

ESTABLISHMENT OF QUALITY PARAMETERS AND PHARMACOGNOSTIC EVALUATION OF LEAVES OF *PEPEROMIA PELLUCIDA* (L.) HBK.

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ABSTRACT

Peperomia pellucida Linn. (Family – Piperaceae) is found throughout in India. The species of Piperaceae show a considerable structural diversity of leaves. No detailed pharmacognostic study or establishment of quality parameters has been done on this species of *Peperomia*. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation. *Peperomia* has epiphyte and herbaceous with whorled leaves phyllotaxis and a multiseriate adaxial leaf epidermis and calcium oxalate monocrystals in parenchyma and collenchyma petiole cells. In *P. pellucida* the stomatal complex is tetracytic and there are calcium oxalate raphide crystals in the parenchyma of the petiole and midrib cells. It has hypostomatic and dorsiventral leaves, oily cells in petiole and mesophyll, and an endodermis with Casparian strips. Chief characters of powder include xylem vessels having reticulate or annular thickening, pericyclic fibres, calcium oxalate crystals, and starch grains. Various physicochemical parameters (leaf constants) were also established. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloids, flavonoids, triterpenoids, saponins and carbohydrates. Such a study would serve as a useful tool in standardization of the leaf material, isolation of medicinally important phytoconstituents, pharmacological investigations and quality formulations in the future. It would also help in distinguishing the plant material of *Peperomia* from its related species.

Keywords: Piperaceae, Pharmacognostical, Physico-chemical screening, Phytochemical study.

INTRODUCTION

Peperomia pellucida belongs to the family Piperaceae, commonly known as Shiny bush, Slate pencil plant, Pepper elder, Rat's ear, Silver bush etc. In Sanskrit, it is known as Toyakandha, Varshabhoo. *Peperomia pellucida* is a herbaceous plant found in many South American and Asian countries. The species develops during rainy periods (often in the spring) and thrives in loose, humid soils under the shade of trees.^{1,2,3,4} It grows in moist habitat and is found throughout the major parts of India. In different parts of India it is known with different names like Lochi pata in Bangali⁵, Mashitandu chedi in Malayalam and Ponoona in Assamese etc⁶. *Peperomia pellucida* is a common fleshy tropical annual, shallow-rooted herb, usually growing to a height of about 15 to 45 cm. It is characterized by fibrous roots, succulent stems, shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. *Peperomia pellucida* is also used in Ayurvedic medicine. According to Ayurveda the whole *Peperomia* plant is described in Ayurveda as – Rasa – Katu and Madhur; Guna- Lakhu, rooksha, Teekshna and Virya- Ushna.



Fig. 1: *P. Pellucida* leaf.

The plant is described to passify vitiated cough, pitta, constipation, kidney diseases, urinary retention, dysuria, urinary tract infections, emaciation, edema and general weakness. Infusion and decoction of leaves and stems of fresh plant are eaten as

salad for the treatment of gout and arthritis⁷. The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material of *P. pellucida* and establishment of its quality parameters, including physicochemical and phytochemical evaluation. This thorough evaluation would be useful in standardization of the leaf material. It would also help in distinguishing *P. pellucida* from *Peperomia* species.

MATERIALS AND METHODS

Collection and authentication of leaves

Leaves of *P. pellucida* were collected from the Trikaripur forest areas, Kasaragod district of Kerala, India, in the month of November 2010 in a quantity sufficient for all the experiments in a single batch. The plant material was authenticated by Dr. Khaleel. Course director, Dept. of Environmental Studies, Kannur University, Payyanor and a specimen was submitted and preserved in the Department of Pharmacognosy Rajiv Gandhi Institute of Pharmacy, Trikaripur (No. RGIP /Cog/01/2010).

Pharmacognostic studies

Morphology of fresh leaves of *P. pellucida* was studied. Photomicrography of unstained transverse sections and stained transverse sections (using phloroglucinol - HCl) of fresh leaves was performed. Leaf constants were established using camera lucida and stage micrometer⁸ (Table 1). The leaves were dried under shade, powdered, stored in airtight containers and used for powder study, physico-chemical evaluation and phytochemical screening.

Physico-chemical evaluation

Various physico-chemical parameters like loss on drying, ash values (total ash, water soluble ash and acid-insoluble ash) and extractive values (water soluble and alcohol soluble extractives), foaming index and swelling index were established using the powdered drug⁹(Table 2).

Phytochemical study

The powder was extracted with 50ml each of water and ethanol at 60°C for two hours. Various phytoconstituents present in the leaves were detected by their respective chemical tests using the appropriate extracts¹⁰⁻¹⁵ (Table 3).

Table 1: Quantitative microscopy

Leaf constant	Mean value (\pm SD)
Stomatal Number (Lower surface)	3.6 \pm 0.55%
Stomatal Number (Upper surface)	Nil
Stomatal Index(Lower surface)	17 \pm 0.71%
Stomatal Index(Upper surface)	Nil
Palisade ratio (Lower surface)	9.55 \pm 0.07/mm ²
Palisade ratio (Upper surface)	13.09 \pm 0.14/mm ²
Vein islet number	3.6 \pm 0.55/mm ²
Vein termination number	1.60 \pm 0.55/mm ²
Phloem fibers (width)	43.68 \pm 0.32 μ
Phloem fibers (length)	237.36 \pm 0.43 μ

Number of observations = 5, SD = Standard Deviation

Table 2: Physicochemical parameters

Parameter	Results (\pm SD) %w/w
Loss on drying	5.2 \pm 0.10%
Total ash value.	901 \pm 0.05%
Acid insoluble ash value	0.358 \pm 0.01%
Water soluble ash value	0.191 \pm 0.01%
Alcohol soluble extractive value	5.6 \pm 0.21%
Water soluble extractive value	9.5 \pm 0.25%
Foaming index	Less than 100
Swelling index	0.4 \pm 0.03

Number of observations = 3, SD = Standard Deviation

RESULTS

Macroscopy

The macroscopic study revealed that leaves are fleshy, shiny, heart-shaped, alternate, blunt tip and smooth cylindrical 50-140mm in diameter, green colour with characteristic odour, acrid taste and waxy soft texture. Petioles are 20-30mm long. Colour of upper surface is dark green and lower surface is light green.

Microscopy

The TS of the petiole cortex possesses parenchyma and sub epidermic collenchymas arranged in irregular striate. There are 3 to 4 bundles in the base and the middle regions and five different dimension bundles in the apex. The cortical region of the petiole is collenchymatous and paranchymatous. (Fig. 2.A).

The leaves are dorsiventral, heterogenous mesophyll with a single palisade parenchyma layer. Furnnet shaped palisade cells. Unlike the petiole, the midrib region of the leaf base consists of just one vascular bundle with a paranchymatous sheath. On the adaxial face of the midrib, a multiple epidermis, palisade parenchyma and a little spongy parenchyma occur. On the abaxial face a uniseriate epidermis, collenchyma with thinner cell wall and parenchyma are observed. In the midrib apex there are few cells in the vascular bundle, the adaxial epidermis presents more cellular layer and there are no collenchyma in the abaxial surface. Leaves species are hypostomatic (one sided) and has an anomocytic stomatal complex (Fig. 3). The blade also has unicellular apex and short pedicle occurs in a small depression in the epidermis. It has an epidermis and sub epidermic layer in both leaf surfaces (Fig. 3.). The blade epidermis has straight or slightly curved anticlinal cell walls (Fig. 2.B).

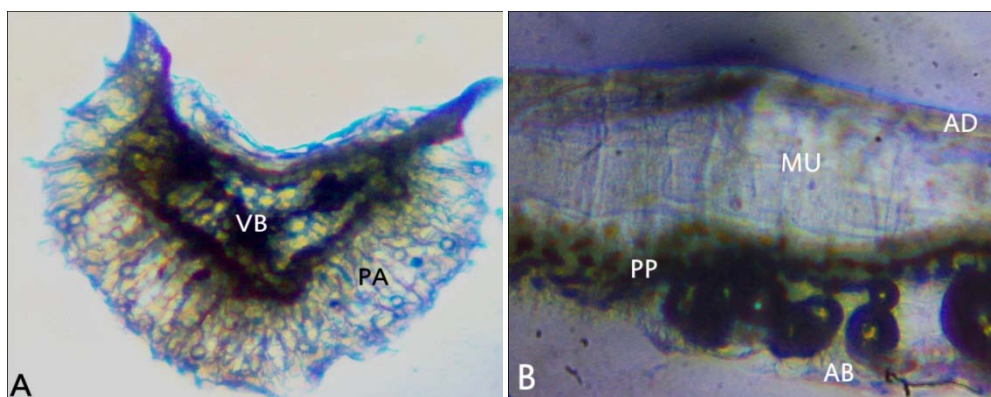


Fig. 2: Microscopy of petiole and leaf blade of *Peperomia pellucida*

Petiole structure in cross-section shows *P. pellucida* petiole base and apex (A) And details of leaf blade in cross-section (B). (VB = Vascular bundle, PA = Parenchyma, PP = Palisade parenchyma, AB = Abaxial face epidermis, AD = Adaxial face epidermis, MU = Multiseriate epidermis)

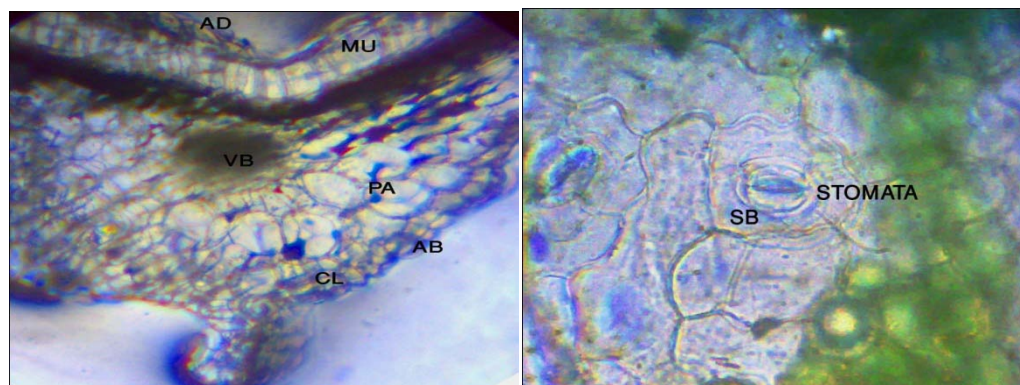


Fig. 3: Microscopy of *Peperomia pellucida* leaf (midrib) and stomata.

Midrib structure in cross-section shows basal and apical regions of *P. pellucida* midrib. (AB = Abaxial face epidermis, AD = Adaxial face epidermis, CL = Collenchyma, MU = Multiseriate epidermis, PA = Parenchyma, VB = Vascular bundle, SB = Subsidiary cell)

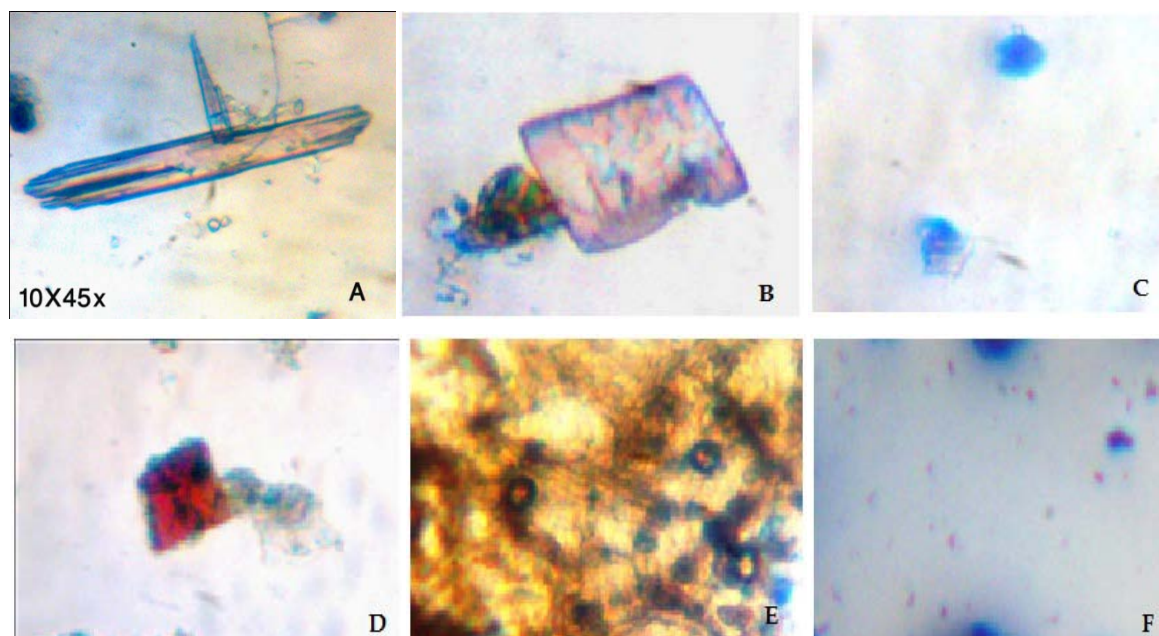


Fig. 4: Powder study (X450)

A, Pericyclic fibers; B, Xylem vessels with reticulate thickening; C, Starch grains; D, Orange matter; E, Oil glands; F, Calcium oxalate cluster crystal. Starch grains and prisms of calcium oxalate are scattered throughout the ground tissue.

Table 3: Phytochemical screening

Phytoconstituents	Result
Carbohydrates	+
Proteins	-
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids	+
Triterpenoids	+

Microscopy

Powder characteristics

The leaf powder of *Peperomia pellucida* has dark green colour with characteristic odour and taste. Diagnostic microscopic features of the powder include the presence of epidermis with wavy anticlinal walls and anomocytic stomata, xylem vessels with annular and spiral thickenings, palisade cells, different fibers, oil glands and starch grains. (figure-3).

DISCUSSION

The present work deals with the microscopic, physicochemical and phytochemical evaluation of the leaves of *Peperomia pellucida*. Main microscopic characters include dorsiventral, heterogenous mesophyll with a single palisade layer, funnel shaped palisade cells, one vascular bundle with a paranchymatous sheath, collenchyma with thinner cell wall on adaxial face and the leaves are hypostomatic (one sided) and has an anomocytic stomatal complex. Powder characteristics include fibres, calcium oxalate crystals. Oil glands and starch grains. Various physicochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, flavonoids, steroids, triterpenoids, saponins and carbohydrates, which may influence the pharmacological actions of the plant. From the foregoing accounts it is evident that the plant *Peperomia* has immense medicinal values that demand further researches towards the development of safe and suitable medications for various human

ailments. In this present studies suggest a methodical approach to quantitative and qualitative evaluation of *Peperomia pellucida* leaf in the field and powdered form which was not earlier reported. The findings may help to proper identification and ensures the quality of the drug and help this plant grown on commercial basis and also conserved in the wild for better use in pharmaceutical industry.

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