

COMPARATIVE PHARMACOGNOSTICAL EVALUATION OF SOME IMPORTANT *Phyllanthus* HERBACEOUS COMPLEX

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Abstract

The genus *Phyllanthus* L. belongs to the family Euphorbiaceae and it includes about 800 species worldwide. In India, the genus represents about 40 species, out of which 12 species from an herbaceous complex group. The species are *P. ajmerians*, *P. amarus*, *P. debilis*, *P. fraternus*, *P. rotundifolius*, *P. scabrifolius*, *P. tenellus*, *P. urinaria*, *P. maderaspatensis*, and *P. virgatus* are very common. Till date no scientific data is available regarding the comparative study of the different species, hence present study three plants has been selected to evaluate comparative morphological, pharmacognostical and phytochemical profile. Pharmacognostical evaluation showed that morphologically similar in appearance. Cork with oil globules, medullary rays uniserriate in *P. urinaria* and *P. fraternus* where as in *P. maderaspatensis* multiserriate medullary rays were observed.

Key words: *Phyllanthus*; Leaves; Root; Stem; Pharmacognosy.

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INTRODUCTION

The correct identification of medicinal herbs used in Ayurveda is one of the most serious problems confronting Ayurvedists as well as botanists. The nomenclatural systems used by the ancient Indians-attributing different names to the same plant and same name for different plants together with the time, have caused considerable confusion in picking the medicinal herbs in various parts of the country by various people. The importance of correct botanical identification of the medicinal herbs used in this system of medicine is now well recognized.

But the difference of opinion as to the identity of genuine drugs among practitioners themselves makes this task extremely difficult. It seems that standardization of raw drugs in Ayurveda is very remote or probably even impossible because neither the present-day practitioners nor raw drug collectors are sure about the genuineness of the herbs that they use. On the part of the Botanists, the only possible help that they can render is to provide proper botanical identification of the herbs used.

The plant genus *Phyllanthus* (Euphorbiaceae) is widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 750 to 800 species and is subdivided into 10 or 11 subgenera: *Botryanthus*, *Cicca*, *Conani*, *Euphorbia*, *Emblica*, *Ericocus*, *Gomphidium*, *Isocladus*, *Kirganelia*, *Jatropha*, *Phyllanthodendron*, *Phyllanthus* and *Xylophylla*.

The genus *Phyllanthus* L. belongs to the family Euphorbiaceae and includes about 800 species worldwide. In India, the genus is represented by about 40 species, out of which 12 species from an herbaceous complex group.

These species are *P. ajmerians*, *P. amarus*, *P. debilis*, *P. fraternus*, *P. rotundifolius*, *P. scabrifolius*, *P. tenellus*, *P. urinaria*, *P. maderaspetensis* and *P. virgatus* are very common. Till date no scientific data is available regarding the comparative study of the three different species, hence present study has been undertaken to evaluate Morphological, Pharmacognostical and Phytochemical profile to have clear vision of the species individually.^[1]

MATERIALS AND METHOD

Samples of whole plants were used for the pharmacognostical evaluation. The study was conducted as per the guidelines of Ayurvedic Pharmacopeia of India (API).^[2]

Collection of Samples

The plant samples of *P. fraternus*, *P. urinaria* and *P. maderaspatensis* were collected personally from its natural habit of Jamnagar and identified with the help of Pharmacognosist and Botany texts and various.^[3]

Processing and Preservations

Herbarium sheet of the plant and sample specimen was deposited in Pharmacognosy lab, I.P.G.T. &R.A., Jamnagar for future references. The root, stem, leaf were washed, shade dried, powdered, sieved through 80# and preserved in an air-tight glass vessel. For microscopical evaluation, fresh sample was preserved in a solution prepared from 70% ethyl Alcohol: glacial acetic acid: formalin (AAF) in the ratio of 90:5:5.^[4]

Morphological Study

The morphological study was carried out which regarding of size, shape of leaf, root, stem of the *P. fraternus*, *P. urinaria* and *P. maderaspatensis*.^[5]

Study of organoleptic characters

The dry sample of whole plant powders of *P. fraternus*, *P. urinaria* and *P. maderaspatensis* was evaluated for their organoleptic characters including touch, colour, taste and odour.^[5]

Microscopical Study

Transverse section microscopy

Free hand transverse sections of rhizomes were first observed with distilled water and then with phloroglucinol and concentrated HCl. Microphotographs are taken by using carl-zeiss trinocular microscope attached with camera. Powder microscopy: Powder of the roots, stems, leaves was studied microscopically and microphotographs are taken by using carl-zeiss trinocular Microscope attached with camera.^[6]

RESULTS AND DISCUSSION

Collection & Authentication of raw drug:

P. fraternus, *P. urinaria*, and *P. maderaspatensis* were collected from natural habit which is free from pollution out spurts and botanical garden of Jamnagar. Pharmacognostical identification and authentication was done in Pharmacognosy laboratory, I.P.G.T. & R.A. Fresh samples were used for various pharmacognostical evaluations. Some of the samples were preserved under AAF (90:5:5) preservative solution. Healthy uninfected samples of *P. fraternus* (Ref. No.: Phm 6018), *P. urinaria* (Ref. No.: Phm 6019) and *P. maderaspatensis* (Ref. No.: Phm 6020) were made into herbarium and preserved in the Lab for future study. Leaves, stem and roots were separated dried in shed powdered at 80 mesh for further powder microscopy and analytical studies.

Macroscopic study of *P. fraternus*

Erect annual herb with many lateral branch lets, leaves simple, oblong, apex obtuse with prominent midrib, inflorescence axillary, unisexual cymose, base female flower ends with male flowers. (Figure 1A)

Transverse section of root of *P. fraternus*

Diagrammatic T.S. of root was circular in outline. Outer cork is followed by cortex, endodermis, pericycle & vascular bundles. (Figure 2A)

Detailed transverse section (T.S.) shows the outer most 3-5 layers are composed of tangentially elongated cork cells, some of the cells filled with brown content and oil globules. (Figure 2A) Cortex region is found to be reduced, loosely arranged & made up of 3-5 layers of simple parenchymatous cells which are sacredly isolated with simple starch grains and tannin content. Some of the isolated 2-3celledpericyclic fibres were observed in cortical region. Cortex ends with single layered of parenchymatous endodermis. (Figure 2A) Vascular radially arranged. Xylem consists of xylem parenchyma and fibres. Here and there xylem vessels were filled with yellowish brown content. Xylem bundles were separated by medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 2A1) Medullary rays uni-serrate and started from central region and extended up to inner layers of the pericyclic region. (Figure 2A1)

Transverse section of stem of *P. fraternus*

Diagrammatic T.S. of stem was circular in outline. Outer epidermis is followed by cortex, endodermis, pericycle, vascular bundle and central parenchymatous large pith.

Figure 1: Live photographs of plants



A: *P. fraternus*



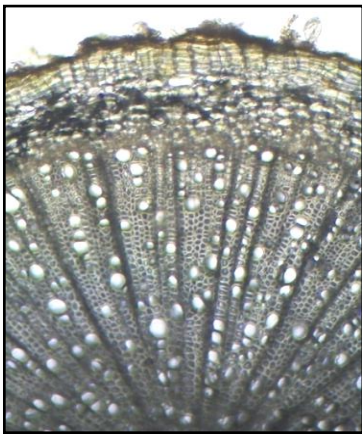
B: *P. urinaria*



C: *P. maderaspatensis*

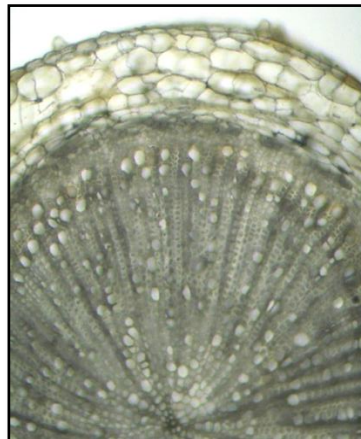
Figure 2: Transverse sections of roots

P. fraternus



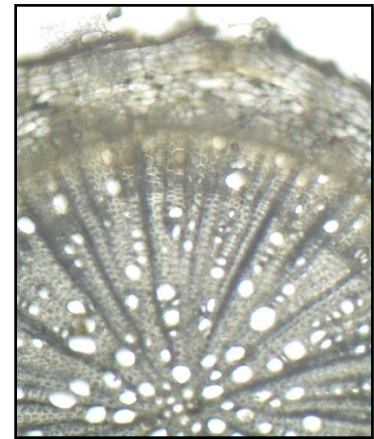
A. Cork and cortex

P. urinaria

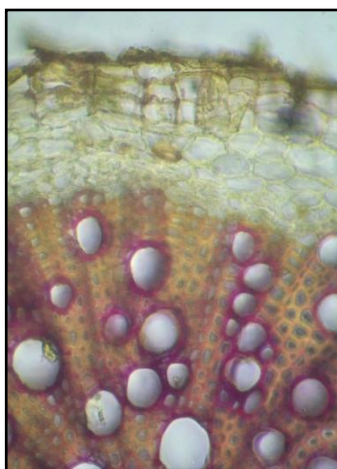


B. Cork and cortex

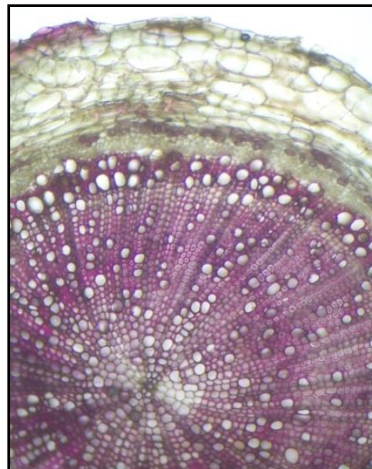
P. maderaspatensis



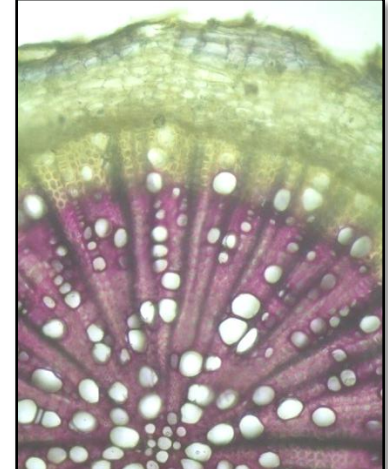
C. Cork and cortex



A. (1). Phloem and xylem



B. (1). Phloem and xylem



C. (1). Phloem and xylem

Detailed T.S. shows the outer most single layer compactly arranged without any interruption of stomata and with simple unicellular trichomes. Epidermis covered with thick cuticle. (Figure 3A) Cortex made up of 3-5 layers of simple parenchymatous cells without any intercellular spaces. Inner cells loaded with rosette crystals of calcium oxalate, oil globules. Cortex is followed by single layer of endodermis. Pericycle is made up of 3-5 layered discontinuously arranged beneath the endodermis. (Figure 3A) Vascular bundles radially arranged. Metaxylem is facing towards cortical region and protoxylem towards central pith region with xylem parenchyma and fibres. Xylem is separated by uni-serrate medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 3A1) Larger region of the section is occupied by pith; parenchymatous cells are compactly arranged in pith region. Pith cells are filled with oil globules, rarely simple starch grains and rosette crystals of calcium oxalate. (Figure 3A1)

Transverse section of leaf through midrib of *P. fraternus*

Leaf is dorsi-ventral type & differentiated into upper palisade parenchyma and lower mesophyll tissue. Epidermis single layered both upper and lower epidermis interrupted by anisocytic stomata. (Figure 4A) Epidermis single layered. Upper epidermis consists of single layered barrel shaped compactly arranged cells. Some of the cells lead to form stomata (anisocytic) and unicellular simple trichomes. Lower epidermis also consists of epidermal cells as in upper epidermis but some epidermal cells interrupted by stomata; stomata are mainly anisocytic in nature. Both the epidermis is covered with thick cuticle. (Figure 4A) Mesophyll is differentiated into upper palisade consists of one layer of elongated compactly arranged cells, below the upper epidermis. Chloroplast, oil globules and Prismatic crystals calcium oxalate are very common in palisade parenchyma. There are 3-

4 layered loosely arranged rounded to oval shaped spongy parenchyma with many air chambers located above the lower epidermis. Oil globules and chloroplast pigments are very common in spongy parenchymatous tissue. Vascular bundle is situated at the centre through the midrib section of the leaf. Vascular bundle is collateral and open type. Xylem differentiated into protoxylem & metaxylem. Metaxylem is found towards lower epidermis & protoxylem towards upper epidermis. Xylem composed of its parenchyma and fibres. Phloem situated beneath the xylem towards lower epidermis and composed of some sieve elements. Vascular bundle is surrounded by parenchymatous cells & covering the bundle sheath. Parenchymatous bundle sheath extended towards both the epidermis. (Figure 4A1)

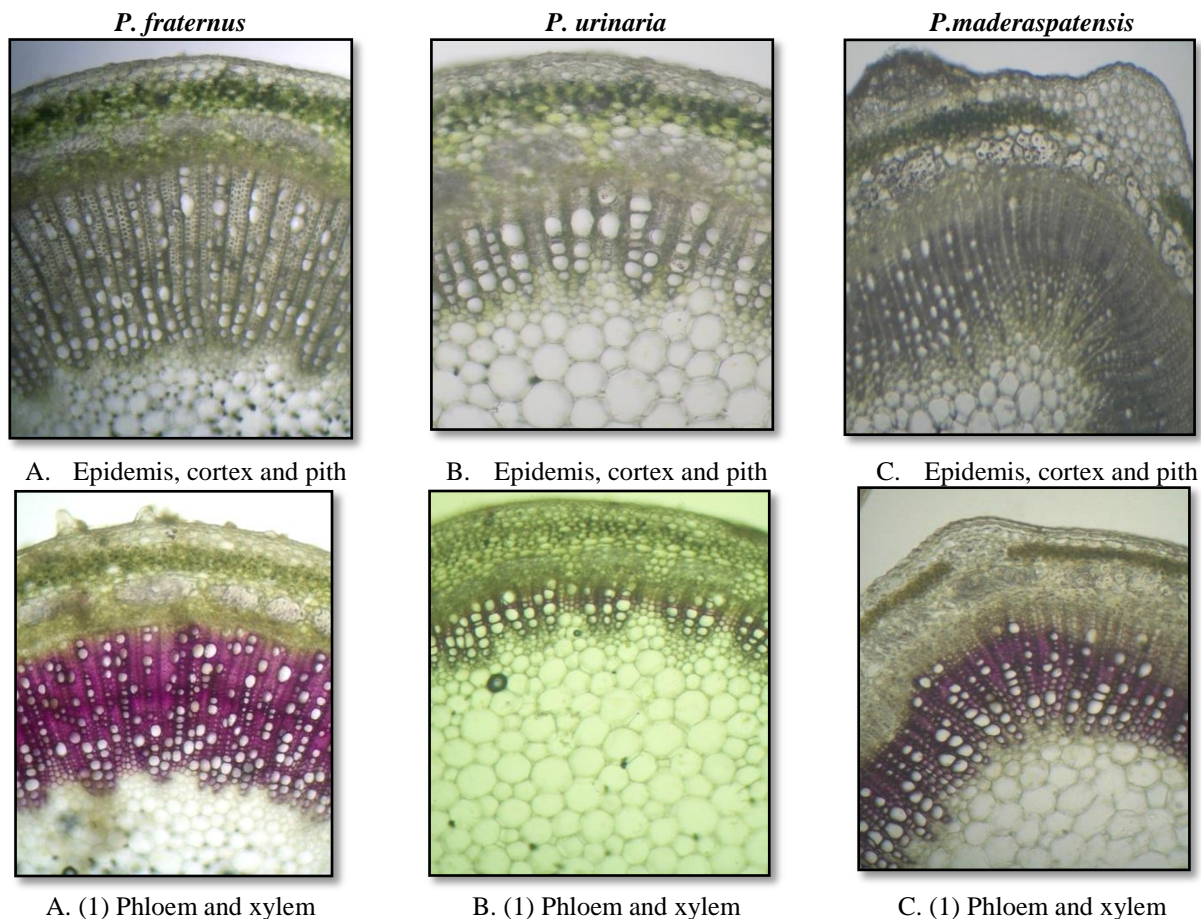
Powder microscopy of whole plant *P. fraternus*

Organoleptic characters of whole plant powder were carried out and the results are depicted in the Table 1. Diagnostic powder microscopy showed that fragments of palisade parenchyma of leaf, oil globule, Rosette and prismatic crystals of calcium oxalate from stem and root, aleurone grains of seeds, fragments of simple fibres of stem and root, fragments of lignified fibres, sclereid, fragments of annular vessels of stem, fragments of pitted vessels of root, anisocytic stomata of leaf epicarp cells of fruit, epidermal cells of leaf, wavy parenchyma cells of leaf, Tannin content, cork in surface view of root, epidermal cells with tannin content. (Figure 5A1- A4)

Macroscopic study of *P. urinaria*

Erect annual herb, with many lateral branchlets, leaves simple, oblong, apex obtuse with prominent midrib, inflorescence axillary, unisexual cymose, base female flower ends with male flowers. (Figure 1B)

Figure 3: Transverse sections of stems



Transverse section of root of *P.urinaria*

Diagrammatic T.S. of root was circular in outline. Outer cork is followed by cortex, endodermis, pericycle & vascular bundles.

Detailed T.S. shows the outer most 2-4 layers are composed of tangentially elongated lignified cork cells, some of the cells filled with brown content and oil globules (Figure 2B). Cortex region is found to be 8-10 layered loosely arranged simple parenchymatous cells which are sacredly isolated with simple starch grains and tannin content. Some of the isolated 2-3 celled lignified pericyclic fibres were observed in the corticle region. Cortex ends with single layered of parenchymatous endodermis. (Figure 2B)Vascular bundles radially arranged. Xylem consists of xylem parenchyma and fibres.

Xylem bundles were separated by medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 2B1) Medullary rays uni to bi-serrate and started from central region and extended up to inner layers of the pericyclic region. (Figure 2B1)

Transverse section of stem of *P.urinaria*

Diagrammatic T.S. of stem was circular in outline somewhat four protruded at different places. Outer epidermis is followed by cortex, endodermis, pericycle, vascular bundle and central parenchymatous large pith.

Detailed T.S. shows the outer most single layer compactly arranged without any interruption of stomata and with simple unicellular trichomes. Epidermis covered with thick cuticle. (Figure 3B)

Epidermis followed by single layered of hypodermis followed by cortex. Cortex made up of 3-4 layers of simple parenchymatous cells. Cortex is followed by single layer of endodermis. Pericycle is made up of 3-5 layered discontinuously arranged beneath the endodermis. (Figure 3B) Vascular bundles radially arranged. Metaxylem is facing towards cortical region and protoxylem towards wide central pith region with xylem parenchyma and fibres. Xylem is separated by uni-serrate medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 3B1) Pith, parenchymatous cells are compactly arranged in pith region. Pith cells are filled with oil globules, rarely simple starch grains and some rosette crystals of calcium oxalate. (Figure 3B1)

Transverse section of leaf through midrib of *P. urinaria*

Leaf dorsi-ventral type & differentiated into upper palisade parenchyma and lower mesophyll tissue. Epidermis single layered both upper and lower epidermis interrupted by anisocytic stomata. Transverse section through midrib shows large vascular bundle present at the center.

Epidermis

Epidermis single layered. Upper epidermis consists of single layered barrel shaped compactly arranged cells. Both the epidermis is covered with thick cuticle. (Figure 4B) Mesophyll is differentiated into upper palisade & lower parenchymatous layers. Palisade consists of one layer of elongated compactly arranged cells, below the upper epidermis.

Chloroplast, oil globules and rosette crystals calcium oxalate are very common in palisade parenchyma. 3-4 layered spongy parenchyma with many air chambers located above the lower epidermis. Oil globules and chloroplast pigments are very common in spongy parenchymatous tissue.

Vascular bundle is situated at the centre through the midrib section of the leaf. Xylem composed of its parenchyma and fibres. Phloem situated beneath the xylem towards lower epidermis and composed of some sieve elements. Vascular bundle is surrounded by parenchymatous cells & covering the bundle sheath. Parenchymatous bundle sheath extended towards both the epidermis. Some of the parenchyma cells with rosette crystals of calcium oxalate. (Figure 4B1)

Powder microscopy of whole plant *P. urinaria*

Organoleptic characters of whole plant powder were carryout and the results are depicted in the Table1. Diagnostic powder microscopy showed that fragments of palisade parenchyma of leaf, oil globule, Rosette and prismatic crystals of calcium oxalate from stem and root, aleurone grains of seeds, fragments of simple fibres of stem and root, fragments of lignified fibres, fragments of annular vessels of stem, fragments of pitted vessels of root, Anisocytic stomata of leaf, epicarp cells of fruit, epidermal cells of leaf, wavy parenchyma cells of leaf, Tannin content, cork in surface view of root, epidermal cells with tannin content. (Figure 5B1- B4)

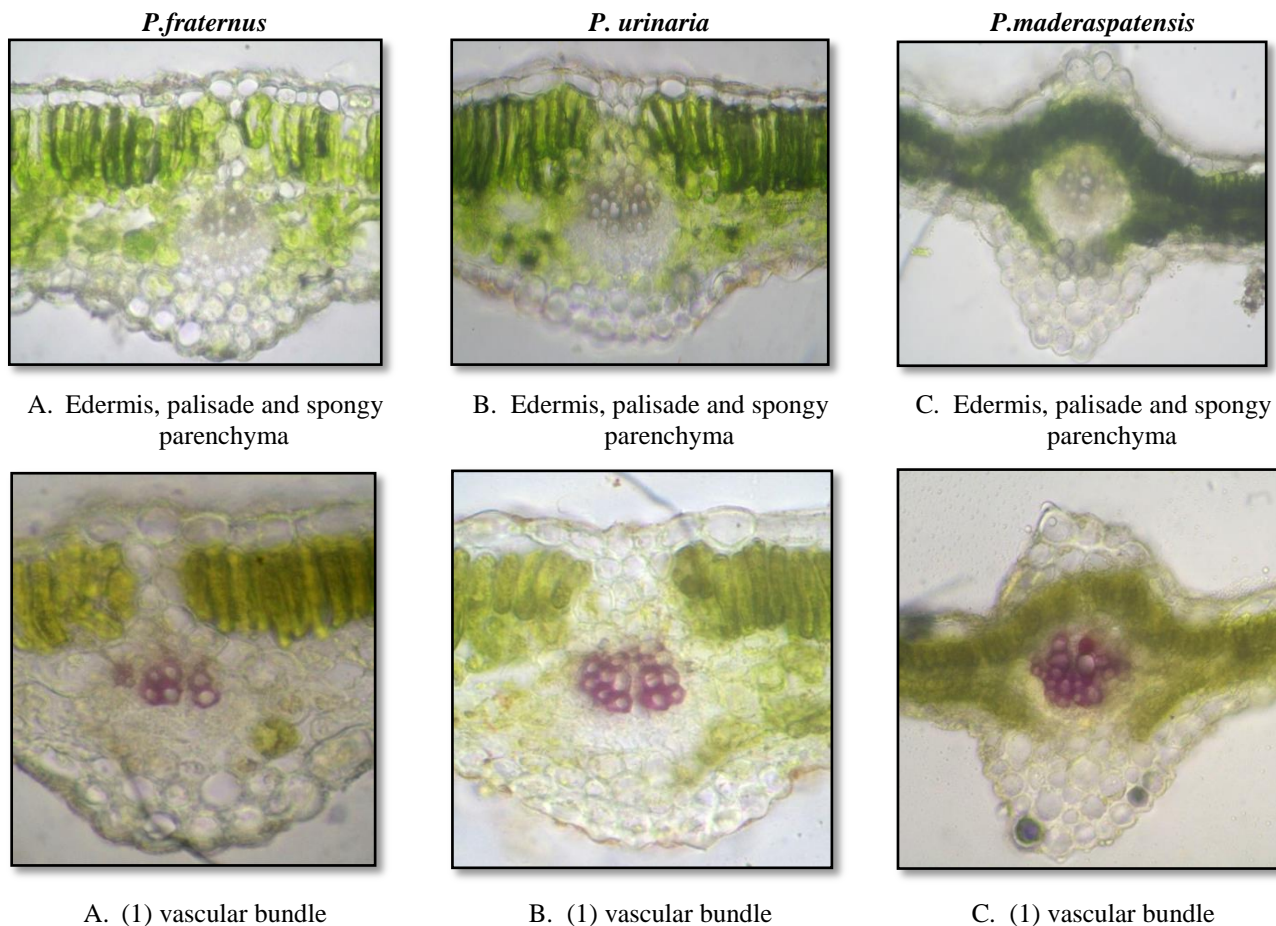
Macroscopic study of *P. maderaspatensis*

Erect herb grows upto 40-60cm, branch and branch lets many, leaf simple stipulate lanceolate to triangular, inflorescence axillary, unisexual cymose, base female flower ends with male flowers. (Figure 1C)

Transverse section of root of *P. maderaspatensis*

Diagrammatic T.S. of root is circular in outline. Outer cork is followed by cortex, endodermis, pericycle & vascular bundles without any pith.

Figure 4: Transverse section of leaves through midrib



Detailed T.S. shows the outer most layers are composed of tangentially elongated 2-3 layered lignified cork cells, some of the cells filled with brown content. (Figure 2C) Cortex region is found to be moderately wide loosely arranged & made up of 4-7 layers of simple parenchymatous cells. (Figure 2C) Vascular bundles radially arranged metaxylem is found towards the cortical region and protoxylem towards center. Xylem consists of xylem parenchyma and fibres. Xylem bundles were separated by medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 2C1) Medullary rays are uni-serrate and started from central region and extended up to inner layers of the pericyclic region. (Figure 2C1)

Transverse section of stem of *P. maderaspatensis*

Diagrammatic T.S. of stem is somewhat pentagonal in outline. Outer epidermis is followed by cortex, endodermis, pericycle, vascular bundle and central parenchymatous large pith.

Detailed T.S. shows the outer most single layer compactly arranged without any interruption of stomata and trichomes. Epidermis covered with suberized thick cuticle. (Figure 3C) Hypodermis made up off single layered but at the region of angle it varies upto 3-5 layers. Cortex made up of 3-5 layers of simple parenchymatous cells. Cortex is followed by single layer of endodermis.

Figure 5: Microphotographs of whole plant powder

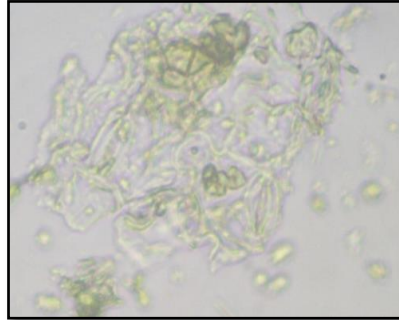
P. fraternus

P. urinaria

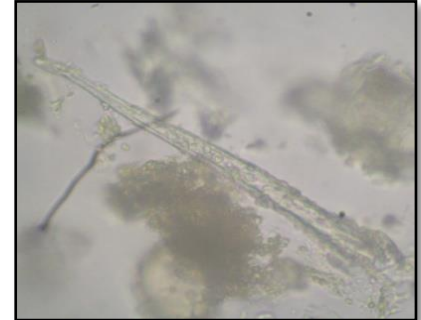
P. maderaspatensis



A. (1) Fragment of palisade



B. (1) Anisocytic stomata



C. (1) Fragment of fibre and oil globules



A.(2) Tannin content



B.(2) Starch grain with hilum



C.(2) Prismatic crystal



A.(3) Prismatic crystal



B.(3) Fragment of spiral vessel



C.(3) Fragment of lignified fibre



A. (4) Fragment of lignified epicarp



B. (4) Fragment of fibre



C. (4) Tannin content

Pericycle is made up of 3-5 celled discontinued pockets which are circularly arranged beneath the endodermis. (Figure 3C) Vascular bundles, Xylem consists xylem parenchyma and fibres. Xylem is separated by uni-serrate medullary rays. Phloem is situated above the xylem with sieve elements and fibres. (Figure 3C) Parenchymatous pith cells are compactly arranged in pith region. Pith cells are filled with oil globules, simple and compound starch grains and sphenoid crystals of calcium oxalate. (Figure 3C)

Transverse section of leaf through midrib of *P. maderaspatensis*

Leaf is dorsi-ventral type & differentiated into upper palisade parenchyma and lower mesophyll tissue. Epidermis single layered upper epidermis devoid of stomata. Transverse section through midrib shows large vascular bundle present at the center.

Epidermis single layered. Upper epidermis consists of single layered barrel shaped compactly arranged cells. Some of the epidermal cells interrupted by stomata (Anisocytic) are present in the upper epidermis. Lower epidermis also consists of epidermal cells as in upper epidermis but some epidermal cells interrupted by stomata; stomata are mainly anisocytic in nature. (Figure 4C) Palisade consists of one layered elongated compactly arranged cells, below the upper epidermis. Chloroplast, oil globules and acicular and cigar shaped calcium oxalate crystals are very common in palisade parenchyma. There is 3-4 layered spongy parenchyma with many air chambers located above the lower epidermis. Oil globules and chloroplast pigments are very common in spongy parenchymatous tissue. (Figure 4C)

Vascular bundle is collateral and open type. Xylem differentiated into protoxylem and metaxylem. Metaxylem is found towards lower epidermis & protoxylem towards upper epidermis. Xylem composed of its

parenchyma and fibres. Phloem situated beneath the xylem towards lower epidermis and composed of some sieve elements. Vascular bundle is surrounded by parenchymatous cells & covering the bundle sheath. Some of the ground tissue consist rosette crystals of calcium oxalate crystals. Bundle sheath extended towards epidermis. (Figure 3C)

Powder microscopy of Whole Plant *P. maderaspatensis*

Organoleptic characters of whole plant powder were carryout and the results are depleted in the Table1. Diagnostic powder microscopy showed that oil globule, loosely arranged parenchyma cells of stem, fragments of spongy parenchyma cells of leaf, Rosette and prismatic crystals of calcium oxalate from stem and root, aleurone grains of seeds, fragments of simple fibres of stem and root, fragments of lignified fibres, sclereids, fragments of annular vessels of stem, fragments of spiral vessels of leaf, anisocytic Stomata of leaf, fragments of pitted vessels of root, epicarp cells of fruit, epidermal cells of leaf, wavy parenchyma cells of leaf, starch grins with hilum, Tannin content, cork in surface view of root, epidermal cells with tannin content. (Figure 5C1- C4)

DISCUSSION

P. fraternus and *P. urinaria* grows upto 30 cm height, whereas *P. maderaspatensis* grows upto 40 cm. Leaves all were simple but *P. fraternus* 0.9 X 0.3 cm, *P. urinaria* 1 X 0.5 cm. whereas *P. maderaspatens* 1.5 X 0.5 cm, both *P. fraternus* and *P. urinaria* ovate to obovate whereas *P. maderaspatensis* lanceolate to obovate. Fruits of *P. fraternus* measures 0.1 cm, *P. urinaria* 0.2 cm, whereas *P. maderaspatensis* 0.5 cm. This showed that from *P. maderaspatensis* may be getting large biomass.

Table 1: Macroscopical characters of *P. fraternus*, *P. urinaria* and *P. maderaspatensis*

Characters	<i>P. fraternus</i>	<i>P. urinaria</i>	<i>P. maderaspatensis</i>
Colour	Yellow greenish	Dark green	Yellow green
Taste	Slightly astringent	Slightly astringent	Slightly astringent
Odour	Characteristic	Characteristic	Characteristic
Nature of powder	Coarse	Coarse	Coarse

All three roots with 3-5 layered cork with oil globules but *P. fraternus* showed that suberized cork. Cortex layers made of 3-5 in *P. fraternus*, 8-10 in *P. urinaria*, where as in *P. maderaspatensis* showed about 4-5 layered & commonly starch and with tannin content. Pericyclic fibres 2-3 layered in both *P. fraternus* & *P. urinaria* but absent in *P. maderaspatensis*. This showed that much fibrous content in *P. fraternus* & *P. urinaria*. Medullary rays uniserriate in both *P. fraternus* & *P. maderaspatensis*, whereas bi-serriate in *P. urinaria*. Vascular bundles radially arranged. Xylem with its fibres and parenchyma, phloem with sieve elements and fibres present in all. Results showed that all were single layered epidermis with thick cuticle and trichomes as well as hypodermis. But *P. maderaspatensis* hypodermis made up of 2-5 layers. Cortex made of 3-5 layered with rosette crystals of calcium oxalate. Isolated 2-5 celled group of pericyclic fibres observed in *P. fraternus* & *P. urinaria*, where as in *P. maderaspatensis* it was 3-5 layered. Commonly single layered endodermis, Vascular bundles open and collateral type observed in all 3 species. Pith cells filled with Oil globules, rarely simple starch grains, rosette crystal in *P. fraternus* & *P. urinaria*, where as in *P. maderaspatensis* filled with microsphenoidal crystals. Results showed that all were both upper and lower epidermis single layered with thick cuticle and stomata, trichomes. Most commonly single layered upper palisade and 3-5 layered lower spongy parenchyma in all 3 species. Vascular bundles made of xylem towards upper epidermis phloem towards lower epidermis.

Prismatic crystals of calcium oxalate observed at ground tissue of *P. fraternus* & *P. urinaria*, where as in *P. maderaspatensis* filled with rosette, acicular and cigar shaped crystals. This variation in types of crystal plays a vital role in identification of individual species. Results showed that the Organoleptic characters like odour, taste, touch are same in all three plants. But colour of powder is different. In *P. fraternus* and *P. maderaspatensis* colour of whole plant powder is yellow green in colour while in *P. urinaria* dark green in colour. The results reveal that the presence of fragment of palisade parenchyma, prismatic crystals of calcium oxalate, anisocytic stomata, spiral and pitted vessels lignified epicarp cells, fibres, starch grains, tannin content, were commonly found all over the three species powder. This shows similar pharmacognostical profile.

CONCLUSION

All the mentioned 3 species which were evaluated, displayed range of characteristic feature that can help in their identification. Some of such features are the nature of stem, leaves, anatomical characters, powder microscopy provided here for easy identification of this complex group. Scientific discussion all over the three species showed that some variation in morphological level but Pharmacognostical study has given much considerable amount of parallel results. These results were crossed over *P. amarus* in the character ie. pharmacognostically showed that merging characteristic features.^[7] Thus *P. fraternus*, *P. urinaria*, *P. maderaspatensis* may be used in the place of *P. amarus*.

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