Pharmacognostical, Physicochemical and Phytochemical Standardization of Petiveria alliacea L.

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ABSTRACT

There are the vast varieties of medicinal plants in the world with therapeutic properties. With increasing popularity of herbal medicine as a curative measure, the need for correct identification and standardization of the plant is also increased. Present work was performed to study the pharmacognostic and phytochemical characters of whole plant of Petiveria alliacea. The whole plant of Petiveria alliacea was investigated for its pharmacognostic parameters viz, macroscopic, microscopic, physicochemical attributes, fluorescence analysis and phytochemical screening and the salient diagnostic features were also documented. The preliminary phytochemical screening of methanol and ethanol extracts of P. alliacea whole plant revealed the presence of alkaloids, coumarins, flavonoids, saponins, steroids, phenols, tannins, terpenoids, glycosides and xanthoproteins. These studies provided referential information for identification of this crude drug.

Keywords: Petiveria alliacea, Microscopic, physicochemical, fluorescence, phytochemical.

INTRODUCTION

Plant based drugs have been used worldwide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world1. Medicinal plants being as an important natural resource and potentially safe drugs can play an important role in assuaging human health by contributing herbal medicines. The high cost of allopathic medicine and their potential side effects, encouraged the people to use the traditional medicine. The increasing demand of plant extracts to be used as cosmetics, foods and pharmaceutical industries suggests that systematic studies of medicinal plants are very important in order to find active compounds and their use as a medicine for curing various diseases2.

There is a need for documentation of research work carried out on traditional medicines3 and also it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical, physicochemical and phytochemical characteristics4,5,6. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

Petiveria alliacea L. (Phytolaccaceae) is claimed to have several medicinal properties. It is used in folk medicine to enhance memory and in the treatment of common cold, flu, other viral or bacterial infections, inflammation, diabetes and cancer7,8,9,10. Previous work on P. alliacea revealed the presence of triterpenoids, saponins, polyphenols, coumarins, benzaldehyde, benzoic acid, flavonoids, fredelinol, pinitol and allantonin, varying their concentrations in the root, stems and leaves11,12. There is no record of pharmacognostical work on the whole plant of Petiveria alliacea. The objective of the present study is to evaluate various pharmacognostic standards like microscopy, physicochemical constant, fluorescence analysis and qualitative preliminary phytochemical analysis of Petiveria alliacea. These findings would be helpful for authentication, purification, quality control and for better use in pharmaceutical herbal formulations.

MATERIALS AND METHODS

The plant specimen (Petiveria alliacea L.) for the proposed study were collected from Agasthiarimalai Biosphere Reserve, Western Ghats, and Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of the collected plant was deposited in the Ethnopharmacological Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu.
Macrosopic studies
The macroscopic characters like surface, shape, size, venation, phyllotaxy, length of the petiole, length of the leaf, etc., were noted.

Anatomical Studies
For anatomical studies, the required samples of root, stem and leaf were cut and removed from the plant and immediately fixed in FAA (formalin- 5 ml + acetic acid- 5 ml + 70% Ethyl alcohol- 90 ml). The specimens were left in the preservative for two days; then the materials were washed in water and processed further. Standard microtome techniques were followed for anatomical investigation. Transverse sections of the materials were made. The microtome sections were stained with 0.25% aqueous Toluidine blue (Metachromatic stain) adjusted to pH 4.7. Photomicrographs were taken with NIKON trinocular photo micrographic unit.

Physicochemical and fluorescence analysis
These studies were carried out as per the standard procedures. In the present study, the powdered whole plant was treated with various chemical reagents like aqueous 1N sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 1N sulphuric acid, concentrated nitric acid, picric acid, acetic acid, ferric chloride and concentrated HNO₃ + NH₃. These extracts were subjected to fluorescence analysis in day light and UV light (254nm and366nm). Various ash types and extractive values were determined by following standard methods.

Preliminary phytochemical analysis
Shade dried and powdered whole plant samples were successively extracted with petroleum ether, benzene, ethyl acetate, methanol and ethanol. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedures.

RESULTS
Exomorphic Features
Petiveria aliaacea is an herbaceous plant, stem cylindrical, erect up to 1 m tall, pubescent to glabrate. Leaves alternate, simple and entire, stipules 2mm; petiole 0.4 – 2 cm; blade elliptic to oblong or obovate, to 20 x 7 cm, base acute to cuneate, acuminate or acute to obround or rounded. Leaves and stems with garlic smell. Inflorescences terminal or supra axillary, often drooping distally, 0.8 – 4 dm, peduncle 1-4 cm. Pedicel 0.5 - 2 mm. Flowers are bisexual, zygomorphic, slightly imbricate to rather remote; sepals white or greenish to pinkish, linear – lanceolate to linear – oblong, 3.56 mm; ovary superior. Fruits are narrowly oblong achenes subented by persistent bracts and perianth, 6-9 mm long, striate with recurved hooks, 1 seed. The roots and leaves have a strong acrid, garlic-like odour which taints the milk and meat of animals that graze on it.

Anatomy of Young (Thin) Root
The young root is circular and measures about 750 µm in thickness. It consists of a uniformly thick and continuous superficial periderm, a narrow homogeneous cortical zone and a wide, thick and circular vascular cylinder measuring about 600 µm in thickness (Plate – 1b). The periderm is fissured and irregular on the outer surface and the remaining periderm zone has 5 to 9 layers of tubular, suberized phellem cells. The cortical zone consists of 3 or 4 layers of wide, angular parenchyma cells which possess dense starch grains (Plate – Ic-f). The secondary phloem looks like a narrow cylinder consisting of sieve elements and phloem parenchyma cells. The secondary xylem cylinder includes regular radial lines of vessels and fibres deviating from the centre. The vessels are sparse, either solitary or less frequently short radial multiples. The vessel elements are up to 25 µm wide. The xylem fibres are also very thick walled, lignified and have wide lumen. The xylem fibres are densely filled with starch grains (Plate - I).

Anatomy of Old (Thick) Root
The old root is measured about 1.9 mm thick. It is circular in outline and has a fairly well defined superficial, continuous periderm, a narrow cortex, a circular, discrete segment of outer vascular cylinder and a compact central vascular cylinder (Plate - Id). The periderm has 4 or 5 layers of thin and tubular cells. The cortex is narrow and is made up of 3 or 4 layers of thin walled parenchyma cells which are arranged compactly. The central vascular cylinder has radially oriented discontinuous, solitary vessels and radial layers of thick walled fibres (Plate – I d, e). The vessels are 30 µm in diameter.

The secondary phloem of the central vascular cylinder occurs along the outer circumference of central xylem cylinder. The phloem elements are narrow and polygonal in outline and include sieve elements and phloem parenchyma cells. The secondary xylem segments of the central vascular cylinder have radial files of lignified fibres and sparsely distributed vessels. The outer secondary xylem cylinder consists of wide and discrete secondary xylem segments with associated secondary phloem. The secondary phloem of the outer vascular cylinder occurs on the outer edge of each secondary xylem segment. The secondary phloem exhibits cambial derivatives of sieve elements and parenchyma cells. The ground tissue of the root contains a large accumulation of starch grains (Plate - If).

Crystals of The Root
Calcium oxalate crystals of prismatic type are sparsely distributed both in the xylem rays and in xylem parenchyma. The crystals differ in size; some of them are large and squarish and the others are small and irregular in shape. The xylem fibres are densely filled with numerous starch grains (Plate - If).

Anatomy of Stem
The stem is more or less circular in outline and measures 1.9 mm in diameter (Plate – Ia). The stem consists of a continuous and distinct epidermal layer of circular thickly cutinized cells. The cortex is wide and it is differentiated into an outer cortex and an inner cortex. The outer cortex is made up of a few layers of collenchyma and the inner cortex is parenchymatous. There is a more or less continuous, thick and thin alternating portion of
sclerenchyma cylinders (Plate –II b, c, d). The sclerenchyma cylinders consist of highly thick walled and lignified cells with narrow lumen. The vascular cylinder consists of an outer thin continuous cylinder of secondary phloem, an inner cylinder of secondary xylem and large parenchymatous pith (Plate – II c). The secondary phloem elements consist of narrow, angular and thin walled sieve elements and phloem parenchyma cells. The phloem rays are slightly dilated and consist of a single row of cells. The secondary xylem cylinder is circular with different thick and thin regions. The thick portion has solitary or short radial multiples of wide, circular or elliptical thick walled vessels and highly thick walled lignified fibres with wide lumen. The xylem vessels are up to 20 µm in diameter.

**Anatomy of Petiole**

In cross sectional view, the petiole appears shield shaped with shallow and wide adaxial concavity. It is 1.35 mm thick and 1.5 mm wide. Calcium oxalate crystals are fairly common in the ground tissue of the petiole. The crystals are prismatic type. The crystals are sparse in distribution and are located in singles with the ground parenchyma cells (Plate – II d). The epidermal layer of the petiole is thin and continuous and it is undulate in outline. The epidermal cells are small, squarish and fairly thick walled. The ground tissue of the petiole consists of an outer thick zone of sclerenchyma cells and an inner zone of compact thin walled parenchyma cells. The central ground tissue is also parenchymatous (Plate – II f).

The vascular system consists of necklace shaped, deep and wide outline of about eight discrete vascular bundles. The vascular bundles are top shaped and collateral. They have circular, thick walled and diffuse cluster of vessels with an outer zone of phloem elements. The median vascular bundle is larger and the bundles become gradually reduced in size towards the lateral wing (Plate – II e, f).

**Anatomy of The Leaf**

The leaf has a thick and broad midrib and thin lamina (Plate – III a). The midrib is 1.2 mm thick and about 1 mm wide. It includes ground tissue and wide and deep bowl shaped vascular strand with incurred end margins (Plate – III a, b). The epidermal layer of the midrib is thin and continuous. The cuticle is thin. The ground tissue is homogeneous comprising of a circular or angular, less compact or compact parenchyma cells. The vascular strand is bowl shaped with a wide opening. The marginal part of the bow is incurred. The vascular strand is wavy with shallow ridges. There are five collateral wedge shaped vascular bundles; a thin continuous sclerenchyma line runs all along the outer part of the bowl shaped vascular strand. The vascular bundle has a wide cluster of circular, thick walled xylem elements. The phloem occurs in thin arcs along the outer boundary of the xylem masses.

**Lamina**

The lamina is dorsiventral, with smooth and even surfaces. It is 90 µm thick. The adaxial epidermis and abaxial epidermis are equally thick. The epidermal cells are cylindrical with thick walls. The mesophyll tissue includes an adaxial band of single row of compact and cylindrical cells and four or five layers of spherical and lobed spongy parenchyma cell (Plate III b).

**Leaf Margin**

The marginal part of the lamina is blunt and semi-circular. The epidermal cells along the leaf margin are much dilated into circular cells with thick cuticle. The marginal part measures 80 µm in thickness. The mesophyll tissue is undifferentiated into palisade and spongy tissues. Only a compact mass of circular thick walled cells is seen in the marginal part (Plate – III b, d).

**Epidermal Cells and Stomata**

The epidermal cells appear smaller in the surface view of the paradermal section of the lamina. They have thin and highly wavy anticlinal walls. The stomata are dense and diffuse. The stomata are paracytic type with two lateral subsidiary cells. One of the subsidiary cells may be smaller than the other. The guard cells are nearly circular in outline measuring 12 x 12 µm in size (Plate – III e). The stomatal aperture is elongated and with parallel ridges.

**Venation of The Lamina**

The lateral veins are fairly thick and straight. The veinlets are thin and form wide rectangular or polyhedral vein-islets. The vein terminations are fairly well developed. The vein terminations are either unbranched or branched once or twice. The terminations are thin, slender and curved (Plate – III f, 8).

**Crystals**

Long and scale like calcium oxalate crystals are abundant in the lamina. They are of styloid type. The central part is broad and the terminal part is tapering. The styloids are random in distribution and are 200 to 250 µm long and 10 µm thick (Plate – III e,d).

**Powder Microscopy**

The powdered preparation of the sample includes vessel elements, fibres and parenchyma cells (Plate – IV a).

**Vessel elements**

The vessel elements are mostly narrow, long and cylindrical. The vessel elements have thick or thin long tapering tails (Plate – IV a, b, c, d). There are also vessel elements without tails. The perforation is mostly elliptical and oblique (Plate – IV b,d). The lateral wall pits are circular, multiseriate and prominent. The vessel elements are 180 to 220 µm long and up to 20 µm wide.

**Fibres**

The fibres either narrow type or wide type. The narrow fibres have reduced lumen and thick walls. They are 350 µm long and 10 µm thick. The wide fibres are comparatively thin walled with wide lumen. The wide fibres have some amorphous inclusions and they are nearly 300 µm long and 20 µm wide (Plate – IV g).

**Parenchyma**

The parenchyma cells are squarish or rectangular in outline. They are thin walled and they are seen in large masses. The protoplast and nucleus are dense in parenchyma cells (Plate – IVh).

**Powder Analysis of the Whole Plant**

**Physicochemical constant**
Table 1a: Ash values of the powdered whole plant of P. alliacea*

| S.No. | Type of Ash         | % of Ash values |
|-------|---------------------|-----------------|
| 1.    | Total ash value of powder | 10.86 ± 1.11    |
| 2.    | Water soluble ash    | 3.64 ± 0.04     |
| 3.    | Acid insoluble ash   | 3.16 ± 0.03     |
| 4.    | Sulphated ash        | 11.02 ± 0.07    |

Table 1b: Extractive values of the powdered whole plant of P. alliacea*

| S.No. | Nature of the extract | % of extractive values |
|-------|-----------------------|------------------------|
| 1.    | Petroleum ether       | 5.68 ± 0.04            |
| 2.    | Benzene               | 6.06 ± 0.02            |
| 3.    | Chloroform            | 6.24 ± 0.02            |
| 4.    | Acetone               | 6.68 ± 0.03            |
| 5.    | Methanol              | 9.08 ± 0.04            |
| 6.    | Ethanol               | 8.86 ± 0.04            |
| 7.    | Water                 | 7.96 ± 0.03            |

* All values are mean of triplicate determination

The physicochemical parameters like ash and extractive values, fluorescence analysis of whole plant of P. alliacea were performed and results are presented below. The powdered whole plant of P. alliacea was investigated for physicochemical constants like total ash value, water soluble ash, acid insoluble ash, sulphated ash and extractive values (Table 1 a & 1b). The total ash content of the powdered whole plant of P. alliacea is 10.86%. The extractive value of methanol is more than the solvents investigated in the present study.

Fluorescent Analysis

The results of fluorescent analysis of whole plant of P. alliacea are shown in Table 2. The powder from the whole plant of P. alliacea emitted pale green under day light, green under short and dark green under long UV light. The whole plant powder shows the characteristic fluorescent green colour when treated with 1N HCl, Conc. HCl, Conc. H3SO4, 50% H2SO4, Conc. HNO3, +NH3, 50% HNO3, petroleum ether and acetone.

Preliminary Phytochemical Screening

Petroleum ether, benzene, chloroform, methanol and ethanol extracts of whole plant of P. alliacea were qualitatively analysed for the presence of different phytoconstituents and the results are presented in Table 3. The methanol and ethanol extracts of whole plant of P. alliacea shows the presence of alkaloid, anthraquinone, catechin, flavonoid, phenol, quinone, saponin, steroid, tannin, sugar, glycoside and xanthoprotein.

DISCUSSION

In recent years, there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical study is still more reliable, accurate and inexpensive. According to the World Health Organization19, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. As a part of authentication of the sample, the macroscopic, microscopical, physicochemical and phytochemical examination of whole plant of P.alliacea was studied. Pharmacognostical evaluation of different parameters is the vital etiquette for standardization of herbs.

Salient Diagnostic Features

Young root exhibits a fairly thick superficial periderm, a wide, starch filled cortex and a circular, dense secondary xylem with thin layer of phloem. The old root has a central, wide and circular secondary xylem and secondary phloem and an outer circle of several discrete, thick

Table 2: Fluorescence analysis of the powdered whole plant of P.alliacea.

| Treatment                        | Under Day Light | Under UV light |
|----------------------------------|-----------------|----------------|
| Powder as such                   |                 | 245 nm         | 365 nm         |
| Powder + 1 N Aqueous NaOH        | Pale green      | Green          | Dark green     |
| Powder + 1 N Alcoholic NaOH      | Yellowish green | Greenish yellow| Dark green     |
| Powder + 1 N HCL                 | Green           | Greenish yellow| Dark green     |
| Powder + Conc. HCL               | Light green     | Fluorescent green| Brown         |
| Powder + Conc. H3SO4             | Green           | Fluorescent green| Dark green     |
| Powder + 50% H2SO4               | Green           | Fluorescent green| Black green    |
| Powder + Conc.HNO3              | Light green     | Green          | Dark green     |
| Powder + 40% NaOH + 10% Lead Acetate | Pale green       | Dark green     | Dark green     |
| Powder + Acetic acid             | Green           | Light green    | Dark green     |
| Powder + Ferric Chloride         | Dark green      | Dark green     | Dark green     |
| Powder + Chloroform              | Yellowish green | Yellowish green| Brown          |
| Powder + Benzene                 | Green           | Pale yellow    | Dark green     |
| Powder + Petroleum ether         | Green           | Fluorescent green| Dark green     |
| Powder + Methanol                | Yellowish green | Pale Yellow    | Dark green     |
| Powder + Ethanol                 | Yellowish green | Green          | Dark green     |
| Powder + acetone                 | Dark green      | Fluorescent green| Brown         |
| Powder + HNO3 + NH3              | Green           | Yellowish green| Dark brown     |
| Powder + 50% HNO3               | Yellowish green | Fluorescent green| Dark brown |
segments of secondary xylem and phloem. The presence of successive cylinder of vascular tissues is an anomalous (unusual) feature. The stem is roughly circular in outline with discontinuous, thick arcs of sclerenchyma cells forming a ring, a prominent layer of phloem cylinder and a thin cylinder of xylem enclosing parenchymatous pith.

Xylem elements include wide and narrow circular thick walled vessels and thick and lignified fibres. Calcium oxalate druses are fairly common in the ground parenchyma cells of the stem. The petiole is shield shaped with flat adaxial side. The vascular system is similar to that of the midrib. It consists of about nine collateral,

| Tests            | Petroleum Ether | Benzene | Ethyl acetate | Methanol | Ethanol |
|------------------|-----------------|---------|---------------|----------|---------|
| Alkaloid         | -               | -       | +             | +        | +       |
| Anthraquinone    | -               | -       | +             | +        | +       |
| Catechin         | +               | +       | -             | +        | +       |
| Coumarin         | -               | +       | -             | -        | -       |
| Flavonoid        | -               | +       | +             | +        | +       |
| Phenol           | +               | +       | +             | +        | +       |
| Quinone          | +               | +       | +             | +        | +       |
| Saponin          | +               | -       | +             | +        | +       |
| Steroids         | +               | -       | -             | +        | +       |
| Tannin           | -               | +       | -             | +        | +       |
| Terpenoids       | +               | +       | +             | -        | -       |
| Sugar            | -               | -       | +             | +        | +       |
| Glucoside        | -               | -       | +             | +        | +       |
| Xanthoprotein    | +               | +       | +             | +        | +       |
| Fixed oil        | +               | -       | -             | +        | +       |

+ Present - Absent
discrete vascular bundles with urn shaped sclerenchyma layer bearing vascular bundles. The leaf has a thick and wide abaxial midrib which is pendent on the lower side of the lamina. The adaxial part of the midrib is wide and short with a hump measuring about 1.2 mm thick. The ground tissue of the midrib exhibits five wedge shaped collateral vascular bundles. Of the five vascular bundles one bundle median abaxial position, two bundles occupy lateral position and the remaining two occupy adaxial lateral position. The bundles are enclosed within deep, bowl shaped and undulate sclerenchyma layer. The lamina is smooth and even, measuring about 90 µm thick and possesses an adaxial, single horizontal row of columnar palisade cells and an abaxial zone of small, circular and less compact spongy parenchyma. Long, flat and conical scale like calcium oxalate stylloid type crystals are common in the mesophyll tissue. The leaf margin has thicker epidermal cells and undifferentiated compact parenchyma cells. The epidermal cells of the lamina are small, thin walled and have highly wavy anticlinal walls. Stomata are of paracytic type with two unequal subsidiary cells which lie parallel to the lateral side of the guard cells. The stomata are circular in outline. Venation of the lamina is reticulate with wide rectangular vein islets which are either simple or dendroid type. Powdered preparation of the plant sample exhibits narrow and wide fibres; long, narrow cylindrical vessel elements with oblique circular perforations and with prominent circular lateral pits and clustered parenchyma cells with prominent nucleus.

Microscopical evaluation is the simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and determination of adulterants and substituents. Physicochemical parameters are important for qualitative standards and useful in determining authenticity and purity of crude drugs. Ash determination is helpful to judge the uniqueness and the cleanliness of the crude material. Water soluble ash in the measure of physiological inorganic components of the crude drug. Elevated water soluble content probably because of hard water supply which has excess high mineral contents20,21. Acid insoluble ash gives an idea about the non-physiological ash produced due to the adherence of...
inorganic dirt, dust to the crude drug. Increased acid insoluble ash means adulteration due to dirt, soil or sand\textsuperscript{22}. Extractive values are useful for the determination of exhausted or adulterated drugs. It gives an idea about the nature of the chemical constituents present in the crude drug.

Fluorescence study is an important parameter for the standardization of crude drugs. Many drugs fluoresce when their powder is exposed to ultraviolet radiation. It is important to observe to all materials on reaction with different chemical reagents under UV light.

The phytochemical screening of the whole plant of \textit{P. alliacea} showed the presence of alkaloids, coumarins, flavonoids, saponins, steroids, phenols, tannins, terpenoids, glycosides and xanthoproteins. The presence of these phytochemicals in the whole plant of \textit{P. alliacea} confer them for their medicinal value\textsuperscript{23}. The pharmaceutical and therapeutic potentials of plants and their products are as a result of the presence of these phytochemicals in them\textsuperscript{24}.

Evaluation of crude drug involves the determination of identity, purity and quality. Purity is the absence of extraneous matter, while the amount of active constituents present in the crude drug is referred to as quality. Macroscopic and microscopic evaluation is an important parameter in accessing the identity of herbal raw material. At the same time, qualitative screening of secondary metabolites focuses on quality of raw material. Moreover, therapeutic potential of plant is solely dependent on the nature and amount of phytoconstituents.
Hence, accessing the quality of herbal raw material in terms of their chemical composition becomes very imperative. To conclude, various macroscopic, microscopic, physical and phytochemical aspects/parameters listed here for *P. alliacea* in the present work can be used with respect to its identification, authentication and standardization.

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