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Research Article

PHARMACOGNOSTICAL STUDIES OF *GUIDONIA TOMENTOSA*

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ABSTRACT
Guidonia tomentosa is a small deciduous tree distributed in Chittoor District of Andhra Pradesh, India. The present study has provided a scientific evaluation such as taxonomical, macroscopical and microscopical characters in identifying the plant drug material to overcome the use of substitutes and adulterants of ayurvedic drugs. Our investigation in the ethnic community of seshachalam forests claimed valuble and important ethnomedico botanical usage and this study revealed the originality of the taxa. The pharmacognostical investigations carried out in terms of organoleptic, macroscopic, microscopic, and fluorescence analysis parameters. As there is no pharmacognostic and anatomical work recorded on this medicinally potent plant, the present work was undertaken. The parameters which are reported could be used for botanical identification of the drug in the crude form, for detecting low grade products and preparation of the monograph of *Guidonia tomentosa*

Keywords*: Guidonia tomentosa, Pharmacognosy, Microscopy*

INTRODUCTION
Ancient Indian literature incorporate a remarkably broad definition of medicinal plants and considers ‘all’ plant parts to be potential sources of medicinal substances (Khare, 2007). Every part of it is credited with its specific medicinal properties. Since no reports on systematic studies of whole plant present, an effort has been made to establish the pharmacognostical parameters (physicochemical), as well as anatomical study (histological characters, micrometric determinations) of *Guidonia tomentosa* thereby facilitating authentication of the correct plant material. Present investigations were planned with an objective toestablish pharmacognostic standards of this plant, there by facilitating authentication of the correct plant material. These standardized parameters would be of immense help in authenticating. If any crude drug which claimed to be as *Guidonia tomentosa* but whose charecters significantly deviate from the character above would then be rejected as contaminated, adultered or downright fake as per results revealed by our study.

Taxonomy of Guidonia tomentosa
Guidonia tomentosa (Roxb.) Kurz.(Flacourtiaceae) is a small deciduous tree or large bushy shrub upto 50-80 cm girth and 7 m tall. Bark is thick, dark brown, exfoliating in square flakes, live bark is 2-3 mm thick, young parts are tomentose. Branchlets are slender, tomentose. Leaves are alternate, 7-15 x 3-7 cm, variable in size, usually ovate or oblong, elliptical, entire or serrulate, acute, obtuse or acuminate, base rounded and asymmetrical, tomentose on both sides, midrib is very prominent, red, lateral veins slender, prominent, 9-12 pairs. petiole is red, 0.5-1.5 cm long, grey tomentose. Flowers are bisexual, whitish green, in axillary clusters. Sepals 5, imbricate. Petals are absent. Stamens vary 6 or 8, basilly connate with 8 staminodes. Ovary is unilocular, ovules numerous, parietal. Fruit is 1-2 cm in diameter, succulent capsule, ellipsoid, 3 valved or ribbed, yellow; seeds numerous embedded in a red pulp (Figure).
Basionym of Guidonia tomentosa is *Casearia tomentosa* Roxb. which is accepted name according to International Plant Name Index (IPNI).
The flowering and fruiting is in February to August. *G.tomentosa* is distributed in Nelakona, Narayanadri and Kanvavanam areas in Tirumala, Sadhumalammakona, Palamaner ghat, Chelluru reserve forest, Horsely hills and Kambakam hills in Chittoor district of Andhra Pradesh, India.

Research Article

Ethnomedicinal uses

Whole plant (root, stem and leaves in equal proportions) is shade dried and made into fine powder. About one teaspoonful of the powder is taken along with honey on empty stomach once a day for 20-30 days to heal pectic ulcers (Gangaiah, Yenadi herbal physician, Chamala forest, near Tirupati, Figure 0.7).

Whole plant (fresh root, stem and leaves in 1:1:2 ratio) is made into paste and prepared a decoction. 20-30 ml of decoction is given once in a day for 5-10 days for dropsy (Pedda Venkatappa, Irula tribal, Talakona).

Root, stem and leaves 20 g each is made into paste with turmeric (10g) and applied topically to the toes and feet to cure fissures and cracks (Boku Suranna, Irula tribal, Chelluru forest).

Equal proportions of root, stem and leaves are shade dried and made into fine powder. About 5 g powder is taken with butter milk for colic pain in the abdomen (Pullachari, Nakkula herbalist, Pavanasanam, Tirumala).

Root, stem and leaves are taken (2:1:2 ratio) and made into paste with water and prepared a pills of soapnut size. Three pills are taken twice a day for one week to cure malarial fever (Jambaiah, Irula tribal, Kambakam hills).

Whole plant juice is mixed with turmeric powder and lime juice is applied externally around the throat for tonsillitis pain (Gadi Somaiah, Yenadi herbal vendor, Sadhumalammakona, near Srikalahasti).

A poultice of leaves is used to heal wounds (Penchulaiah, Irula tribal Horsely hills).

Leaf paste is used for severe bone fractures as a plaster (Gelikamma, Sheep keeper, Talakona).

Figure A: Tree Habit

Figure B: Leaf Twig

Figure C: Fruits

Figure D: Dried Specimen

Figure E: Gangaiah, Yenadi herbal physician preparing the drug

Figure 1 A-D : Taxonomic and Ethnobotanical Description of *Guidonia tomentosa*

MATERIALS AND METHODS

Whole plant (Mixture of root, stem and leaf).is used for the study. Plant specimen was collected from Talakona Reserve Forest. Deposited at the Herbarium, Department of Botany, SV University, Tirupati

with voucher no: SVUTY-FL/2106. Taxonomic characters and identification was done by referring the regional floras (Gamble, 1967; Chetty *et al*., 2013).

Sample preparation

Samples of root, leaf, stem, bark were taken in equal ratios by air-dried in shade, powdered and passed through a 70mm mesh sieve and stored in light-protected tight container. For the microscopical studies, transverse sections were prepared and stained according to the method followed by Rao et al., (2013) and Sivaji et al., (2013). The powder microscopy was performed according to the methods of Kokate (2008) and Khandelwal (2002). Microscopic descriptions of tissue are supplemented with micrographs wherever necessary.

Photomicrographs were taken using binocular photomicroscopic apparatus Leitz microscope (24DSLR camera integrated-Nikon made, Japan.) of different magnifications in microscopic units. For normal observation bright field was used. For the study of crystals, lignified cells polarized light was employed. Magnifications of the Figure s are indicated by the scale-bars. For microscopic studies and macroscopic characterization methods adapted by Johansen, (1940) was considered. Anatomical Studies was referred from standard books such as Fahn (1982) and Easu (1964).

Physicochemical studies The ash value (total ash, acid insoluble ash, water soluble ash) done according to Evan and Trease (2007). Extractive values (petroleum ether, chloroform and methanol) were determined according to the official methods of WHO guidelines (2002), Wallis (1953). Flouresence analysis was carried out as per Kokoski *et al*., (1958).

RESULTS

Macroscopic characters (Organoleptic characters)

The root is thick, woody, bark brown, aromatic odour and bitter in taste. Stem is woody, brown. Leaf is oblong, tomentose, brittle, aromatic odour and bitter in taste.

Microscopic Characters

Anatomy of Leaf (Figure 1.1& 1.2)

The leaf is dorsiventral with prominent midrib and smooth and even lamina (Figure 1.1). The midrib consists of wide, short adaxial hump and wide semicircular abaxial part. The midrib measures 950 µm thick and 900 µm wide. The epidermal layer of the midrib is thin comprising small squarish cells. The ground-tissue is aerenchymatous in the upper portion and compact parenchymatous in the lower part. The vascular strand is single wide and bowl-shaped with incurved margins (Figure 1.1, 2). The vascular strand extends 750 µm horizontally. The vascular strand includes uniseriate, several parallel short rows of xylem elements; there are 5-7 cells in each row. Running along outer part of xylem occurs a thick and continuous zone of phloem which in turn is unsheathed by a thick and continuous layers of sclerenchyma cells (Figure 1.2).

Lamina (Figure 1.3)

The lamina is 230 µm thick. It is smooth on both surfaces. The adaxial epidermis is slightly thicker comprising rectangular thick walled cells which are 20 µm thick. The abaxial epidermis is thin, the cells being small and squarish. The mesophyll tissue is differentiated into adaxial zone of two layers, thick, cylindrical columnar palisade cells and abaxial 6 or 7 layers of spherical or lobed aerenchymatous spongy parenchyma cells. The palisade zone is about 70 µm in height (Figure 1.3).

Petiole (Figure 2, 3, 4)

The petiole is vertically rectangular with wide and thick adaxial-lateral wings (Figure 2). It is 2 mm thick in vertical plane and 1.5 mm in horizontal plane. The adaxial wings are 200 µm in height and 500 µm in thickness. The petiole consists of a thin epidermal layers of small squarish cells. The ground-tissue is homogenous and parenchymatous, the cells are thin walled, circular to angular and compact.

The vascular strand is wide and thick. It consists of wide and deep main vascular strand and two small arc-shaped strands situated in the inner-upper ends of the main strand (Figure 2, 3). The two adaxial arc-shaped strands appear as though they are pinched of from the pre upper ends of the main strand. The main

strand as well as the two accessory strands are collateral; they possess several, uniseriate long parallel row of xylem elements with narrow space in between the rows. Phloem occurs in narrow zone, outer to the xylem strands (Figure 3). A few layers of cells external to them phloem possess dense tannin contents. Tannins are also wide spread in many of the ground cells.

The proximal part of the petiole is slightly different from the distal part. The proximal petiole is semicircular with shallow adaxial concavity (Figure 4). Adaxial wings are lacking. Petiole has thin epidermal layers, homogenous parenchymatous ground tissue and wide circular vascular strand. Several wide circular secretary cavities diffusely distributed in the ground tissue. Cavities are upto 60 μm wide. A single layer of narrowly rectangular epithelial cells is situated around the cavity (Figure 6.1).

Vascular strand is almost circular with a narrow split at the adaxial end. These are two, small accessory strands placed within the main strand. The main strand consists of numerous long, narrow parallel lines of xylem elements with thick shealth of phloem enclosing the xylem. Accessory strands are also collateral and are small rectangular blocks with outer phloem and inner xylem rows (Figure 4).

Venation of the leaf (Figure 5.1, 2)

As seen in paradermal section, the leaf consists of reticulate venation with less distinct vein-islets. The islets are rectangular to squarish in outline (Figure 5.1). Vein-terminations are either simple (unbranched), Short and thick or long and slender (Figure 5.2). Secretary cavities are seen scattered within the vein-islets.

Stomata (Figure 6.2)

Stomata occur on the abaxial epidermis and are predominantly paracytic type having two subsidiary cells, one on either side of guard cells, which are parallel to the stomata. Epidermal cells are small, squarish to rectangular, fairly thick walled, slightly wavy. Guard cells are 10 x 12 μm in size.

Stem (Figure 7.1, 2; 8.1, 2)

The stem is roughly squarish in outline in cross sectional view consisting of an epidermal layer followed by cortex and thick hallow cylinder of vascular tissues enclosing wide pith (Figure 7.1).

Cortical cells are parenchymatous and most of them are filled with tannins (Figure 7.2). Along the inner boundary of the cortex occurs a thin, discontinuous layer of gelatinous fibres, their inner walls consists of cellulose and mucilage and stain purple with toluidine blue (Figure 8.1).

Secondary phloem is wide continuous cylinder around the xylem (Figure 8.1). Secondary phloem consists of short radial compact elements which includes sieve-elements and phloem parenchyma (Figure 7.2; 8.1). Secondary xylem is dense and compact. It includes long radial multiples of vessels, xylem rays and radial lines of liberiform fibres (Figure 8.2). Vessels are upto 20 μm wide. The fibres are thick walled and lignified. Xylem rays are thin and straight (Figure 8.2).

Crystal distribution

Calcium oxalate crystals are widely distributed in different organs of the plant. In the leaf the crystals are exclusively druses or sphaerocrystals. They occured in the lamina in single straight line beneath the palisade zone (Figure 11.1, 2). The crystals are also distributed along the veins (Figure 6.2). In the veins, the crystals are located in the bundle sheath parenchyma.

Druses are also seen in the midrib and petiole. In the midrib, the druses are located in the ground tissue outer to the vascular strand (Figure 11.1, 2). The druses are seen in the phloem tissue as well. The druses in the phloem zone are smaller than those in outer ground tissue.

In the petiole, druses are found in the outer ground tissue as well as in the phloem parenchyma cells (Figure 10.2). The druses in the phloem zone are much smaller than those in the outer ground parenchyma. The crystals in the phloem zone are 10 μm wide, while those in the ground parenchyma is 30 μm.

Crystals in the stem

Calcium oxalate druses are also seen in the phloem parenchyma cells of the stem (Figure 11.1). Druses are sparse in distribution and are located in ordinary cells and are 30 μm wide. In the secondary xylem, calcium oxalate crystals of prismatic are located in the xylem parenchyma cells (Figure 11.2). The crystals are cuboidal or rhomboidal in shape which is solitary and scanty.

Root

Both thin and thick roots were studied.

Thin root **(**Figure 8.1 & 8.2, 2)

The thin root is 750-800 µm in diameter. The roots exhibit well developed secondary growth. The surface of the root is folded forming irregular ridges and furrows. There is a wide periderm which is deep in origin and a few outer layers of the cortex are broken and exofoliate. Secondary phloem is fairly thick and continuous all around. Secondary xylem is thick and dense. It consists of narrow circular vessels which scattered and solitary xylem fibres are narrow and thick walled and are in regular radial rows.

Thick root (Figure 10)

The thick root is more than 3 mm in diameter. It consists of thick and continuous periderm which is 100 µm in thickness. The periderm is homocellular comprising regular parallel radial lines. Inner to the periderm is a narrow cortex of parenchyma cells. Secondary phloem is thick and includes small groups of circular sclereids and phloem elements.

Secondary xylem includes radial lines of rays, mostly solitary, wide angular, thin walled vessels and thin walled fibres (Figure 10). The vessels are diffuse in distribution and are 40-120 µm wide. The xylem fibres are filled with dense starch grains which are simple type, spherical with concentric hilum.

Powder Microscopy

The following elements were observed in the powder when examined under the microscope

(i) *Fragments of lamina* (Figure 9.1, 2.)

Small pieces of lamina are frequently seen in the powder. These pieces were cleared to observe the venation pattern. From the main vein, the lateral vein originate at right angles and run parallel to each other. Thus, the major veins are parallel and minor veinlets form dense with definite boundaries. The islets vary in shape and size. One or more vein-terminations occurs in each islet. These terminations are short, thick, unbranched or branched once.

(ii) *Epidermal trichomes* (Figure 11.3, 4)

Non-glandular, unicellular unbranched, they are chip-like. The walls are thick and the lumen is narrow. The trichomes are 250-900 µm long and 10 µm thick.

(iii) *Crystals* (Figure 11.3, 4)

Calcium oxalate prismatic types of crystals are abundant and densely adhering on the trichomes. The crystals are also seen free from the trichomes. The crystals are amoeboidal or rhomboidal in shape and are 10-13 µm thick.

(iv) *Fibres* (Figure 3)

Liberiform fibres are abundant in the powder. They are long narrow cells with thick walls and narrow lumen. Pits are not evident on the walls. The fibres are 600 µm long, 12 µm thick.

(v) *Vessel elements* (Figure 3)

There are two types of vessel elements found in the powder. Some of the vessel elements are narrow, long with long tapering tails on both ends. They resemble the fibres in size and shape, they have simple, oblique small end wall perforations, which are less distinct.

The observation of cell inclusions and measurements of different tissues and cells are provided in Table 1.

Table 1: **Cell inclusions**

Compounds	Stain / Reagents used	Results - Localisation
Starch	Potassium iodine solution	Present in xylem fibres of root, turn blue colour
Tannin	Ferric chloride solution	Present in the spongy parenchyma, ground tissue of the petiole, peripheral cells of the pith in stem, turn light blue
Crystals	Polarised light	Druses in the leaf mesophyll along the veins midrib, petiole-phloem zone
Lignin	Toluidine blue solution	Stem-prismatic crystals in phloem, fibres and xylem elements, turn blue
Mucilage	Toluidine blue solution	Cortex inner walls consists of mucilage and stain purple with toluidine blue

Table 2: **Powder characteristics of the drug**

Name of the plant	Colour	Appearance	Odour	Taste
Guidonia tomentosa	Pale brown	Fine powder	Aromatic	Bitter

Table 3: **Powder analysis of the drug**

Treatment	Observation
Powder treated with water	Non-sticking
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Pale brown
Powder treated with 60% aqueous sulphuric acid	Pale brown
Powder pressed between filter paper for 24 hours	No oil stain

Table 4: Ash values of the drug

Name of the plant	Total ash (% w/w)	Water soluble ash (% w/w)	Acid soluble ash (% w/w)	Alkalinity of water soluble ash (ml)
Guidonia tomentosa	5.1	3.11	4.98	0.1

Extractive values of the drug *(% w/w)*

The Extractive values of the drug of Ethanol soluble extract is 23.98%, Water soluble extract is 15.81%, Hexane soluble extract is 3.81% and Chloroform soluble extract is 2.6478%

Solubility values of the drug*(% w/w)*

In Ethanol the solubility is recorded as 56.86% , in Water 20.45% and in Methanol 60.82%.

Table 5: **Fluorescence analysis of various extracts of the drug powder**

Extract	**Treatment**	**Observation**
Ethanol	Day light	Brown
	Short UV	Brown
	Long UV	Dark green
Water	Day light	Brown
	Short UV	Dark green
	Long UV	Brown
Hexane	Day light	Dark green
	Short UV	Green
	Long UV	Brown
Chloroform	Day light	Brown
	Short UV	Green
	Long UV	Brown

Table 6: **Fluorescence analysis of the drug**

Experiments	**Visible / Day light**	**UV light**	
		254 nm	**365 nm**
Drug powder (D.P)	Pale brown	Green	Pale brown
D.P + 1 N NaOH (aq.)	Brown	Green	Pale brown
D.P + 1 N NaOH (alc.)	Brown	Pale green	Colourless
D.P + 1 N HCl	Brown	Pale green	Pale brown
D.P + 50% H_2SO_4	Brown	Green	Colourless
D.P + 50% HNO_3	Brown	Green	Pale brown
D.P + Picric acid	Brown	Pale green	Pale brown
D.P + Acetic acid	Brown	Green	Pale brown
D.P + Ferric chloride	Brown	Pale green	Colourless
D.P + HNO_3 + NH_3	Brown	Blue	Pale brown

Research Article

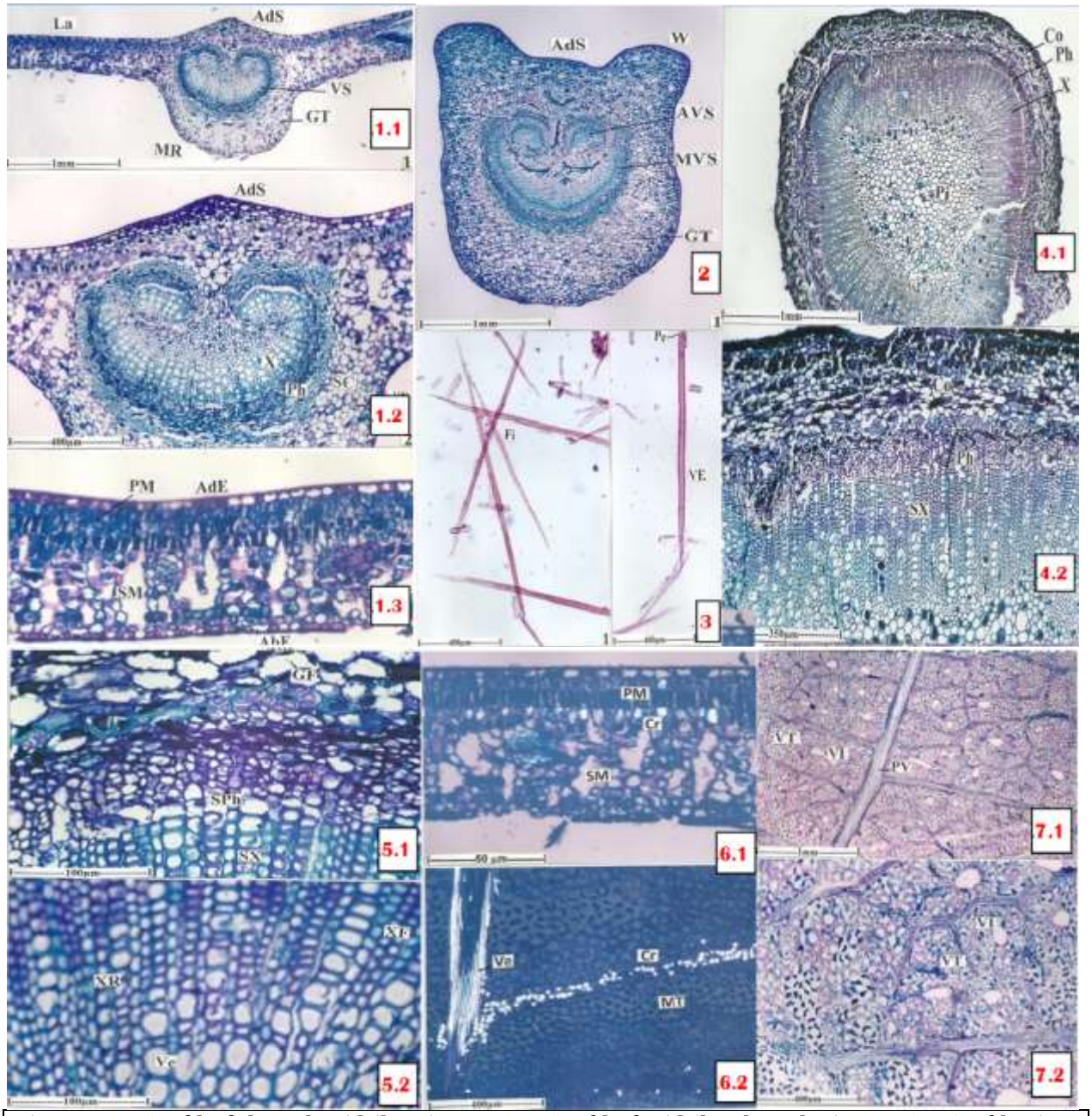

Figure 1.1:T.S. of leaf through midrib , Figure 1.2: T.S. of leaf midrib enlarged, Figure 1.3:T.S. of lamina; AdS: Adaxial side, AdE: Adaxial epidermis, GT: Ground tissue, Ep: Epidermis, AbE: Abaxial epidermis, La: Lamina, MR: Midrib, SC: Sclerenchyma, SM: Sponge mesophyll, PM: Palisade mesophyll, VS: Vascular strand, Ph: Phloem, X: Xylem; **Figure 2: T.S of petiole through distal part;** AVS: Adaxial vascular strand, MVS: Median vascular strand, GT: Ground tissue, W: Wing. Figure **3: Fibres and vessels.** Figure **4.1 &4.2 :T.S of Stem and enlarged** Co: Cortex, SX: Secondary xylem, Pi: Pith, Ph: Phloem, X: Xylem**:** Figure **5.1 &5.2:Sec. Xylem &Sec.Phloem** GF: Gelatinous fibre, Ve: Vessel, SX: Secondary xylem, SPh: Secondary phloem, XF: Xylem fibre, , XR: Xylem ray; **Figure 6.1: T.S. of lamina showing crystals in the mesophyll tissue, Figure 6.2:** Crystals located along the vein**;** Cr: Crystal, SM: Spongy mesophyll, PM: Palisade mesophyll, MT: Mesophyll tissue, Ve: Vessel, **Figure 7.1**: Lamina showing cleared venation pattern, **Figure 7.2:** Vein-islet and vein-termination enlarged.VI: Vein-islet, T: Vein-termination.

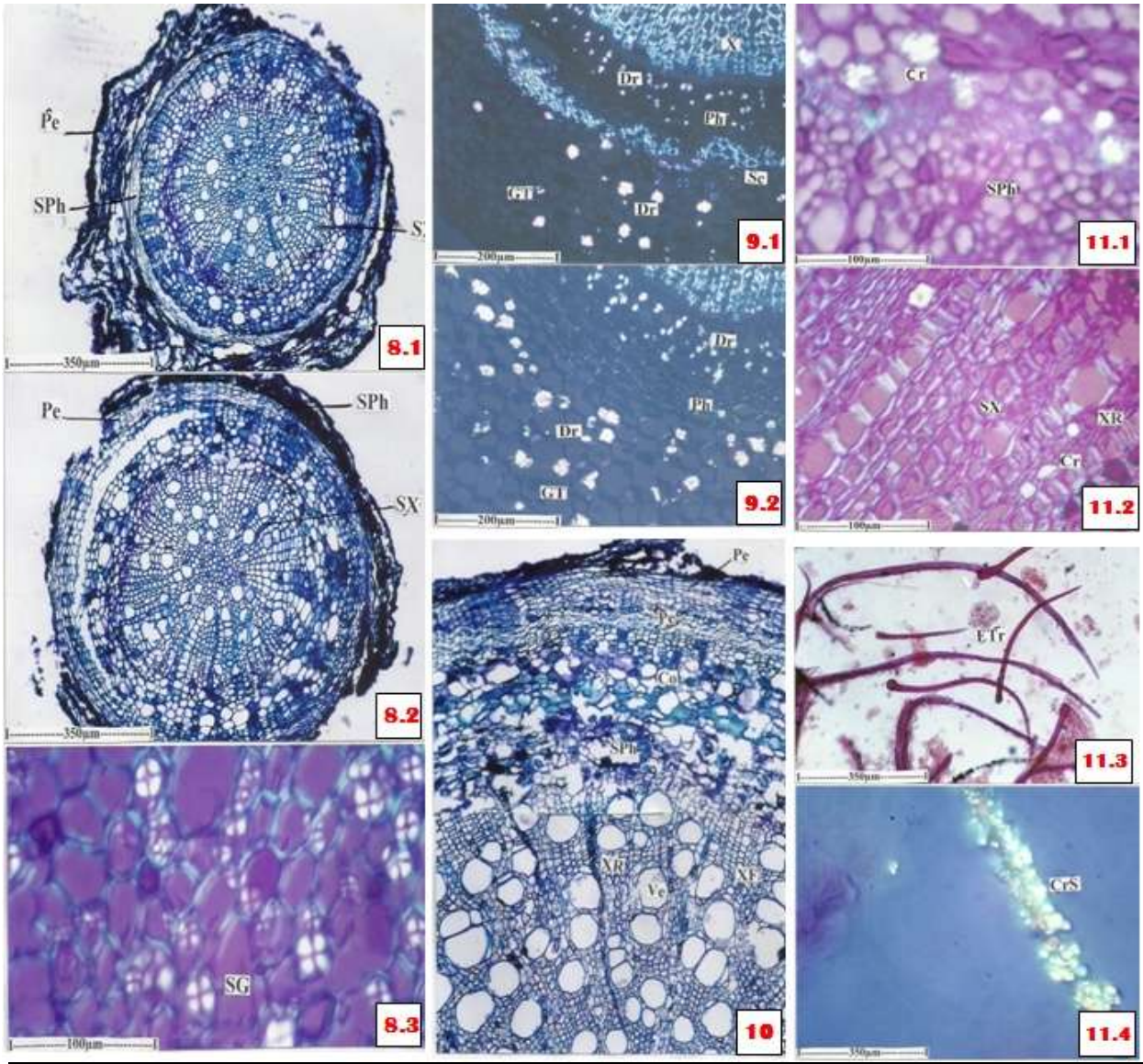

Figure 8.1 &8.2: T.S. of thin root ;**Figure 8.3: Starch grains in the xylem fibres** ; Fi: Fibre, Pe: Periderm, SG: Starch grain, SPh: Secondary phloem, SX: Secondary xylem**. Figure 9.1: Crystal in the midrib under polarized microscope Figure 9.2 Crystal in the petiole under polarized microscope. Figure 10: T.S. of thick root** Co: Cortex, Pe: Periderm, SC: Sclerenchyma, SPh: Secondary phloem, SX: Secondary xylem XF: Xylem fibre, Ve: Vessel; **Figure 11.1 & 11.2:** Crystals in the cortical region and xylem GT: Ground tissue, Dr: Druses, SC: Sclerenchyma, Ph: Phloem, X: Xylem **Figure 11.3 & 11.4 :**Epidermal trichomes , Crystals on the trichome; Cr: Crystal, SPh: Secondary phloem, XR: Xylem ray, SX: Secondary xylem ETr: Epidermal trichome, CrS: Crystal strand.

The taxonomical, macroscopical and microscopical characters and phytochemical analysis of the above studied taxa help in identifying the plant drug material to overcome the use of substitutes and adulterants for these plant drugs. It also helps in identifying the germplasm of medicinal plants and aid in conserving the important medicinal plants of the area (Ramesh *et al*., 2013).

In conclusion, the present study has provided a scientific evaluation of *Guidonia tomentosa* present widely in Chittoor district of Andhra Pradesh representing the pharmacognostical features, photochemical and physicochemical studies and fluorescence analysis which resolve to be useful for deciding the

identity, quality, purity and strength of the plant material and also for fixing pharmacopoeial standardization profiles of phytodrugs. Thus the results of this present investigation certainly helpful in the preparation of medicinal monograph for these plants in the Indian herbal pharmacopoeia.

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