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Review Article

EVALUATION OF TAXO-CHEMICAL STANDARDIZATION AND QUALITY CONTROL PARAMETERS OF *PEPEROMIA PELLUCIDA* (FAMILY: PIPERACEAE): A MULTI VALUABLE MEDICINAL HERB Pulak Majumder*

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ABSTRACT

Evolving methods of standardization and establishing quality control parameters for herbal drugs calls for a well-planned approach for establishing standards and systemic evaluation of plant material using modern analytical techniques. The present work reports the standardization of this traditional herb *peparomia pellucid* which is widely used to treat a wide variety of ailments like Analgesic, Anti-inflammatory, chemotherapeutic, broad spectrum antibiotic, refrigerant, anticancer, CNS depressant activities, lower cholesterol levels and diuretic. In Ayurveda, used as – Rasa – Katu and Madhur; Guna- Lakhu, rooksha, Teekshna; and Virya- Ushna and to passify vitiated cough, pitta, constipation, kidney diseases, urinary retention, dysuria, urinary tract infections, emaciation, edema and general weakness, gout and arthritis. The plant material was standardized based on Ayurvedic pharmacopeia and WHO guidelines. The detailed studies on pharmacognostical, phytochemical, physicochemical properties, fluorescence studies and development of TLC profile of different parts of *Peperomia pellucida* plant had been carried out. In this context the studies suggest a methodical approach to quantitative evaluation of crude drug in the underground and powdered form. The limits obtained from the different physicochemical parameters of this herbal drug could be used as reference standard for standardization of this plant parts in a quality control laboratory.

Keywords: Peperomia pellucida, Microscopy, Phytochemicals, Standardization, TLC

INTRODUCTION

The effectiveness of these traditional medicines only depends upon the proper use and sustained availability of genuine raw materials. Evolving methods of standardization and establishing quality control parameters for herbal drugs calls for a well-planned approach for establishing standards and systemic evaluation of plant material using modern analytical techniques. Standardization of herbal drug is essential to validate the quality of drugs and to ensure that the consumers are getting medication, which guarantees purity, safety, potency and efficacy. The present work reports the standardization of this traditional herb *Peparomia pellucida* in various ways.



Fig. 1. Peperomia pellucida plant

Taxo-Botanical Status of the plant.^{1,2, 3} Taxonomic classification

| Kingdom | : Plantae |
|----------------|-----------------|
| Subkingdom | : Tracheobionta |
| Super division | : Spermatophyta |
| Division | : Magnoliophyta |
| Class | : Magnoliopsida |
| Subclass | : Magnoliidae |

| Order | : Piperales |
|---------------|--|
| Family | : Piperaceae |
| Genus | : Peperomia Ruiz & Pav. |
| Species | : Peperomia pellucida (L.) Kunth |
| Vernacular na | ame |
| English | : Shiny Bush, Slate pencil plant, pepper elder rat's ear shiny bush silver bush |
| Sanskrit | : Toyakandha, Varshabhoo. |
| Malayalam | : Mashitandu chedi. |
| Assamese | : Pononoa. |
| Bengali | : Lochi pata. |
| | |

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. It has a mustard-like odor when crushed.

The plant has a thread like but angular trailing stem. Those growing in rich habitats have fleshy and stout stems. They are translucent pale green, erect or ascending, usually 15-45 cm long, internodes usually 3-8 cm long, glabrous and hairless. Leaves are alternate, blunt, heart shaped and as transparent and smooth as candle wax grows as a long shrubby looking creeping cover or as an epiphyte. They are medium green on upper surface, lower surface whitish green, thinly fleshy, drying papery, broadly ovate, 1.5- 4 (-5) cm long, 1-3.3 cm wide, palmately 3-nerved or 5-nerved, glabrous, apex acuminate, base sub cordate to truncate, petioles 0.5-2 (-3) cm long, glabrous. The elongated stems look like a vine with leaves rising 6 to 9 cm above the surface. Both leaves and stems have shiny waxy surfaces. The foliage of the plant looks ornamental. Flowers are very small, well-spaced, unnoticeable and bi-sexual growing in the form of cord-like spikes arising from the leaf axils, 1 to several, terminal and axillaries or leaf-opposed, filiform, 3-6 cm long, the rachis 0.4-0.6 mm in diameter and glabrous. The peduncles are 0.6-1 cm long, glabrous; ovary ovoid and stigmas terminal. Inflorescence consists of compact; erect spikes of minute creamy white flowers and maturing gradually from the base to the tip; turning brown when ripe. The fruits are also tiny, dot like smooth, oval or round to oblong, ridged, first green later black. They have one single seed. Their sub-globose, size 0.5 mm long, longitudinally ridged, apex beaked. These develop partially embedded in the spike with their hooked beaks protruding outside and maturing gradually from the base to the tip; turning brown when ripe.

USES ^{4,5,6}:

Leaves and stems may be eaten as vegetable. In salads, the fresh plant has the crispness of carrot sticks and celery. Ethnomedicinal uses for the plant has been used for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders.

Infusion and decoction of leaves and stems are used for gout and arthritis. Externally used as a facial rinse for complexion problems. Pounded whole plant used as warm poultice for boils, pustules and pimples. Used for headaches, rheumatic pains, impotence.

Peperomia pellucida is also used in Ayurvedic medicine. According to Ayurveda the whole Peperomia plant as medicinal. It is described in Ayurveda as – Rasa – Katu and Madhur; Guna- Lakhu, rooksha, Teekshna; and Virya-Ushna. 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 arthritis.

According to Ethno-botanical studies the whole plant has been in medicinal use since long. It is crushed and mixed with water to form a mixture, heated and administered orally to cure hemorrhage. The extract of the aerial part of the plant has been applied to cure wounds. It is also been applied against coughing, fever, common cold, headache, sore throat, diarrhea, against kidney - and prostate problems and against high blood pressure.

Peperomia pellucida plant possesses Analgesic, Antiinflammatory, chemotherapeutic, and broad spectrum antibiotic, refrigerant, anticancer, depressant of Central Nervous System. There are popular descriptions of *P*. *pellucida* to lower cholesterol levels or used on proteinuria and as diuretic has been scientifically proven.

MATERIALS AND METHODS

Collection and Authentication of plant

P. pellucida plant were collected from the Trikaripur semi 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.

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Morphology of fresh plant of *P. pellucida* was studied. Photomicrography of unstained transverse sections and stained transverse sections (using phloroglucinol -HCl) of fresh parts of the plant was performed. The plant material were dried under shade, powdered, stored in airtight containers and used for powder study, physico-chemical evaluation and phytochemical screening.

Physico-Chemical Evaluation ^{8,9}

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 according to standard procedure by using the powdered drug.

Preliminary phytochemical studies ^{10,11,12}

Preliminary phytochemical screening which is performed to establish a chemical profile of a crude drug. The phytochemical tests done according to recommended procedure.

Fluorescence analysis of extract and drug powder¹³

The powders as such, after treatment with various solvents were subjected to fluorescent analysis. Observations were made under visible light and then under UV light of short wave length and long wave length separately.

Behavior of different plant parts powder with treatment of different reagents or solvents¹⁴

Colour behavior of the drug powder was performed using different parts of the plant powder as such, and after being separately treating with 10 % Ferric chloride, Conc. Sulphuric acid, Conc. Hydrochloric acid, Conc. Nitric acid, Glacial acetic acid, Picric acid solution, Iodine solution. The observations were made in visible light.

Thin Layer Chromatography¹⁵

Slurry of silica gel G was prepared in distilled water and poured over a glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at $100-120^{\circ}$ C (30min) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (1cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase, and allowed to run 3/4th of the height of the prepared plate.

RESULTS AND DISCUSSION

Collection and Authentication of the plant

Drugs are collected suitably when they contain maximum concentration of active ingredients. *Peperomia pellucida* were collected from the road sides of Trikaripur area, kasaragod district of Kerala, India, in the month of November 2010 in a quantity sufficient and authenticated by a Botanist. One voucher specimen was submitted and preserved in the Department of Pharmacognosy, Rajiv Gandhi Institute of Pharmacy, Trikaripur.

PHARMACOGNOSTICAL EVALUATION Morphological/Organoleptic evaluation

The morphological of evaluation crude drugs can be carried out by using special sense organs. The morphological features of the drug are presented table-1.

Roots were 4-6 cm, buff coloured, fibrous and multiple sub branches. Stems are succulent, translucent pale green, erect or ascending, internodes usually 3-8 cm long, glabrous and hairless. Leaves are fleshy, shiny, heart-shaped, alternate, blunt tip and smooth cylindrical, 50-140mm in diameter,

| Sl. no. | Character | When fresh | After drying | Powder |
|---------|-----------|-----------------|--------------------|-----------------|
| | Colour | Buff | Straw | Yellowish brown |
| | Odour | Characteristic | Characteristic | Characteristic |
| Root | Taste | Bland | Bland | Bland |
| | Texture | Thin soft | Fibrus | Fibrus |
| | Fracture | Greenish Yellow | Yellowish green | Yellowish brown |
| | Colour | Yellowish green | Green to yellowish | Gray |
| | Odour | Characteristic | Characteristic | Characteristic |
| Stem. | Taste | Acrid | Acrid | Acrid |
| | Texture | Thin soft | Fibrus | Fibrus |
| | Fracture | Pale green | Straw green | Dark green |
| | Colour | Green | Straw | Dark green |
| Lasf | Odour | Characteristic | Characteristic | Characteristic |
| Lear | Taste | Acrid | Acrid | Acrid |
| | Texture | Waxy soft | Fibrus soft | Soft |

Table- 1: Organoleptic evaluation of Peperomia pellucida plant

Histology of the aerial parts of *Peperomia pellucida* plant The TS of the root was circular in outline. Cork- outer cuticular layer was followed by thin walled epidermal cells. Phellogen- bilayered, immediately below the cork and cells tangentially elongated. Cortex- composed of 16-18 layers of horizontally elongated parenchyma with small intercellular spaces and subepidermic collenchyma arranged in irregular strate. The parenchyma cells contain starch grains which were both simple and compound. Oil cells also visible. The inner collenchymatous cells differentiated in the fibers. Endodermis consists of irregular vascular bundles, 3-5 in no which contains both phloem and. Xylems. Phloem- was an irregular ring consists of phloem parenchyma. Phloem was seen in several thin patches around the well developed xylem. Xylem- occupied the central portion in different patchs and was traversed regularly by rows whose cells were non lignified. Each ray cell was radially elongated and contains starch. The root T.S found to be absence of pith (Fig. 3a).

The TS of the stem has a polystelic structure. Each collateral vascular bundle and fascicular cambium is covered with a uniseriate parenchymatous pericycle and an endodermis with casperin strips. The epidermis is unilayered with cutical, secretory trichroms and periclinal thick walled cells. The cortex possesses parenchyma and subepidermic collenchyma

arranged in irregular strate. Endodermis contains 2-4 vascular bundles (Fig, 3b).

The TS of the petiole resembles the stem except for the number and arrangement of vascular bundles in the petiole. There are 3 to 4 bundles in the base and the middle regions and five different dimentioned bundles in the apex. The cortical region of the petiole is collenchymatous and paranchymatous (Fig, 3c). The leaves are dorsiventral, heterogenous mesophyll with a single palisade parenchyma layer. Furnnet shaped palisade cells. The midrib region of the leaf base unlike the petiole, present 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. 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, 3d.A). The blade epidermis has straight or slightly curved anticlinal cell walls (Fig, 3c.B)



Figure 3a. Microscopy of *Peperomia pellucida* root. Root structure in cross-section shows overall diagram detail and vascular bundle of *P. pellucida* (EP = epidermis, CO= cortex, EN= endodermis, ST= starch, XY=xylem, PH= phloem).

Pulak Majumder: Evaluation of quality control parameters of Peperomia pellucida



Stem structure in cross-section shows overall diagram detail and vascular bundle of *P. pellucida* (CL = collenchyma, EP = epidermis, FA = fibers; PA = parenchyma, VB = vascular bundle, XY=xylem, PH= phloem).



Figure 3c. Microscopy of petiole and leaf blade of Peperomia pellucida

Petiole structure in cross-section shows *P. pellucida* petiole base and apex. And details of leaf blade in cross-section. (VB = vascular bundle, PA = parenchyma, PP = palisade parenchyma, AB = abaxial face epidermis, AD = adaxial face epidermis, MU = multiseriate epidermis)



Figure 3d. 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).

Powder Microscopy

The powder microscopy of the root powder of *Peperomia pellucida* showed the presence of cork- about two to three layers, thin walled, tubular, polygonal cells. Wood elements-xylem vessels and fibres inter lock with each other to form a spindle shaped structure, starch grains- simple as well as compound. The powder microscopy of the stem powder of *Peperomia pellucida* showed the presence of cork cells 2 to 3

layers, round shaped oil glands, sleder fibres, starch grains, vessels accociated with fibres, xylem cells. The powder microscopy of the leaf powder of *Peperomia pellucida* showed the presence of epidermis with wavy anticlinal walls and anomocytic stomata, xylem vessels with annular and spiral thickenings, palisade cells and starch grains.

Pulak Majumder: Evaluation of quality control parameters of Peperomia pellucida

| Table-2: Quality control parameters | | | | |
|--|--------------------|------------------|-----------------|---------------|
| Sl. No. | Particulars | Root Stem Leaf | | |
| | | Values (%) | Values (%) | Values (%) |
| 01 | Ash values | | | |
| | Total ash | 0.95±0.01 | 0.49 ± 0.02 | 1.901±0.05 |
| | Water soluble ash | 0.328±0.01 | 0.274±0.01 | 1.70 ± 0.01 |
| | Acid insoluble ash | 0.019 ± 0.01 | 0.025±0.01 | 0.358±0.01 |
| 02 | Extractive values | | | |
| | Ethanol (90%) | 9.3±0.15 | 7.25±0.02 | 5.6±0.21 |
| | Aqueous | 10±0.20 | 15.05±0.03 | 9.5±0.25 |
| 03 | Loss on drying | | | |
| | Dry matter content | 79.6±0.15 | 77.7±0.01 | 94.8±0.15 |
| | Moisture content | 20.4±0.20 | 22.3±0.15 | 5.2±0.10 |
| 04 | Foaming index | Nil | Nil | < 100 |
| 05 | Swelling index | 0.8±0.03 | 0.8±0.03 | 0.4±0.03 |
| N= Three experiments for each parameter. The values shown are mean \pm S.E | | | | |

Table-3. Description of Panaromia pollucida plant extracts

| Plant parts | Extracts | Colour | Consistency | %yield |
|-------------|----------|----------------|----------------|--------|
| Root | Ethanol | Dark brown | Semisolid mass | 4.5 |
| | Aqueous | Brown | Semisolid mass | 4.9 |
| Stem | Ethanol | Greenish brown | Semisolid mass | 7.25 |
| | Aqueous | Greenish brown | Semisolid mass | 15.0 |
| Leaf | Ethanol | Deep green | Semisolid mass | 5.6 |
| | Aqueous | Blackish Green | Semisolid mass | 9.5 |

Table-4: Preliminary phytochemical analysis of various plant part extracts of Peperomia pellucid plant

| SI no. | Chemical Constituents | Root | Stem | leaf |
|--------|-----------------------|------|------|------|
| 01. | Carbohydrates | + | + | + |
| 02. | Proteins | - | - | - |
| 03. | Alkaloids | + | + | + |
| 04. | Saponins | - | - | + |
| 05. | Tannins | + | + | + |
| 06. | Flavonoids | + | + | + |
| 07. | Steroids | + | + | + |
| 08. | Triterpenoids | + | + | + |

+ = Present, - = Absent.

Table-5: Fluorescence characters of plant powder in different solvents

| | | Under UV light | | V light |
|-------|-------------------------------|----------------------|----------------|----------------|
| Plant | Particulars of treatment. | Under visible light. | Short | Long |
| part | | | Wavelength. | Wavelength. |
| | | | (254nm) | (366nm) |
| Root | Root as such | Straw | Black | Grayish brown |
| | Root Powder as such | Light brown | Dark blue | Pale yellow |
| | Powder + 50% H_2SO_4 | Greenish black | Deep green | Intense Green |
| | Powder + 1N HCl | Greenish brown | Deep green | Green |
| | Powder + 50% HNO ₃ | Golden yellow | Black | Bluish black |
| | Powder + 5% KOH | Yellowish brown | Deep green | Intense green |
| | Powder +MeOH | Dark brown | Deep green | Grayish black |
| Stem | Stem as such | Greenish yellow | Black | Grayish brown |
| | Stem Powder as such | Gray | Grayish yellow | Greenish white |
| | Powder + 50% H_2SO_4 | Black | Green | Green |
| | Powder + 1N HCl | Brown | Greenish black | Green |
| | Powder + 50% HNO ₃ | Golden yellow | Black | Light green |
| | Powder + 5% KOH | Greenish black | Greenish black | Green |
| | Powder +MeOH | Dark brown | Brown | Light green |
| Leaf | Leaf as such | Straw | Black | Greenish black |
| | Leaf Powder as such | Grayish brown | Brown | Greenish white |
| | Powder + 50% H_2SO_4 | Black | Reddish brown | Greenish black |
| | Powder + 1N HCl | Dark brown | Black | Green |
| | Powder + 50% HNO ₃ | Golden yellow | Black | Greenish black |
| | Powder + 5% KOH | Deep brown | Black | Black |
| | Powder +MeOH | Black | Black | Black |

Table-6: Behavior of the drug powder (*Peperomia pellucida*) with different chemical reagents/ solvents.

| | The behavior of the drug powder with different chemical reagents will also be neipiul in characterization of the crude drug. | | | | |
|--------|--|-----------------|-----------------|---------------|--|
| Sl no. | Drug treatment | Leaf | Stem | Root | |
| 01 | Powder as such | Green | Pale green | Brown | |
| 02 | Powder + Picric acid | Greenish yellow | Yellowish green | Yellow | |
| 03 | Powder + 50% HNO ₃ | Orange | Orange | Yellow | |
| 04 | Powder + 1N HCl | Light green | Brown | Light brown | |
| 05 | Powder + 50% H ₂ SO ₄ | Greenish black | Brown | Black | |
| 06 | Powder + Glacial acetic acid | Green | Yellowish green | Light brown | |
| 07 | Powder +10% FeCl ₃ | Green | Green | Golden yellow | |
| 08 | Powder + Iodine | Reddish brown | Brown | Reddish brown | |

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Pulak Majumder: Evaluation of quality control parameters of Peperomia pellucida

| Plant parts | Extract | No. of Spot observed under uv (366nmn) | Rf value | | |
|-------------|-----------------|---|---|--|--|
| | Methanol | 12 | 0.049, 0.147, 0.229, 0.295, 0.442, 0.524, 0.606, 0.737, 0.852, 0.978, 0.950, 0.983 | | |
| Leaf | Chloroform | 10 | 0.049, 0.131, 0.213, 0.311, 0.377, 0.475, 0.573, 0.721, 0.852, 0.934 | | |
| | Petroleum ether | 8 | 0.065, 0.098, 0.262, 0.311, 0.409, 0.491, 0.622, 0.967 | | |
| | Methanol | 8 | 0.067, 0.203, 0.237, 0.355, 0.440, 0.559, 0.694, 0.915 | | |
| Stem | Chloroform | 13 | 0.050, 0.084, 0.152, 0.237, 0.305, 0.355, 0.423, 0.474, 0.542, 0.627, 0.745, 0.915, 0.983 | | |
| | Petroleum ether | 7 | 0.052, 0.087, 0.122, 0.350, 0.491, 0.894, 0.982 | | |
| Root | Ethyl acetate | 7 | 0.079, 0.126, 0.349, 0.428, 0.666, 0.746, 0.952 | | |
| | Chloroform | 4 | 0.090, 0.163, 0.236, 0.400 | | |

Fig. 2: TLC of *P. pellucid*a chloroform extracts (Leaf) B (Stem) C (Root)



CONCLUSION

These qualitative and quantitative approaches to understand the plant *Peperomia pellucida*, does help in better identification and medicinal importance of this unused multi valuable traditional herb in depth. The adulterants in drug obtained from this plant can be identified by this investigation and ensure the proper use of this plant in pharmaceutical field.

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