shows distinct tip and acute base. The earliest leaves on the branch have short and cuneate leaf bases. It has stiff and rough feel. The dried leaf blades were hairy, wrinkle and greenish black in colour on both sides.

Figure 1: **The morphology of *Plumeria obtusa* L. plant shows A:The fresh *Plumeria obtusa* L. plant, B: Leaf of plant, C: Flowers of plant with green synsepalous sepals and white colored petals, D: Growing seed pods of plant which will change from green colour to black and then brown at maturity.**

3.2.2 Flowers

Plant has the compound cymose inflorescence, which is a cluster of stalks bearing flowers. White petals and green synsepalous sepals make up each flower. All base-fused petals create a tube with a funnel shape. The ovary is in superior position. There are five petals, sepals and anthers on each flower. Petal length and width was 4.1 and 2 centimeters, respectively. The petiole is rigid, the dried petals are a light brown colour and the aroma is comparable to that of the original fresh flower. Flowers appear in clusters, mostly at branch tips. Each blossom measures about 5 cm in diameter. It is creamy-white and has an orange center. The calyx is 5 lobed, with equal to sub-equal lobes (Kamran *et al.*, 2020).

3.2.3 Seed pods

After pollinated, *P. obtusa* flowers are begins to fade and then grow into seed pods. Seeds develop in further as pods that form under the base of pollinated flowers. *P. obtusa* seed pods look like short green beans and may reach 12 inches long or more at the end of their growth. A single pod may contain around 25 to 60 seeds and it usually grows in pair. Each *P. obtusa* seed is about 5 mm across, with a small tail that acts as a propeller, helping the seeds spiral gently to the floor. Most importantly, the seed wing almost always lands facing up. As they ripen they dry out, turn brown, and eventually split open to drop seeds that float gently from their casing. Morphological/organoleptic characteristics of plant are presented in Table 1.

3.3 Anatomical evaluation

3.3.1 Quantitative microscopy

The average number of stomata in 25 fields (400×) and Stomatal index (4000×) were determined. In addition to this, lower surface of leaf Vein-islet number (50×) and Vein-termination number [(50×)-*99] were examined. The results are shown in Table 2.

Table 1: Morphological characteristics of *Plumeria obtusa* L. seed pods, leaves and flowers

S.No.	Organoleptic character	Seed pods	Leaves	Flowers
1.	Color	Brown to dark brown	Glossy green	White
2.	Odor	Strong sweet	Slight	Strong sweet
3.	Taste	Pungent	Sweet	Sweet
4.	Shape	T shape with double pod	Obovate rounded at tip	Funnel shaped tube
5.	Size	10-30 cm	60-75 cm	4.1 cm
6.	Texture	Course	Smooth	Smooth
7.	Fracture	Rough	Rigid	Hard

Table 2: Leaf constant of *Plumeria obtusa* L.

S.No.	Parameters	Value
1.	Average stomatal number in 25 fields (400×)	7-9 sq. mm
2.	Stomatal index (4000×) lower surface	21.88-24.32%
3.	Vein-islet number (50×)	12-15/mm^2
4.	Vein-termination number (50×)-*99	16-20/mm^2

3.3.2 Qualitative microscopy

The presence of tissue system was discovered in the transverse section of seed pod. Below is a list of several seed pod traits that were seen under a microscope shown in Figures 2 and 3.

3.3.2.1 Chlorenchyma

Plants have a sort of persistent tissue called collenchyma. Its walls thicken both during and after elongation and serve as the primary supporting tissue of developing organs. When cell wall composition changes in older organs, collenchyma may become stiffer or experience sclerification; a result of lignification of freshly deposited cell wall material. Besides acting as a photosynthetic component, chlorenchyma is also engaged in storage of photosynthetic products such as carbohydrates and metabolites like calcium oxalate crystals. It is located at the center.

3.3.2.2 Lignified peripheral fibers

Lignin is often present in cells that have secondary cell wall thickening. Lignin and cellulose work together to give strength. Longitudinally arrange bundles of lignified fibers are present in bundle that provides mechanical support along the length of the seed pod.

3.3.2.3 Epidermis

The epidermis is single layer of cells which covers many parts of plant like leaves, flowers, stems and roots. Circularized epidermis that can be identified using Sudan red III was prominently seen at the periphery of the transverse section and also in the surface view.

3.3.2.4 Cuticle

All land plants have a plant cuticle, an extra cellular hydroph obic coating that protects aerial epidermis from desiccation and other environmental stressors. Waxy coat of cuticle checks water evaporations and also serves as water resistant layers. The thickness of this layer is dependent upon geographical and seasonal variation.

3.3.2.5 Cyclocytic stomata

Our observations underline the fact that the seed pod bears cyclocytic type of stomata, wherein guard cells are surrounded by many subsidiary cells that are arranged in cyclic manner.

3.3.2.6 Sunken stomata

Stomata that have sunk below the surface of the leaf cannot be seen. Both epidermis and underside of plant leaves contain them. It reduces the rate of transpiration, which ensures less water loss, *i.e.*, presence of sunken stomata is the evidence of the geographical adaptation of the plant in arid climatic conditions. Such stomata can be observed between the two bundles of peripheral fibers.

3.3.2.7 Calcium oxalate crystals

A typical biomineral found in plants, calcium oxalate appears as crystals in a variety of forms. Oxalates are organic substances that many plants naturally contain. Oxalates are used by plants to control their internal mineral content and to fight against predators. Oxalic acid, which we acquire from eating plants, interacts with several minerals inside the body. It is a substance that exists in all plant tissues and organs and is regularly generate in vacuoles of specialized cells known as crystal idioblasts. Rhomboidal calcium oxalate crystals were present in the chlorenchyma. The toxic metabolite, oxalic acid, is neutralized by combining it with the calcium absorbed from soil in order to form calcium oxalate crystals.

3.3.2.8 Sclerenchyma

Simple permanent tissue called sclerenchyma typically comprises dead cells. The morphology of the sclerenchymatous cells is long and thin. The sclerenchyma's cell walls contain lignin and are evenly thick there at corners. The tissue of the sclerenchyma lacks cytoplasm. In order to withstands the external mechanical stress, the seed pods are innervated with a layer of dead sclerenchymatous sheath that offer substantial rigidity.

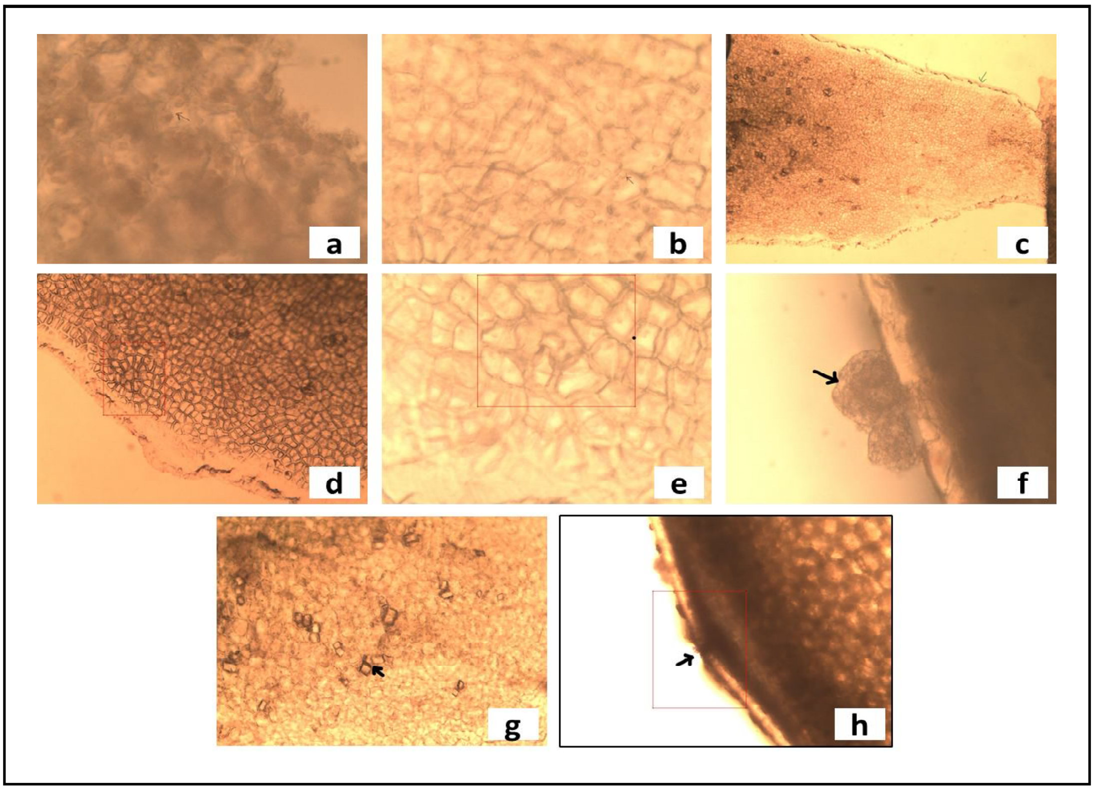

Figure 2: Transverse section of *Plumeria obtusa* L. seed pod (40X & 100X resolution); a: Chlorenchyma (100X); b: Chloroplast (100X); c: Cuticle (40X); d:Cyclocytic stomata (40X); e:Cyclocytic stomata (100X); f: Lignified peripheral fibers (40X); g: Rhomboidal prisms of calcium oxalate (100X); h: Sunken stomata (100X).

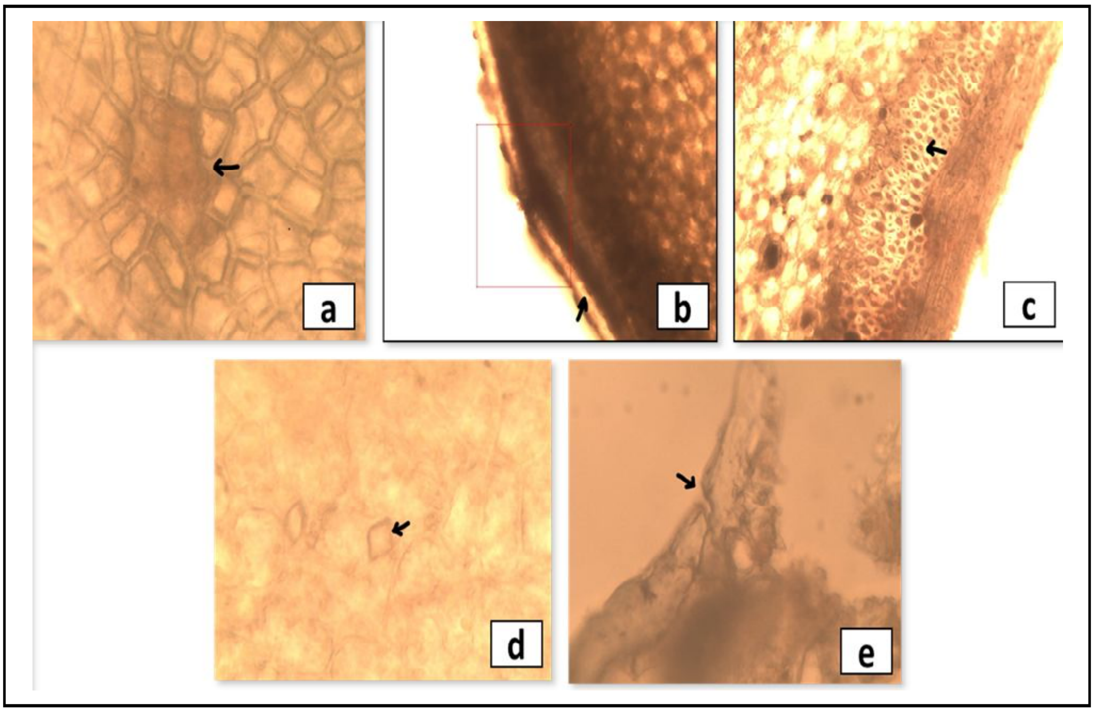

Figure 3: Transverse section of *Plumeria obtusa* L. seed pod (400X resolution); a:Cyclocytic stomata; b: Cuticle; c:Sclerenchymatous sheath; d:Rhomboidal prisms of calcium oxalate; e: Sunken stomata.

4. Discussion

Plants have been important contributors to human welfare since the beginning of civilization (Sharma and Alam, 2022). Modern research instruments for evaluating plant medications are now accessible, but the microscopic method remains one of the easiest and most cost-effective ways to begin determining the correct identify of the parent materials (Singh *et al.*, 2010). Many herbal medications, including *Zanthoxylum armatum* DC, *Ficus* species, *Dillenia indica* L. leaf, and *Memecylonum bellatum* Burm. f. leaves have been standardized using microscopy (Kamalesh *et al.*, 2013; Babu *et al.*, 2010; Kumar *et al.*, 2011; Killedar *et al.*, 2014). *Aloe vera* (L.) Burm.f., *Radix astragali* and *Alium cepa* L. are also mentioned in WHO monographs (World Health Organization, 2022). Furthermore, for herbal standardization, the majority of regulatory procedures and pharmacopoeias recommend morphological and microscopic examination (Patel *et al.*, 2017).

Characterization of morphological and anatomical parameters are essential for correct identification (Kumar *et al.*, 2021). Morphological studies of the leaf, flower and seed pod and seed pod microscopy will be helpful for identification of crude drug. For defining specific standards of crude drug, quantitative evaluation of various pharmacognostic parameters is helpful. Leaf constants studies are significant in standardization of herbs (Khan *et al.*, 2016). These values will help to evaluate purity of drugs. The microscopic characteristics disclosed in this study are first histological analyses for *Plumeria obtusa* L. herbal materials that have ever been published. The achieved data of present study can be used as referral standards.

As a result, the study reaffirms the importance of traditional approaches in herbal medicine quality control. The key organoleptic features, cells and tissues of investigative relevance for *P. obtusa* seed pods were discovered by morphology and microscopic examinations. The morphological evaluation depicted that plant is angiosperm, having leathery, dark green leaves and a T-shaped seed pod with two pods (Lim, 2012; Singh *et al.*, 2019). The presence of established tissue organization comprised of epidermis, vascular bundles (concentric), xylem, phloem arteries and lignified fibers was revealed by microscopic characteristics of *P. obtusa* seed pod.

5. Conclusion

Several past and scientific discoveries in realm of cell biology have backed up microscopic evaluation of plants (Mineyuki and Otegui, 2019). The data obtained is usually helpful due to the simplicity and affordability of the approaches used. Furthermore, the technique is sustainable because the equipment required is minimal, readily available, inexpensive and simple to apply. Morphological and microscopic characteristics of the *P. obtusa* will be valuable information to ascertain identity of the plant and ensure quality and purity of plant in further studies. Although, such study cannot evaluate the herbal drug completely, sufficient supporting evidences can be provided. Advancements in microscopy method may aid in the goal of standardization of this herbal plant, which is important for monograph development and decrease in the adulteration.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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