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A Comprehensive Study on Morpho-anatomy, Embryogeny and Histochemical Localisation of Traditional *Siddha* Medicinal Plant *Senkizhanelli* (*Phyllanthus urinaria* L.)

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Abstract

Background: *Phyllanthus urinaria* L. is an important plant used to treat oral diseases, diarrhea, hepatitis, jaundice and many other health problems related to the urinary system in *Siddha* and various traditional medical systems. **Aim:** The present study describes the morpho-anatomical characteristics and histochemical localisation of *P. urinaria* L. (family *Phyllanthaceae*) for precisely identifying crude drugs procured from its leaf, stem, root, and fruit through microscopic study. **Methods:** Anatomical studies and determination of leaf constants, as well as histochemical localisation of metabolites, were determined as per standard protocols. **Results:** This study showed the presence of paracytic amphistomatous leaves with trichomes throughout leaf blades. The anatomical study of different parts of the plant revealed that the ground tissue of the petiole with various cellular contents including calcium oxalate crystals, brown contents and oil globules, branchlet with angular projections, significant secondary growth in both stem and root, the node with unilacunar stele, and tri carpellate dehiscent fruit showed axile placentation with six seeds with bitegmic coat layers and Capsella variation of the onagrad type of embryogeny. Histochemical study disclosed the major localising zones of various phytochemicals in *P. urinaria*. **Conclusions:** The histo-anatomical profile obtained through the present study may help in the authentication and establishment of the monograph standards of *P. urinaria*.

Major Findings: The distribution of paracytic stomata and non-glandular trichomes were significant as observed in quantitative microscopic analysis of the leaf. The anatomical differences in primary and secondary stems and roots revealed remarkable growth of vascular elements. The embryonic development is an important characteristic in the ontogenic developmental pathway of fruit and seed.

Keywords: Anatomy, Embryogeny, Fruit, Histochemical Localisation, *P. urinaria* L. Seed

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1. Introduction

Phyllanthus urinaria L. (Synonyms: *Diasperus urinaria* (L.) Kuntze, *Phyllanthus alatus* Blume, *Phyllanthus cantoniensis* Hornem, *Phyllanthus croizatii* Steyerm) is a rarely available species of the family *Phyllanthaceae* and distributed in various tropical regions of the world[1]. The genus *Phyllanthus* is comprised of herbs, shrubs and trees and are medicinally valuable as they contain active phytoconstituents[2,3]. Several studies indicate that more than 500 phytochemicals have been isolated from various species of the genus *Phyllanthus*[3].

All parts of this plant are used extensively in various traditional medicinal systems as single drugs and formulations. As a Chinese folk medicine, *P. urinaria* is used for the treatment of liver ailments, hepatitis,

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jaundice, dropsy, enteritis, renal disorders and diarrhea[4]. *P. urinaria* has been traditionally used in northern Thailand against hypoglycemia[5]. Furthermore, juice of *P. urinaria* is used as a tongue cleaner, appetite stimulator in children and for malaria treatment in Malaysia. The roots and stem of this plant have been used as a natural remedy for the treatment of diabetes, kidney as well as urinary bladder problems in Korea[6]. Moreover, *P. urinaria* is widely used in Indonesia to treat diseases related to urinary problems, such as kidney stones, and urinary tract infections and malfunction[7]. Meanwhile, *P. urinaria* is used for diabetes and diarrhea treatment in Trinidad and Tobago[8]. *P. urinaria* has long been used in traditional medicine to facilitate the expulsion of gallstones and kidney stones and as an immunity booster[9,10]. Studies indicate that *P. urinaria* extracts possess numerous bioactivities, including cardioprotective, anti-hypertensive, antiplasmodial, and antioxidant effects[11-14]. Recently, anti-tumour, anti-inflammatory and anti-microbial activities of *P. urinaria* were reported[1,15,16]. In addition, *P. urinaria* extracts possess antiviral action against Hepatitis B Virus (HBV), Herpes Simplex Viruses (HSV), Dengue virus, Enteroviruses, Hepatitis C Virus (HCV), and Human Immunodeficiency Virus (HIV)[17-30].

However, there are many studies reported on the comparative anatomy of different *Phyllanthus* species including *P. urinaria*. A detailed anatomical profile of the whole plant of *P. urinaria* is not reported yet. To lay down a scientific basis for the identification of *P. urinaria* as well as to avoid the risk of adulterations in different medications, this study aims to analyse the pharmacognostic aspects of the whole plant of *P. urinaria*.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The fresh plant specimens were collected from the premises of Siddha Regional Research Institute (SRRI), Thiruvananthapuram, Kerala, India (8.49647° N, 76.97630° E) in June 2024. Followed by the collection, the plant specimens were preserved by standard herbarium techniques and preserved in the Department of Pharmacognosy, SRRI (Voucher no. 179).

2.2 Macroscopy

The macroscopic features like the shape, size, and type of different parts of the plant samples were analysed by using a Stereomicroscope and data were recorded.

2.3 Microscopy

Transverse Sections (T.S) of the parts of plant samples were made using a sharp section cutter. Sections were stained with Safranin stain and mounted using glycerin on a glass slide and observed under the 10X and 40X objectives of the Brightfield microscope attached to an image analyser. Photographs of observed microscopic characters were captured and stored for further referencing.

2.4 Quantitative Microscopy (Determination of Leaf Constants)

Stomatal number, stomatal density, frequency and stomatal index, vein termination and islets were calculated[31-34].

2.4.1 Stomatal Number

To study the morphotype of stomata and number of stomata as well as stomatal index of the leaf, the leaf was subjected to partial maceration of epidermal peeling. A fragment of peeling was placed on a microscopic slide, and the mount of the lower and upper epidermis was prepared with a drop of glycerol solution. The slide was examined under a 10X objective eyepiece to which Camera lucida was attached. A circle (O) -like mark was marked on the drawing paper for each stoma. The average number of stomata/mm^2 for each surface of the leaf was calculated.

2.4.2 Stomatal Index

It is defined as the numbers of stomata divided by the total number of stomata and epidermal cells in percentage. For the stomatal index, the glycerin-mounted leaf peeling prepared as above and a circle (O)-like mark for each stoma and a cross (X) like mark for each epidermal cell were marked on the drawing sheet. The stomatal index was calculated as follows.

$$S.\,I = \frac{S}{S+E} \times 100$$

Where,

S = Number of stomata per unit area.

E = Number of epidermal cells in the same unit area.

2.4.3 Vein Islet and Vein Termination Number

The fragment of leaf lamina with an area of not less than 5 sq. mm, excluding the midrib and the margin of the leaf was taken. The fragments of leaf lamina were cleared by heating in a test tube containing sodium hydroxide solution in a boiling water bath until clear. The cleared fragments were stained with safranin solution, and a temporary mount was prepared with glycerol solution. The stage micrometer was placed on the microscopic stage, examined under 10X objective eyepiece, and an area of 5 sq. mm was drawn. The cleared leaf piece was placed on the microscope stage, and the vein islets and vein terminals included in the square were drawn. The number of vein islets and terminals within the square, including those overlapping on two adjacent sides and excluding those intersected by the other two sides, was counted.

2.4.4 Palisade Ratio

The palisade ratio is the average number of palisade cells per epidermal cell. It is one of the important criteria for the identification of plant species. Since it is constant for a plant species, it is useful to differentiate the species. Epidermal peeling was done by partial maceration, and the sample preparation was done the same as above. It was then examined under 10 X objective eyepiece. Four adjacent epidermal cells were recorded. The palisade cells under the epidermal cells were counted, and the palisade ratio was calculated using the following formula;

$$Palisade\ ratio = \frac{The\ average\ number\ of\ palisade\ cells\ beneath\ the\ 4\ epidermal\ cells}{4}$$

2.5 Histochemical Localisation

Freehand sections of the different parts of *P. urinaria* (leaf, petiole, branchlet, stem, node, root and fruit) were taken and treated with specific reagents to localise components.

2.5.1 Lignin

The section was moistened with a small volume of Phloroglucinol and allowed to stand for 2 minutes or until almost dry. 1 drop of HCl (50%) was added. Lignified cell wall will be stained pink to cherry red[35].

2.5.2 Suberised or Cuticular Cell Wall

Few drops of Sudan red III were added and allowed to stand for few minutes and warm gently if necessary. Suberised or cuticular cell wall is stained orange red or red[35].

2.5.3 Fatty Oils or Volatile Oils or Resins

1-2 drops of Sudan IV were added to the section and allowed to stand for few minutes. Fatty oil substance- orange to red/pink/red coloured globules, resin- red coloured irregular red contents[36].

2.5.4 Phenolic Compounds

A drop of alcoholic ferric chloride was added. Phenolic compounds (flavanoids or tannins) will appear as bluish black coloured[35].

2.5.5 Mucilage

A drop of Ruthenium red was added. Mucilage -pink to red coloured content[35,37].

2.5.6 Alkaloid

A drop of Wagner's reagent (1g Iodine and 2g KI in 50 ml dd water) was added. Alkaloids - yellow to reddish brown contents[38].

2.6 Organoleptic Characters

The basic physical properties such as odour, colour, taste and texture of powdered samples were evaluated using the senses (sight, smell, touch, taste) which help to establish identity, quality, and purity.

2.7 Powder Microscopy

Samples were shade-dried and powdered. About 0.5 gm powder was mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objectives of a bright field microscope for the identification of various diagnostic characters.

3. Results

3.1 Macroscopic Characters

Phyllanthus urinaria is a monoecious, annual, small, slender, or short-lived perennial herb, erect or sprawling, growing up to 60 cm tall, with reddish branchlets (Figure 1).

3.1.1 Leaves

Simple, alternate, subsessile (Figure 1c and 1d); stipules ovate to lanceolate, long acuminate. Leaves are crowded along lateral branchlets with blade oblong to elliptical oblong.

3.1.2 Petiole

Petiole is short, glabrous, compressed, stipules broad; articulate at the base and narrowed into a filiform point.

3.1.3 Stem

Reddish-green in colour, horizontally branched, smooth, angled, woody at base, glabrous to pubescent.

3.1.4 Root

Cluster of small thin rootlets at the end, light reddish brown in colour (Figure 1f).

3.1.5 Flowers

Flowers are solitary, subsessile, monoecious with a warted ovary, greenish red with sepals 6, minute and appear at the axils of the leaves 1-2 in the axils of leaves. Male flowers are seen towards the apex or branchlets, female flowers at the basal part of branchlets, unisexual, regular, 6-merous; male flowers with obovate perianth, disk lobes 6, stamens 3, filaments fused, anthers free; female flowers with triangular-ovate perianth lobes, disk cup shaped, ovary superior; ovoid, warty, 3-belled, styles fused at base into a triangular plate, 2-fid at apex (Figure 1h).

3.1.6 Fruit

Fruits are numerous, small, round capsules, green-red in colour, found along the underside of the leaves, erect. Fruits are usually warty, hanging, 6-seeded (Figures 1i and 1 j).

3.1.7 Seeds

1 mm long, with sharp transverse ridges (Figure 1k).

3.2 Microscopic Characters

3.2.1 Leaf

T.S of the leaf through the midrib showed a single-layered epidermis covered with a smooth cuticle (Figure 2a). Mesophyll tissues are biphasic and clearly differentiated into palisade and spongy parenchyma layers. Palisade parenchyma is single-layered and composed of closely packed columnar cells, and rosette crystals are embedded within it. A prominent midrib vascular bundle is present at the centre, which forms an abaxial hump. Vascular bundles consisting of the 3-4 radially arranged xylem elements contain starch grains (Figure 2b), followed by 3- 4 layers of phloem. A single layer of phloem fibres is prominently visible, and 3-4 layers of cells just beneath phloem fibres contain rosette crystals (Figure 2b and 2c).

3.2.2 Lamina

Lamina is dorsiventral, having a single-layered upper and lower epidermis (Figure 2c). Well-differentiated palisade tissues are single-layered (Figure 2d) and composed of tangentially elongated cells and unicellular and multicellular trichomes are present at the marginal end (Figure 2e).

3.2.2 Leaf Constants

The quantitative parameters of epidermal peelings of leaf of *P. urinaria* L. are recorded in Table 1. The leaf

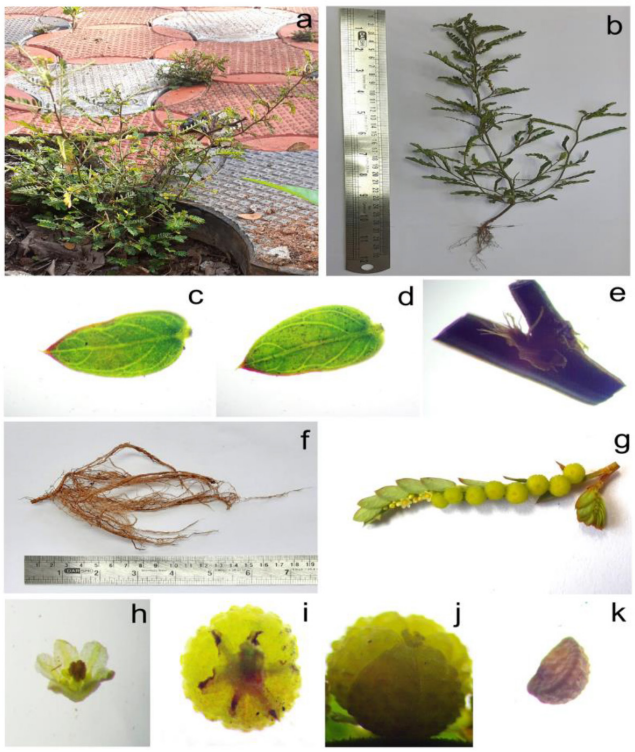


Figure 1. Morphological characters of *P. urinaria* L. (**a**, **b**). Habit; **(c).** Adaxial surface of leaf; **(d).** Abaxial surface of leaf; **(e).** Nodal region shows cataphylls; **(f).** Root; **(g).** Branchlet showing flowers and fruits; **(h).** Flower; **(i).** Dorsal view of fruit; **(j).** Ventral view of fruit; **(k).** Seed.

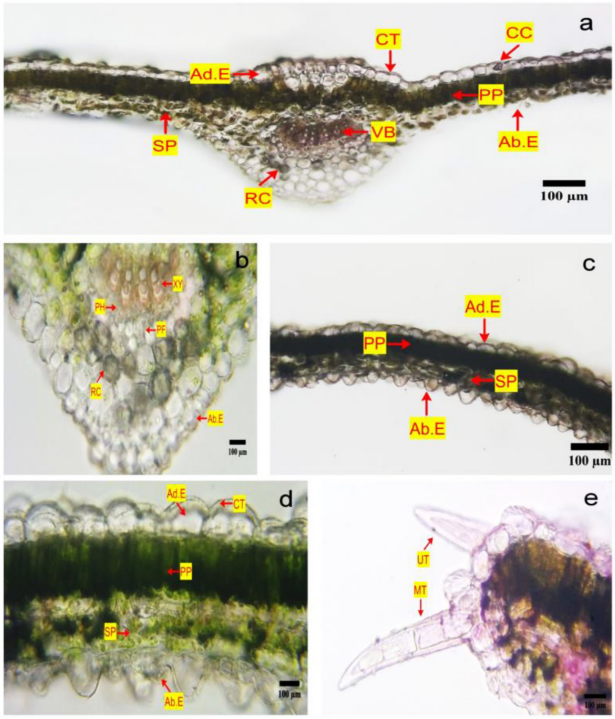
showed paracytic stomata and trichomes on both surfaces (Figure 3).

3.2.3 Petiole

T.S of petiole has a circular outline with quadrangular and rounded epidermal cells with a thin cuticle (Figure 4a). The ground tissue is formed by rounded parenchymatous cells of varying sizes containing starch grains, and some of them have calcium oxalate crystals and brown contents and oil globules (Figure 4b). The vascular bundles are collateral in an open arch surrounded by a pericyclic fibre sheath (Figure 4c). Pith is absent.

3.2.4 Branchlet

T.S of the branchlet showed a circular to oval outline with two ridges or angular projections with non-glandular multicellular trichomes (Figure 5a and 5b). Single layered epidermis is covered with smooth cuticles. Hypodermis is single-layered and made up of round, compactly arranged parenchymatous cells in distinguishable size and showed the presence of oil globules (Figure 5d). Cortex is 5-6 layered, and the first 3 layers are composed of collenchymatous cells, and the remaining layers are of loosely arranged

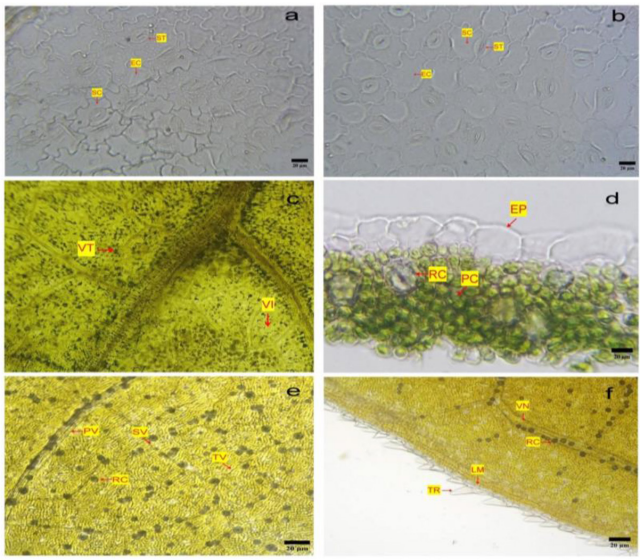


(CT- Cuticle, EP- Epidermis, AdE- Adaxial Epidermis, AbE- Abaxial Epidermis, VB- Vascular Bundle, PP- Palisade Parenchyma, SP- Spongy Parenchyma, RC- Rosette Crystals, XL- Xylem, PH- Phloem, PF- Phloem Fibre, TR- Trichome, OG- Oilglobule)

Figure 2. Transverse section of leaf **(a).** The transverse section of the leaf through the midrib; **(b).** Enlarged view of midrib; **(c).** Abaxial cells contain rosette crystals; **(d).** The transverse section of the leaf lamina; **(e).** Enlarged view of Adaxial epidermis of lamina; **(f).** Laminal margine shows non-glandular, unicellular and multi-cellular trichomes.

Table 1. Leaf constants of *P. urinaria*

Parameters	Upper epidermis (/mm^2)	Lower epidermis (/mm^2)
Epidermal number	318-323	402-454
Stomatal number	265-279	290-298
Stomatal index	29.62-31.90	35.35-36.34
Palisade ratio	14.5-15	
Vein islets number	27-35	
Vein termination number	13-17	



(ST- Stomata, SC- Subsidiary Cell, EC- Epidermal Cells, VI- Vein Islet, VT- Vein Termination, EP- Epidermis, RC- Rosette Crystals, PC- Palisade Cells, VN- Vein, PV- Primary Vein, SV- Secondary Vein, TV- Tertiary Vein, LM- Leaf Margin)

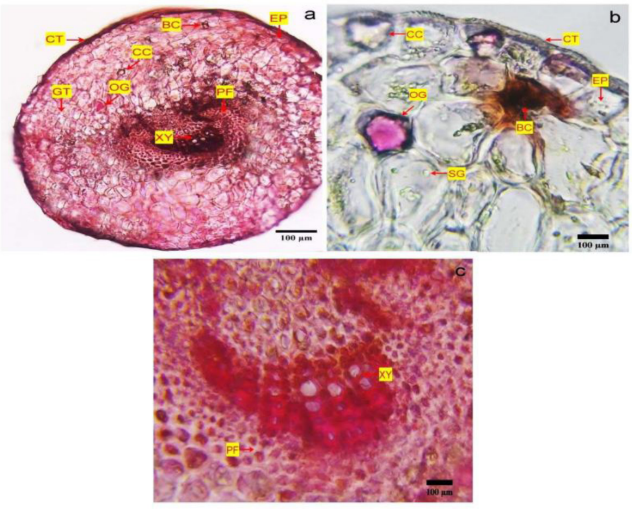
Figure 3. Leaf constants of *P. urinaria* L. **(a).** Paracytic stomata on upper epidermis; **(b).** Paracytic stomata on lower epidermis; **(c).** Vein islet and vein terminations; **(d).** Surface view of lamina shows palisade cells and starch grains; **(e).** Epidermal view of leaf showing rosette crystals distributed over the either side of the veins; **(f).** Leaf blade (margine) showing non-glandular trichomes.

parenchymatous tissues with intercellular space (Figure 5c). Pericyclic fibre patches are present outside of radially arranged vascular bundles (Figure 5c). Vascular elements form a complete circle at the centre. Phloem is represented by a few layers just above the vessel's dominant xylem elements (Figure 5f). Pith is made up of loosely arranged parenchymatous tissue with starch grains (Figure 5g).

3.2.5 Stem

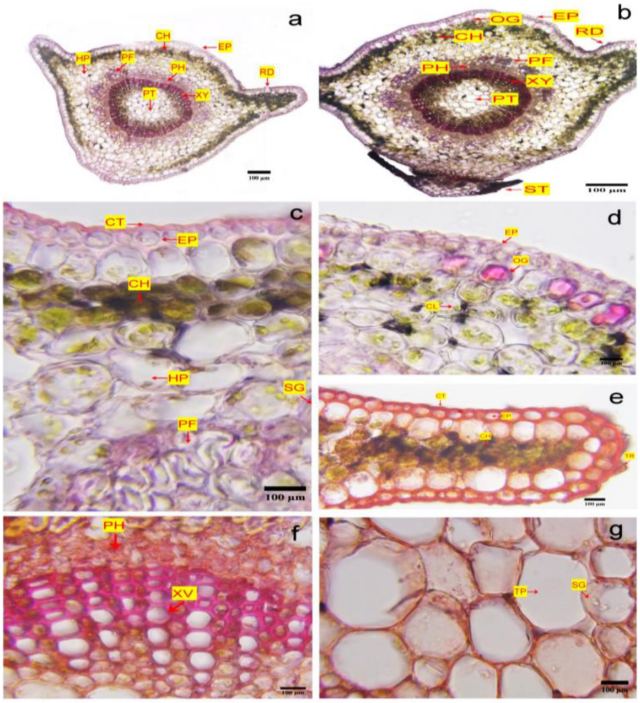
The transverse section of the young stem is circular in outline with angular projections or ridges at various regions of the section (Figure 6). Epidermis is single-layered and thickly cuticularised (Figure 6d). Hypodermis and cortex are the same as seen in the branchlet (Figure 6b). Endodermis was indistinct, and the pericyclic fibre is arranged as patches in three to four layers outside the vascular bundles (Figure 6g). Endarch vascular bundles have phloem outer xylem. Phloem is 3-5 layered, and xylem vessels range from 2-5 cells thickened (Figure 6h). The pith is wide and composed of loosely arranged parenchymatous cells filled with some simple starch grains (Figure 6j).

The anatomy of a mature stem differs from a young stem by the development of secondary growth (Figure 6c). The outline is similar to the young stem. Epidermis is replaced by periderm and shows lenticels frequently (Figure 6e and f). And 2-3 layers of cork followed by cork cambium are present (Figure 6f). Hypodermis is crushed due to pressure, and the cortex becomes a narrow zone with polygonal cells of collenchyma (Figure 6c). Endodermis is indistinct from the cortex layers. Pericyclic fibres are thickened. The phloem ranged from 3-5 layers, almost the same as in young stems. The secondary development is more evident in the xylem region. Xylem possesses comparatively narrow vessels opposite the ridges and wider vessels in the other regions. Uni to biseriate medullary rays are present with the starch grains (Figure 6i). Pith becomes narrow due to the secondary growth of xylem elements and is made up of polygonal parenchymatous cells filled with clusters of starch grains (Figure 6k).



(CT- Cuticle, EP- Epidermis, BC- Brown content, CC- Calcium oxalate crystal, GT-PF- Pericyclic fibre, XY- Xylem, OG- Oil globule, SG- Starch grains)

Figure 4. Anatomy of petiole *P. urinaria* L. **(a).** T.S. of petiole; **(b).** Enlarged view of epidermal and ground tissue of petiole; **(c).** Vascular bundles.



(CT- Cuticle, EP- Epidermis, RD- Ridge, CH- Chlorenchyma, HP- Hypodermis, PF- Pericyclic Fibre, PH- Phloem, XL- Xylem, PT- Pith, ST- Stipule, OG- Oil Globule, SG- Starch Grains, CL- Chloroplast, TR- Trichome, TP- Thin-walled Parenchyma)

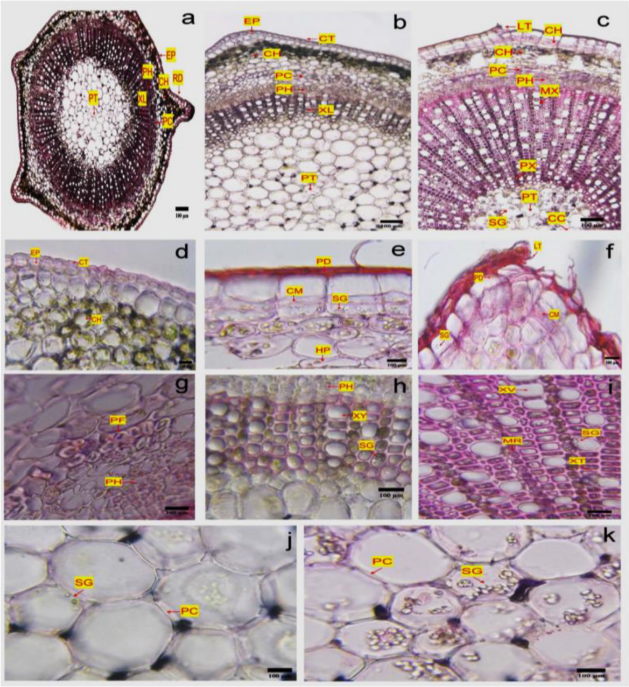
Figure 5. Anatomy of branchlet of *P. urinaria* L. **(a).** T. S. of branchlet; **(b)**.T.S. of tender region of branchlet; **(c).** Enlarged view of epidermal and ground tissue of branchlet; **(d).** Enlarged view of epidermal region of branchlet; **(e).** Enlarged view of ridge; **(f).** Vascular bundles; **(g)** Pith.

3.2.6 Node

The nodal anatomy of *P. urinaria* L. is much similar to the anatomy of young stems (Figure 7a-7b). The outline is more or less oval with prominent angular projections (Figure 7a). Stipule is present at the nodal region with a wing-like appearance and is composed of closely packed parenchymatous cells with rosette crystals (Figure 7d-7f). The stele is unilacunar with a single leaf gap and leaf trace. Chloroplasts are distributed over to the pith region (Figure 7c).

3.2.7 Root

T.S of root is circular in outline (Figure 8a). Periderm is not well-differentiated (Figure 8b). Cork is 2-4 layered and made up of tangentially elongated lignified cells filled with starch grains (Figure 8d). In the young root, a wide cortex region is composed of 8-10 layers of loosely arranged simple parenchymatous cells, sacredly isolated with simple starch grains (Figure 8b). While the mature root showed a narrow cortex due to the secondary thickening of the root (Figure 8c). Endodermis and pericycle are indistictable in both young and mature roots (Figure 8b and8c). Vascular bundles are radially arranged and show secondary thickening in mature root (Figure 8f). Xylem vessels and tracheids being more prominent; medullary rays are present between the vascular bundles (Figure 8g). Pith occupies one-third portion of the young root and parenchymatous whereas narrow to indistinctable in mature root and filled with starch grains (Figure 8h).

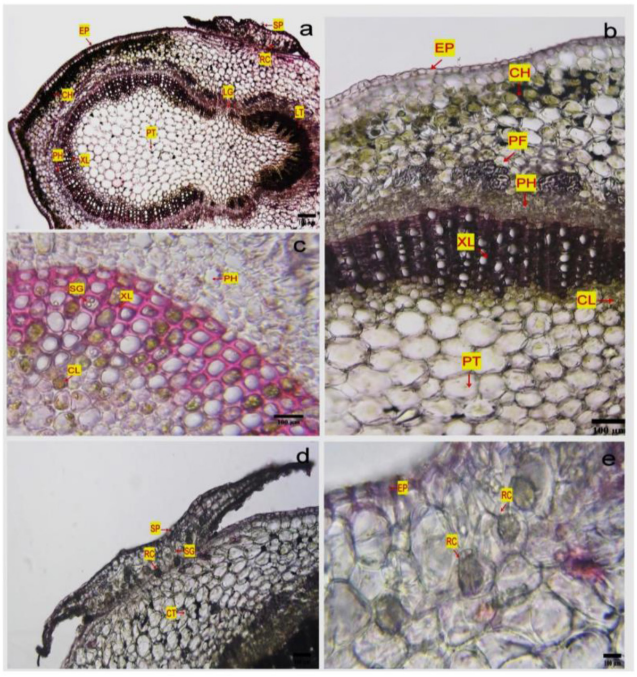


(CT- Cuticle, LT- Lenticel, CK- Cork layers, PD- Periderm, EP- Epidermis, RD- Ridge, CH- Chlorenchyma, CM- Cambium, HP- Hypodermis, PH- Phloem, XY- Xylem, MX- Metaxylem, PX- Protoxylem, XV- Xylem Vessel, XT- Xylem Tracheids, PT- Pith, PC- Pericycle, PF- Pericyclic Fibre, SG- Starch Grains, CC- Calcium oxalate Crystals, OG- Oil Globule, TP- Thin-walled Parenchyma)

Figure 6. Anatomy of stem of *P. urinaria* L. **(a).** Transverse section of stem; **(b).** T.S. of young stem; **(c).** Enlarged view of T.S. of mature stem; **(d).** Enlarged view of epidermal region of young stem; **(e).** Enlarged view of epidermal region of mature stem; **(f).** Enlarged view of cork layers; **(g).** Hypodermal region with pericyclic fibres; **(h).** Vascular bundle of young stem; **(i).** Vascular bundle of mature stem; **(j).** Pith of young stem; **(k).** Pith of mature stems.

3.2.8 Fruit

The transverse section of the fruit showed axile placentation and trilocular with two seeds per locule.



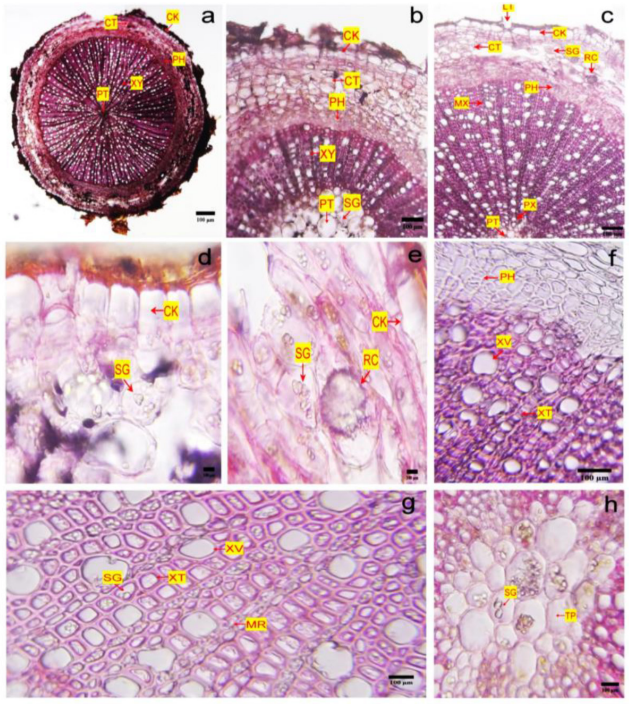
(SP- Stipule, EP- Epidermis, RC- Rosette Crystal, CH- Chlorenchyma, CL- Chloroplast, LG- Leaf Gap, LT- Leaf Trace, PF- Pericyclic Fibre, PH- Phloem, XL- Xylem, PH- Phloem, PT- Pith)

Figure 7. Anatomy of the node of *P. urinaria* L. **(a).** Transverse section of the nodal region; **(b).** Enlarged view of T.S. of node; **(c).** Enlarged view of vascular bundle; **(d).** Epidermal region of node showing stipule; **(e).** Enlarged view of the stipule.

Zones of separation (abscission region) along each of the septa and a prominent central column (Figures 9a and 9b) were observed. T. S of pericarp shows a lot of ridges that are not in a uniform pattern. In immature pericarp or fruit, the exocarp is composed of a uniseriate epidermal layer followed by loosely arranged small parenchymatous cells in 3-4 layers with abundant crystals and the mesocarp consists of 2-3 layers of chlorenchymatous cells (Figure 9c to 9e). The endocarp is the prominent layer in pericarp region which is composed of macrosclereids oriented longitudinally or obliquely to the longitudinal axis of the fruit and that has significant borders all along the wall and completely separates from outer pericarp regions (Figure 9f). When the fruit matures, a notable change is seen in the mesocarp. The chlorenchymatous cells of the mesocarp become much thickened, and the border of the endocarp towards the mesocarp is indistinguishable (Figure 9e). Each of the six septae is bipartite, formed by the invagination of the columnar endocarp layers of adjoining locules. In mature fruit, the septae do not join at the centre of the fruit. Instead, a central column is developed with three to four vascular bundles (Figure 9i). The central column is separated from the septum through the abscission tissue of the abscission region present between the septum (Figure 9g). Each seed is attached to the central column by means of an axicorn (Figure 9i).

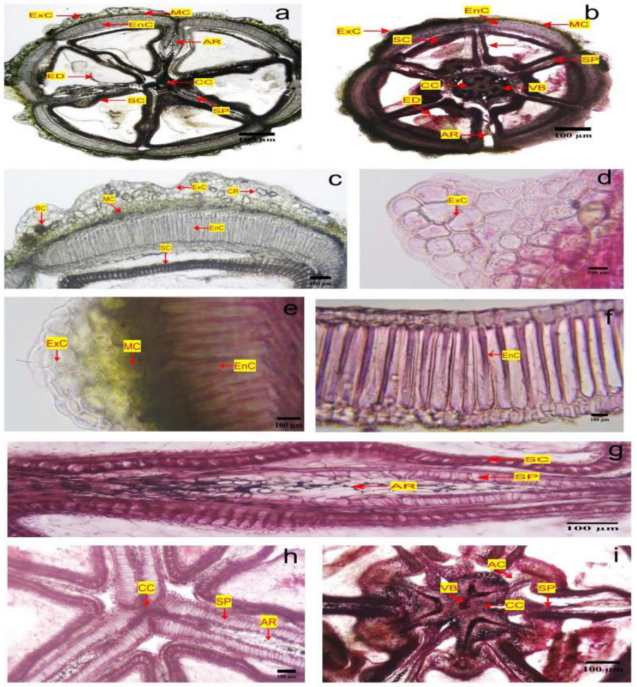
3.2.9 Seed

Seeds are small, non-arillate, bitegmic, and ellipsoidal to somewhat trigonous in transverse sectional view (Figure 10a). Seed coats are differentiated into testa



(CK- Cork, CT- Cortex, PH- Phloem, XY- Xylem, XV- Xylem Vessel, XT- Xylem Tracheids, MR- Medullary Rays, SG- Starch Grains, RC- Rosette Crystals, TP- Thin-walled Parenchyma)

Figure 8. Anatomy of root *P. urinaria* L. **(a).** Transverse section of root; **(b).** T.S. of young root; **(c).** T.S. of mature root; **(d).** Enlarged view of epidermal region; **(e).** Cortex contains rosette crystal and starch grains; **(f).** Enlarged view of vascular bundle; **(g).** Enlarged view of xylem elements; **(h).** Pith.



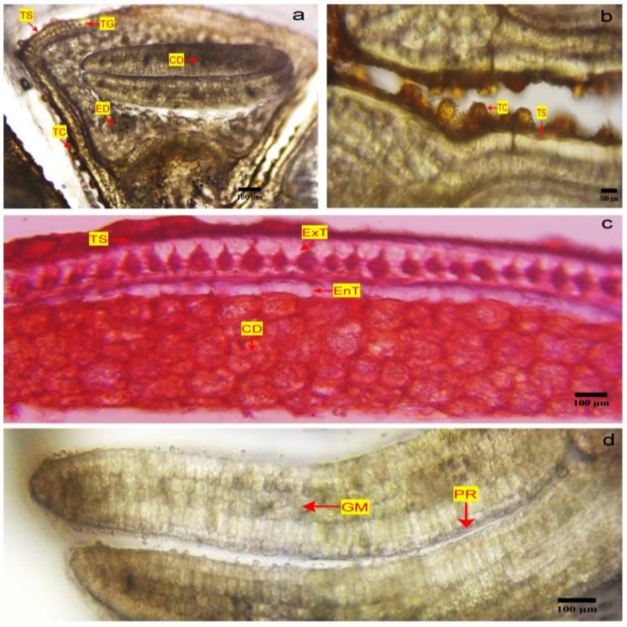
(CT- Cuticle, EC- Exocarp, MC- Mesocarp, MS- Macrosclereids, EnD- Endocarp, RC- Rosette Crystrals, RP- Replum, SP- Septum, AR- Adhession region, LC- Locule, ED- Endosperm, CC- Central Column, VB- Vascular Bundle, OG- Oil Globule, SG- Starch Grain, AC- Axicorn, EM- Embryo, MS- Membraneous Seed coat)

Figure 9. Anatomy of fruit and seed development of *P. urinaria* L. **(a).** Transverse section of young fruit; **(b).** Transverse section of mature fruit; **(c).** Enalrged view of exocarp of young fruit; **(d).** Enlarged view of exocarp of mature fruit; **(e).** Exocarp contains rosette crystals; **(f).** Enlarged view of macrosclereids of mesocarp; **(g).** Septum with adhesion point and replum; **(h).** Enlarged view of central column of young fruit; **(i).** Enlarged view of central column of mature fruit; **(j-o).** Different stages of embryo development.

and tegmen. Testa is non-multiplicative, brown, and more or less crushed and covered with epicuticular wax containing tannin (Figure 10b). Followed by testa, well-differentiated tegmen is composed of exotegmen and endotegmen. Exotegmen is composed of anticlinal columnar to hourglass-shaped sclerotic cells with simple pits (Figure 10c). The configuration of these cells varies from more or less isodiametric over the circular chalaza to radially and periclinal elongate cells. Endotegmen is a thin inner layer with U-shaped thickenings in cells and has separated from the prominent mechanical layer that borders the endosperm (Figure 10c). Embryo is curved, well preserved with two cotyledons surrounded by the endosperm and has uniseriate protoderm surrounding homogenous ground meristem (Figure 10d). The embryo axis consists of short radicles and hypocotyl.

3.3 Embryogenesis

Embryonal development in the seed of *P. urinaria* is depicted in Figure 11. Both T.S and L.S of the seed showed different stages of embryonal development. In young fruit, the seed is in the proembryonic stage and filled with endospermic tissues (Figures 11a and 11b). The globular stage of the embryo can be seen in Figures 11c to 11e, and the early torpedo stage is evident in Figure 11f. The lateral walls of seed coat invaginated and start to thicken, and a reduction in width is visible in the entire process of embryonal development. In mature fruit, the embryo is transformed into an elliptical body with two cotyledons (Figure 11 l). This pattern of development confirms the Capsella variation of the onagrad type of embryogeny- late globular, heart-shaped, torpedo-shaped and dicotyledonous embryos. The mature embryo is slightly curved and is spread along the entire length of the seed (Figures 11o and 11i).

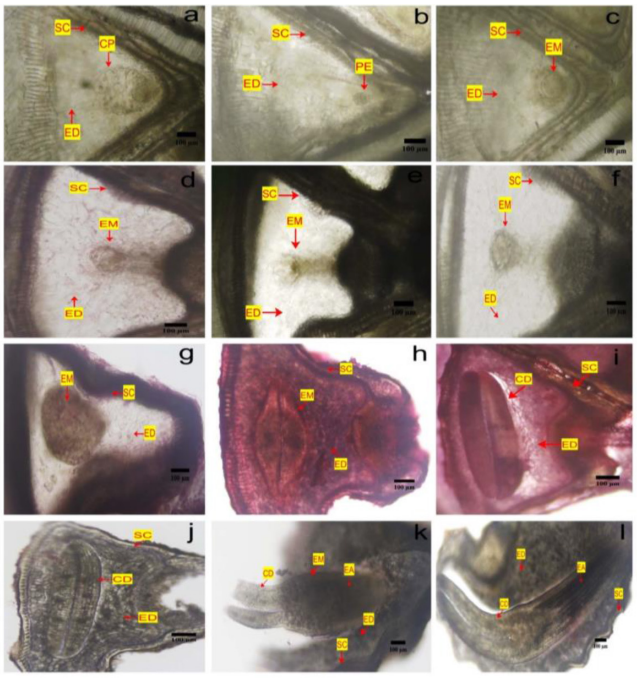


(TS- Testa, TG- Tegmen, ExT- Exotegmen, EnT- Endotegmen, ED- Endosperm, CD- Cotyledone, TC- Tannin Content, GM- Ground Meristem, PR- Protoderm)

Figure 10. Anatomy of seed of *P. urinaria* L. **(a).** Transverse section of mature seed; **(b).** Enlarged view of testa covering with epicuticular wax containing tannin; **(c).** Enlarged view of layers of tegmen; **(d).** Enalrged view of cotyledone.

3.4 Organoleptic Characters

The organoleptic characters of dried plant powder of *P. urinaria* are investigated and showed in Table 2.



(CP- Cellularised proembryo; PE- Proembryo; EM- Embryo; ED- Endosperm; SC- Seed Coat; EA- Embryonal Axis; CD- Cotyledone)

Figure 11. Anatomy of developing seeds of *P. urinaria* L. **(a-j).** Transverse section of developing seed; (**k-i).** Longitudinal sections of developing seeds; **(a).** Cellularised proembryo; **(b).** Developing proembryo; **(c-e).** Globular embryo; **(f-h).** Torpedo embryo; **(i-j).** Cotyledonous stage; **(k-l).** Longitudinal sections of seed showing bending torpedo stages.

3.5 Powder Microscopy

The powdered sample of the whole plant of *P. urinaria* showed characters like epidermal cells with paracytic stomata, trichome with base cells, uniseriate unicellular trichome, uniseriate multicellular trichome, warty trichome, a fragment of elongated cells, calcium oxalate crystal, rosette crystals, brown content, a cluster of starch grains, pollen grain, stone cell, fruit epidermal fragment, a lignified vessel with wide lumen, spiral fragment with parenchyma cell, lignified vessel with narrow lumen, aseptate fibre, fibre bundle, simple pitted vessel, reticulate vessel bundle (Figure 12).

3.6 Histochemical Localisation

The results of histochemical localisation study in various parts of *P. urinaria* are shown in Table 3 and Figures 13-18.

3.6.1 Suberised or Cuticular Cells

Table 2. Organoleptic characters of dried plant powder of *P. urinaria*

Properties	Result
Texture	Slightly rough, free flowing.
Colour	Pale brown
Odour	Pungent
Taste	Slightly astringent



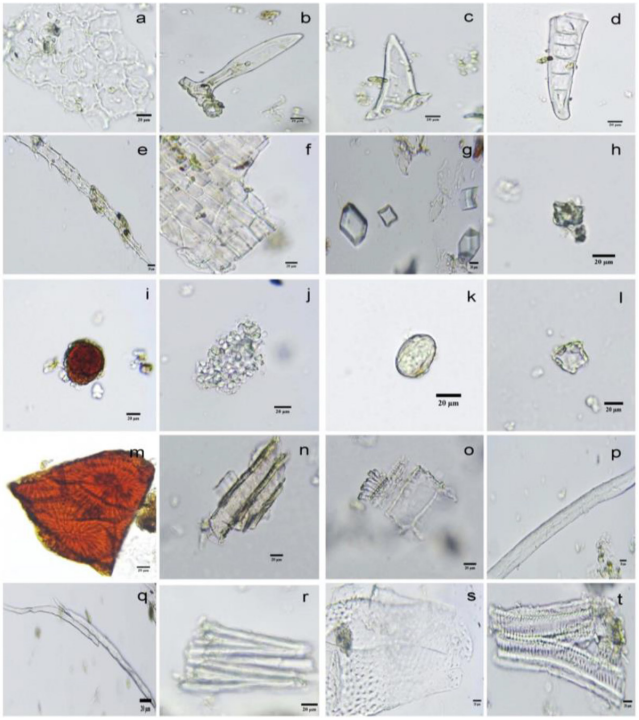


Figure 12. Powder microscopy of *P. urinaria* L whole plant **(a).** Epidermal cells with paracytic stomata; **(b).** Trichome with base cells; **(c).** Uniseriate unicellular trichome; **(d).** Uniseriate multicellular trichome; **(e).** Warty trichome; **(f).** Fragment of elongated cells; **(g).** Calcium oxalate crystal; **(h).** Rosette crystals; **(i).** Brown content; **(j).** A cluster of starch grains; **(k).** Pollen grain; **(l).** Stone cell; **(m).** Fruit epidermal fragment; **(n).** Lignified vessel with wide lumen; **(o).** Spiral fragment with parenchyma cell; **(p).** Lignified vessel with narrow lumen; **(q).** Aseptate fibre; **(r).** Fibre bundle; **(s).** Simple pitted vessel; **(t).** Reticulate vessel bundles.

Table 3. Histochemical localisation in various parts of *P. urinaria*

Plant part	Lignin	Suberised/ Cuticular cell wall	Fatty oil/ Volatile oils and Resins	Phenolic compounds (Flavanoids/Tannin)	Mucilage	Alkaloids
Leaf	Xylem elements	Epidermis	-	Ground tissue	-	-
Petiole	Xylem elements	Epidermis		Hypodermis, Cortex, Pericycle	-	-
Branchlet	Xylem elements	Epidermis	-	Hypodermis, Pith	-	Hypodermis
Stem	Xylem elements	Epidermis	Hypodermis	Hypodermis, Cortex, Pith	Near to phloem tissue	Phloem and Pith
Node	Xylem elements	Epidermis	Cortex	Hypodermis, Cortex, Pith	Hypodermis, Phloem	Hypodermis and Phloem
Root	Xylem elements	-	Xylem elements	Phloem	Phloem	Pericycle to Phloem
Fruit	Testa and vascular bundles.	-	Endosperm	Mesocarp, Adhession region	-	-

3.6.2 Alkaloid Contents

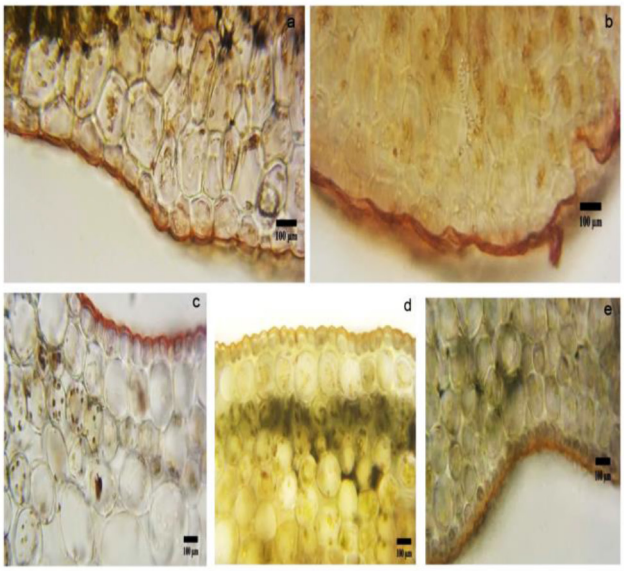


Figure 13. Suberised/cuticularised cell walls of various parts of *P. urinaria* **(a).** Leaf; **(b).** Petiole; **(c).** Branchlet; **(d).** Stem; **(e).** Node.

3.6.3 Fatty oils or Volatile oils or Resin

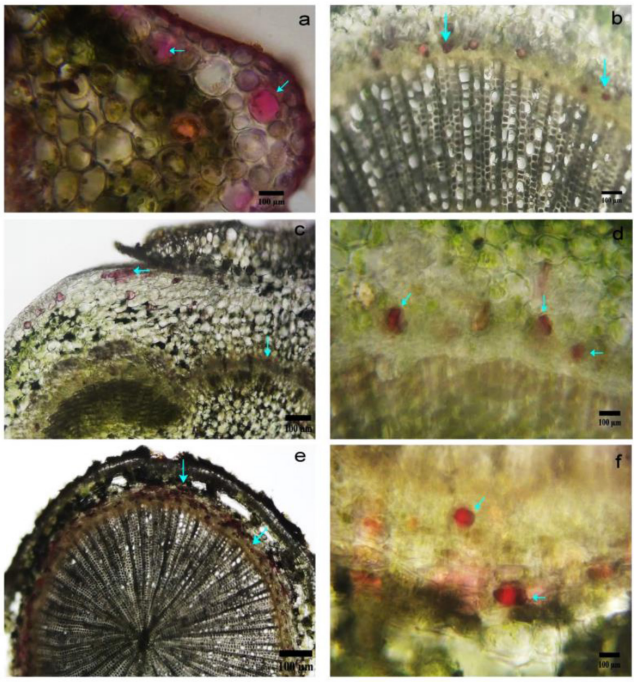


Figure 14. Alkaloid content in various parts of *P. urinaria* L. (arrow) **(a).** Branchlet; **(b).** Stem; **(c).** Node; **(d).** Phloem cells of node; **(e).** Root; **(f).** Pericycle and phloem cells of root.

3.6.4 Mucilage

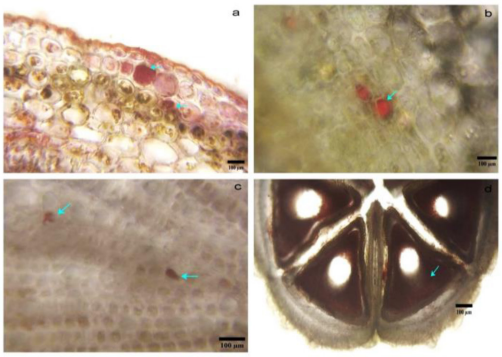


Figure 15. Fatty oils or volatile oils or resin contents in various parts of *P. urinaria* L. (arrow) fatty oils or volatile oils; **(a).** In stem; **(b).** In cortex of node; **(c).** In root xylem elements; **(d).** In endosperm of fruit.

3.6.5 Lignin

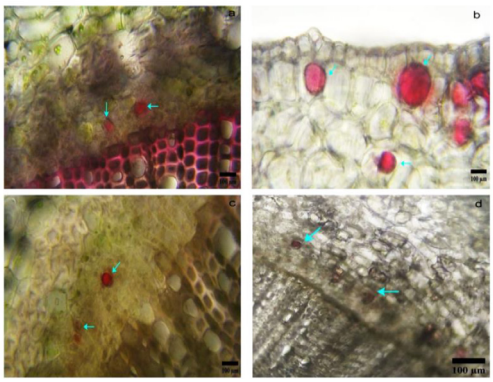


Figure 16. Mucilage in various parts of *P. urinaria* L. (arrow); **(a).** Phloem of the stem; **(b).** Epidermal region of node; **(c).** Phloem tissues of node; **(d).** Phloem of root.

3.6.5 Phenolic Content

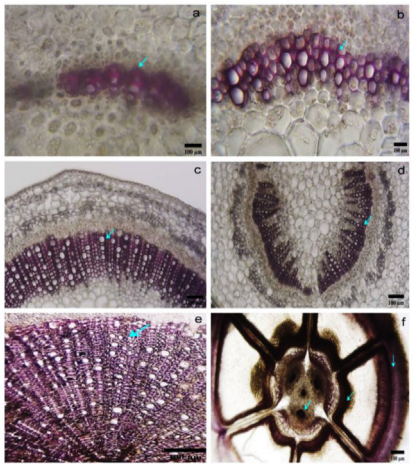


Figure 17. Lignin in various parts of *P. urinaria* L.; **(a).** Leaf; **(b).** Branchlet; **(c).** Stem; **(d).** Node; **(e).** Root; **(f).** Fruit.

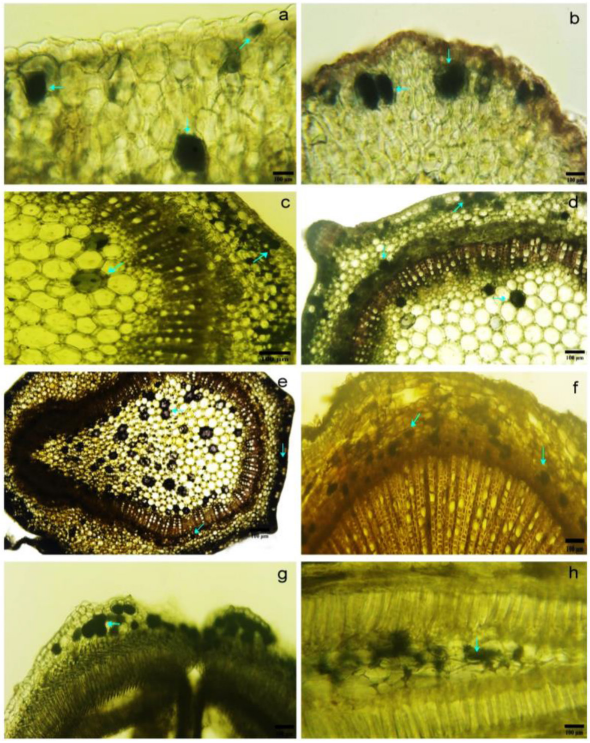


Figure 18. Phenolic content in various parts of *P. urinaria* L. **(a).** Leaf; **(b).** Petiole; **(c).** Branchlet; **(d).** Stem; **(e).** Node; **(f).** Root; **(g).** Pericarp of Fruit; **(h).** Septa of fruit.

4. Discussion

The present study evaluated the macro-microscopical characters of various parts of *P. urinaria* including embryogeny and distribution of phytochemicals in various parts of the plant. Even though the anatomy of the leaf, stem, and root of *P. urinaria* has already been studied in comparison with some other *Phyllanthus* species, there were no detailed descriptions available about each parts like petiole, branchlet, nodes, fruit and seed to give a clear idea about the whole plant of *P. urinaria*[39-41]. Furthermore, the embryogenesis and distribution of phytochemicals in different parts of *Phyllanthus* species are reported for the first time in the present study.

Leaf is oblong or linear, oblong in shape in corroboration with earlier studies[41]. The tip of the leaflet of *P. urinaria* is pointed out which confirms the observation reported in earlier work[40]. The present study revealed a very short petiole which agreed with the findings of a previous study and showed dissimilarity with another study where leaf was found sessile[42,43]. Reddish and laterally free stipules in some *Phyllanthus* species including *P. urinaria* were reported earlier[39]. Cataphylls were reported in *Phyllanthus* species, *P. amarus* Schum. and Thonn. and *P. debilis* Klein ex Wildand trichomes on leaves in *P. fraternus* G.L. Webster[44,45].

The present study revealed that palisade parenchyma was single-layered in *P. urinaria* which was contrary to the findings of a previous work, where two layered palisade parenchyma was found[39]. The presence of rosette crystals in the palisade parenchyma, which agrees with the result of a study in *Phyllanthus pterocaulis* as well as idioblasts containing druses were found among cells of the palisade parenchyma[46]. Furthermore, various morphotypes of crystals (prismatic and styloid crystals, crystal sands and druses) were observed on both the adaxial and abaxial sides of *P. amarus* Schum. and Thonn and *P. physocarpus* Mull. Arg. while these were found only on the abaxial surfaces of *P. acidus* (L.) Skeels, *P. capillaris* Schumach. and Thonn., *P. floribundus* Kunth, *P. muellerianus* (Kuntze) Exell, *P. niruri* L., *P. odontadenius* Mull.Arg. and *P. rotundifolius* J.G.Klein ex Willd. However, the crystals and druses were observed only on the adaxial surface of *P. fraternus* G.L. Webster[43]. The distribution of the collenchyma cells within the midrib of the *P. urinaria* and the nature of the xylem cells within the midrib agreed with the finding of an earlier work[39].

The studies on various quantitative aspects of the leaf (stomatal number, stomatal index, palisade ratio, vein islet and termination numbers) have a remarkable taxonomic as well as pharmacognostic value in the accurate identification of different plant taxa[47]. The result of the present study agrees with the findings of a few previous studies where leaf of *P. urinaria* showed paracytic stomata with two subsidiary cells[40,48]. *P. urinaria* is differed by rosette-type crystals distributed along the veins and marginal cells forming a serrate margin[49].

The branchlet of *P. urinaria* showed the presence of oil globules in the hypodermis while the same was present in the root epidermis of *P. amarus* Schum. and Thonn. and pith cells stem of *P. maderaspatensis* L[41]. In the case of another *Phyllanthus* species, *P. acidus* (L.) Skeels, oil droplets were observed only on the adaxial surface of the leaf[43].

The nature, colour, outline, and polygonal shape of cortical cells of the stem of *P. urinaria* agreed

with results of the former study[41]. Comparatively, a wide pith is found in *P. urinaria*[40]. Crystal-like black depositions are found in the pith of *P. urinaria*. A study reported about the anatomy of the stem of *P. urinaria* was more or less in line with the result of the present study[39]. However, the presence of starch grains in the stem was not reported earlier. The same study confirms the unilacunar arrangement of stele in the nodal region of *P. urinaria*.

The nature of the root periderm was contrary to the findings of a previous study, *i.e.*, a well-differentiated periderm with a wavy margin in the root section[40]. Microscopical studies indicated the presence of brown content in root sections of *P. urinaria*[41]. The cortex of the species was seen as conspicuous as rays of cells rising from the middle which are composed of a layer of thin walled, colourless parenchymatous cells with vascular bundles at the centre of the root[39].

The characteristic feature of *Phyllanthaceae* fruit is dehiscent capsular, tricarpellate with two seeds per chamber[50]. The present study revealed that the fruit of the *P. urinaria* is a capsule with wart-like projections appearing on its rough surface, and the axile placentation indicates a similar finding of the previous studies[40,51]. The outer integument is two cells thick in *Phyllanthaceae*. The anatomy of the seed of *P. urinaria* was not reported earlier. Whereas the morphology was reported as a seed coat with transverse ribs on the dorsal face, and longitudinal ribs on the lateral side[52]. In *Phyllanthus*, non-arillate and non-multiplicative testa were already reported, and seed coat layers of *P. urinaria* were almost similar to *P. niruri* and one of the extinct *Phyllanthus* species *Phyllanthocarpon singpurensis* sp. nov[51,53]. The embryonic development in a *Phyllanthus* species is reported for the first time and which is also in corroboration with that of another genus *Phyllanthaceae*, *Bischofia javanica* Blume and a genus of order *Malpighiales*, *Mascagnia cordifolia* (A. Juss.) Griseb[54,55].

The identification and distribution of chemical compounds in biological cells were studied using different stains and indicators[56]. In this study, phytochemicals like lignin, suberised or cuticular cell walls, fatty oil or volatile oils and resins, phenolic compounds, mucilage, and alkaloids were found in various tissues of different parts. There is only one study reported on the phytochemical localisation in anatomical sections of *Phyllanthus* species, *P. reticulatus* Poir. (stem bark). The study revealed the secondary phloem, cortex, few cells of phelloderm and cork cells as the major localising zones of phytochemical classes of compounds[57]. The presence of a cuticular cell wall in all the parts of *P. urinaria*, facilitates the defense against pathogen attack or more like solute transport across the apoplast. The major deposition of lignin was in xylem elements which resulted in the formation of the secondary cell wall[58-60]. The test for fatty oil or volatile oils and resins in *P. urinaria* were positive in cortical region of node, xylem elements of root, and in endosperm of the fruit. This was supported by the findings of previous studies[56,61]. Alkaloids are the degradation product of protein and are produced in various parts of plants such as the leaves, roots, seeds, *etc* and in the case of *P. urinaria*, it was seen in the hypodermis of branchlet, phloem and pith of stem, hypodermis and phloem of node, pericycle and phloem of the root[62]. Mucilages are physiological products obtained from the cell wall of various plant parts, such as bark, stem, leaves, seed, and roots[63]. Here, the presence of mucilage is mainly observed in phloem tissues of stem, node, and root. Whereas a study reported in a *Phyllanthus* species named *P. maderaspatensis* L. contains mucilage in defatted seed cake[64]. Moreover, an anatomical study in another *Phyllanthus* species, *P. emblica* L. and *P. pectinatus* Hook.f. confirmed the presence of mucilage cells[65]. In *P. urinaria* phenolic contents were seen in the hypodermis, cortex, and pith of aerial portion while phloem tissues of roots as well as in mesocarp and adhesion region of fruit.

The powder microscopic studies of whole plant of *P. urinaria* highlighted presence of some distinguishable characteristics such as epidermal cells with paracytic stomata, warty trichome, calcium oxalate crystal including rosette crystals, pollen grain, stone cell, fruit epidermal fragment, lignified vessel with wide lumen, spiral fragment with parenchyma cell which will serve as specific anatomical markers for identification of the *P. urinaria*.

5. Conclusion

The anatomical characteristics of different parts of *P. urinaria* obtained from this study are noteworthy. From the quantitative microscopic analysis of the leaf,

it was found that few distinguishable characteristics like the presence of paracytic stomata, and distribution of non-glandular trichomes throughout leaf margins and rosette crystals around the veins. The primary and secondary anatomical variations in stem and root are reflected as significant growth in vascular bundles and especially in xylem elements. Moreover, the anatomy of minute plant parts like petioles, branchlets, and nodes showed the presence of various contents (oil globules, brown contents, and crystals) in different tissues. The embryonic development acts as an extra identification tool, which gives more insight into the development of fruit and seeds. The characters obtained through organoleptic and powder microscopic studies support the anatomical findings. Various phytochemicals identified in different parts of *P. urinaria* L. indicate its therapeutic properties.

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Author Contribution

SK carried out the laboratory experiments and drafted the manuscript. SGK and NKB guided in performing the research and writing and reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.