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Pharmacognostic Studies of *Didymocarpus gambleanus* C. Fischer (Gesneriaceae)

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ABSTRACT

Didymocarpus gambleanus C. Fisher is an endemic species of Western Ghats of Tirunelveli, Tamil Nadu. Folklore claims attribute some medicinal properties to this plant. In spite of its pharmacological values, this taxon remains unstudied so far. The present Paper gives detailed account of anatomical features of leaf, midrib, petiole and rhizome. The results of the study showed some of the microscopic features of the chosen plant are specific for this taxon. It is suggested that the microscopic data of the plant may be useful for those who opt this plant for any other scientific studies.

INTRODUCTION

Gesneriaceae Rich. & Juss. ex DC. includes 141 genera and 2900 species at global level. The genus ***Gesneria*** includes 70 species which are characterised by pseudostipules and mucilage secretion. Some species are cultivated and ornamental (Mabberly, 2005). In South India 17 genera have been reported by Gamble, 1921. Of these genera in India, 12 taxa have been reported. The species ***Didymocarpus pedicellata*** which is common in Northern part of India has been studied for its pharmaceutical activities. However, the South Indian species of *Didymocarpus* remain unstudied for their pharmacognostical, phytochemical and pharmacological aspects. Recently we have published a paper or "Pharmacognostical standardisation of ***Didymocarpus humboldtianus*** Gard". Barring this study no papers have been published on many aspects of this species of ***Didymocarpus*** available in South India. We feel that the available species of ***Didymocarpus*** distributed in Western Ghats of Tamil Nadu are worthy of investigation for the biological activities. Taxonomic circumscription of the species of *Didymocarpus* of Western Ghats may throw light on diagnostic aspects of the taxa. Based on the above perusal it is planned to undertake pharmacognostical parameters of ***Didymocarpus gambleanus*** *C.* Fischer. It is a common element Western Ghats and a co-existing plant often found along with ***Didymocarpus tomentosus*** and ***Didymocarpus humboldtianus.*** We hope that the microscopic studies of ***Didymocarpus*** are most essential aspect of analysis of the family. The present paper provides different parameters of botanical (Anatomy and Taxonomy) studies of ***D. gambleanus.***

REVIEW OF LITERATURE

We made much extensive survey of literature pertaining to different aspects of the species of ***Didymocarpus.*** We were able to find some papers on ***Didymocarpus pedicellata.*** Virtually no work has been carried out on other species of ***Didymocarpus.*** So we planned that pharmacognostical analysis of ***Didymocarpus gambleanus*** C. Fisher will fill the gap in our knowledge of the taxon which is restricted in distribution to Western Ghats of Tamil Nadu.

AIM AND SCOPE

Microscopic parameters of medicinal plants provide highly reliable clues for the identification of crude drugs. The study also helps to detect the adulterants or sometimes substitutes of the herbal drugs. It has been generally accepted that anatomical studies have been playing a crucial role in diagnosis of fragmentary plant materials. So in the present

study of ***Didymocarpus gambleanus***, we will be dealing with detailed anatomical character of leaf (lamina and midrib), petiole, rhizome and root. Stomatal morphology and trichome characters will also come under the present purview. Histochemistry deals with the localisation of different chemical substances in the plant cells. This enables an investigator to know the particular chemical compound is present or absent if present which part of the plant organ or which type of tissue possess the particular chemical compound. Thus, histochemistry also plays a prominent role in the diagnostic studies of herbal drugs. We have aimed at histological and histochemical studies of the selected plant.

The scope of the study obviously will be useful to who want to identify a plant and to eliminate the adulterants when mixed with original herbs. Thus, the anatomical and cytochemical studies have immense scope for investigator to identify the plants in their hands.

MATERIALS AND METHODS

Didymocarpus gambleanus occurs in the natural condition in the Western Ghats of Tamil Nadu, Tirunelveli. It is usually found on shady crevices of rocks. It is fairly common during a particular season. The plants were collected during the months of November and December. Flowers were not available during this season. Different parts of the plant namely leaf, petiole, rhizome and root were cut and removed from the plant and fixed in FAA (Formalin 5 ml + Acetic acid 5 ml + 70% Ethanol 90 ml) in the field itself. Field details of the plant were noted and the plants in the natural condition were photographed.

The materials were dehydrated through graded series of tertiary butyl alcohol and paraffin wax was infiltrated gradually into the specimens which are in pure TBA (Tertiary butyl alcohol). The above process is as per the schedule given by Sass, 1940. After supersaturation of TBA with wax, the materials were cast into paraffin blocks.

The materials in the wax blocks were cut into individual fragments and subjected to Rotary Microtome sectioning at a thickness of 10 µm. The section were stretched and fixed on microscope slides. After the sections were dry they were subjected to dewaxing and then stained with Toluidine Blue O stain which was prepared by dissolving 0.05 gm of powder in 100ml of distilled water. The sections were dehydrated by passing through graded series of TBA (Tertiary Butyl Alcohol). The dehydrated sections were stained with 0.05% aqueous

Toluidine blue (O. Brein et.al., 1964). The sections were sealed with DPX mounting medium and covered with square shaped coverslips.

The slides were photographed under Nikon microscope attached with Nikon digital Coolpix-8400 camera. The photographs were prepared in different magnifications. Magnification scales were given at the bottom of the photographs. Descriptive terminology of the anatomical features was followed as given in the standard anatomical textbooks (Esau 1964; Metcalfe & Chalk 1979).

RESULTS

1. EXTERNAL PROFILE

Didymocarpus gambleanus is a scapigerous herb. The leaves have thick Cinnamomum tomentose trichomes. The plant bear basal rosette of leaves. There are some morphological differences between the younger and older leaves. The older leaves have short winged petiole. The young leaves are central in position and are wingless and almost sessile. The lamina is thick, ovate and broadly decurrent. The leaf margins are somewhat crenulate (Fig.1.1, 2, 3). The adaxial surface of the leaf has blister like raised spots (Fig.2.1 & 2; 3.5). The abaxial surface of the leaf has dense nonglandular trichomes (Fig.3.2 & 3). The scape is sparingly branched and the branching is dichotomous (Fig.4.1, 2 & 3). The fruits are cylindrical with longitudinal ridges alternating with furrow. The dehiscence of the fruit is loculicidal (Fig.5.1 & 2). The seeds are minute, elliptical or ovate. They are reddish brown with white spots. The seeds have short beak at the micropylar end (Fig.5.3 & 4).

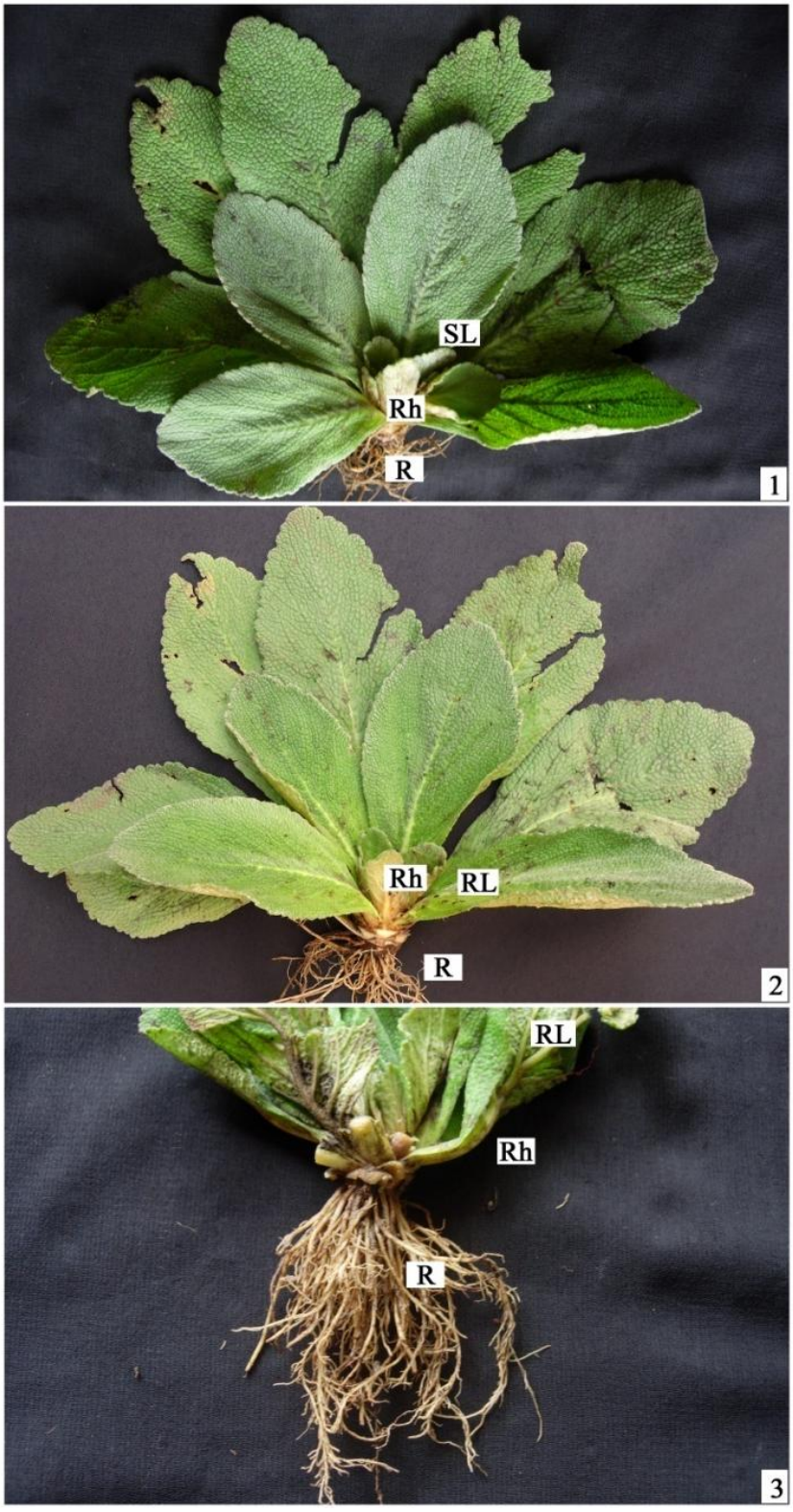


Fig. 1.1, 2, 3 *Didymocarpus gambleanus*

1. A shoot with rosette of sessile leaves on the rhizome.

2. Rhizome bearing short sessile young leaves in the centre and subsessile leaves along the

periphery.

3. Rhizome bearing radical leaves and thick bunch of adventitious roots

(R: Root; Rh: Rhizome; RL: Radical Leaves; SL: Sessile Leaves).

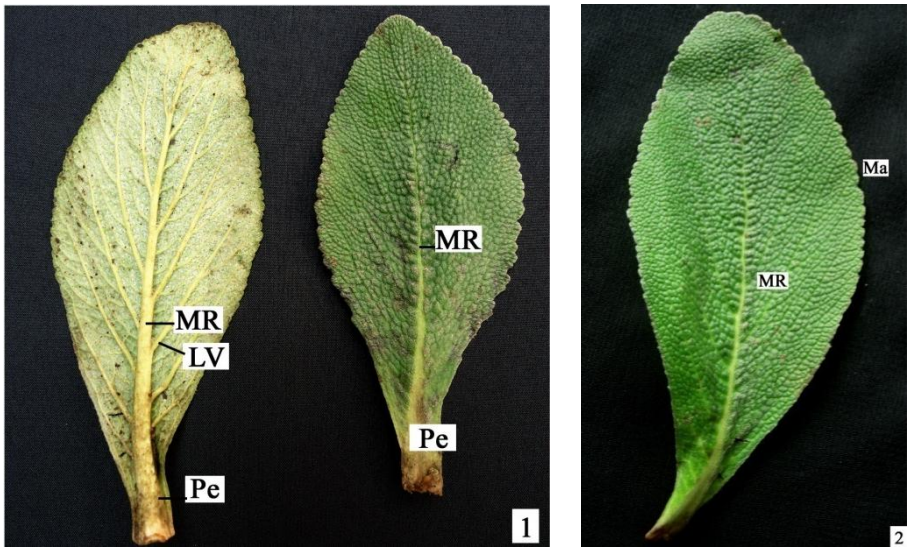
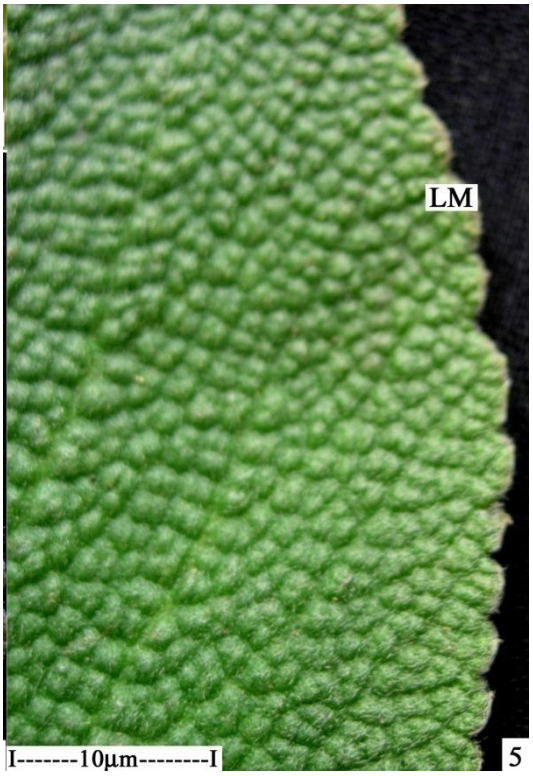


Fig.2.1 & 2.2

1. Two leaves (Leaf in the left side in abaxial view, leaf on the right side in adaxial view).

2. Adaxial view of the leaf enlarged showing blister like appearance of the lamina.

(LV: Lateral Vein; Ma: Marginal part of leaf; MR: Midrib; Pe: petiole)



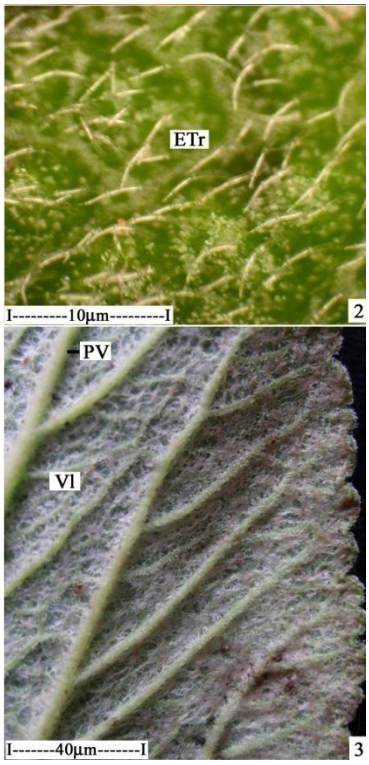


Fig. 3.5 **Fig. 3.2 & 3**

2. Abaxial surface of the lamina showing dense non glandular trichomes.

3. Abaxial view of the lamina showing thick primary veins and thin veinlets.

5. Adaxial surface of the lamina showing blister like appearance.

(Etr: Epidermal Trichome; LM: Leaf Margin; PV: Primary Vein; Vl: Veinlet)

Fig.4.1, 2 & 3

1. An old scape bearing dry follicles.

2 & 3.Follicles enlarged

(Fo: Follicle)

Fig.5.1 & 2

1. A dry fruit with longitudinal ridges and furrows.

2. A dehisced fruit.

(Fo: Follicle; FW: Fruit Wall; S: Seeds)

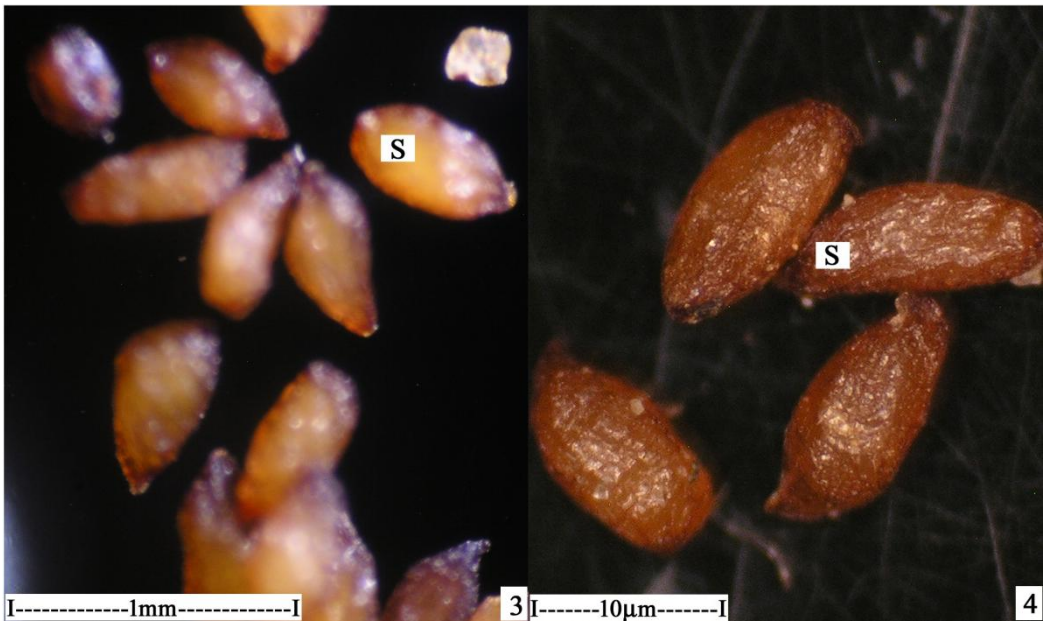


Fig.5.3 & 4

3. A dehisced fruit with seeds attached on the parietal placenta.

4. Seeds showing the colour and surface features.

(S: Seeds)

2. ANATOMICAL FEATURES

(i) LEAF

The leaf has thick midrib and highly wavy lateral lamina. The lamina is undulate because of the raised abaxial vein (Fig.6.1). The midrib is plano-convex with flat adaxial side and thick vertically oblong abaxial part. The midrib is 800µm thick and 700µm wide. The adaxial epidermis of the midrib has wide vertically oblong cells. The abaxial epidermal cells are variable in size. The ground tissue of the midrib is homocellular, compact and parenchymatous (Fig.6.1, 2 & 3). The vascular system of the midrib consists of one larger fan shaped collateral vascular bundle and the other is smaller and adaxial in position. The xylem elements of the vascular bundle are angular, very thick walled with narrow lumen. The phloem elements occur in thin layer along the lower part of the xylem strand (Fig. 6.2). The lateral veins are also planoconvex with parenchymatous ground tissue and single collateral small vascular bundle (Fig.7.1).

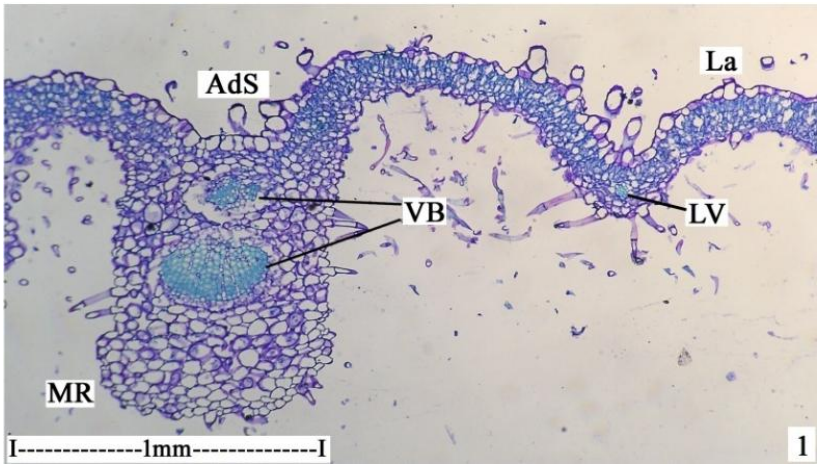


Fig.6.1 T.S of leaf through midrib and lamina

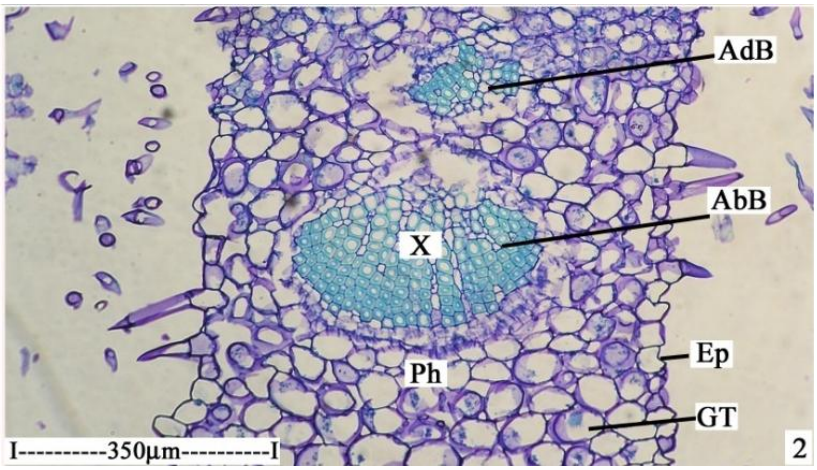


Fig. 6.2 T.S of midrib with two vascular strands

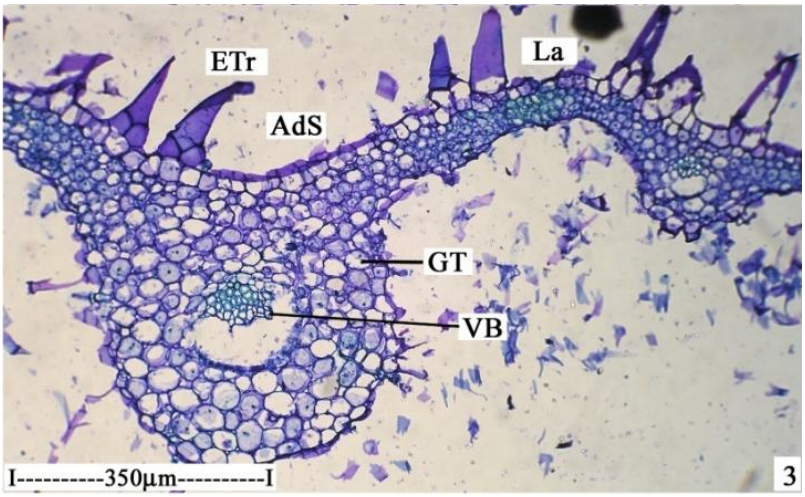


Fig.6.3 Lateral vein with single vascular strand

(AbB: Abaxial Bundle; AdB: Adaxial Bundle; AdS: Adaxial Side; Ep: Epidermis; ETr: Epidermal Trichome; GT: Ground Tissue; La: Lamina; LV: Lateral Vein; MR: Midrib; Ph: Phloem; VB: Vascular Bundle; X: Xylem).

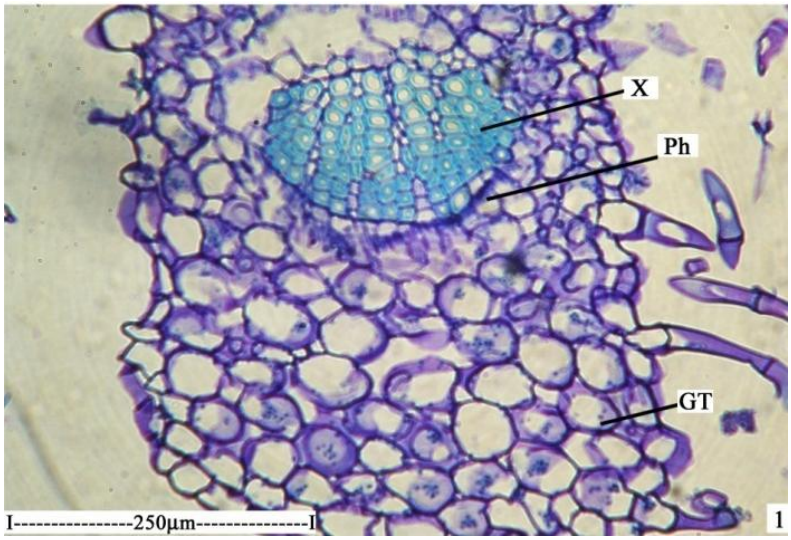


Fig.7.1: T.S. of lateral vein showing single collateral vascular bundle

(GT : Ground Tissue; Ph: Phloem; X: Xylem).

Lamina has dense epidermal trichomes on both upper and lower sides. The leaf is amphistomatic. The adaxial epidermis of the lamina has large thin walled, squarish cells. The abaxial epidermal cells are smaller and thin walled. The mesophyll tissue is differentiated into adaxial zone of two layers of palisade cells and two or three layers of spherical loosely arranged small spongy mesophyll cells. The lamina is 180µm thick.

The stomata (Fig.8.1& 2) are located within shallow stomatal pits. The stomata are cyclocytic type. In this type, each stoma is encircled by four or five subsidiary cells which are equal in size and shape.

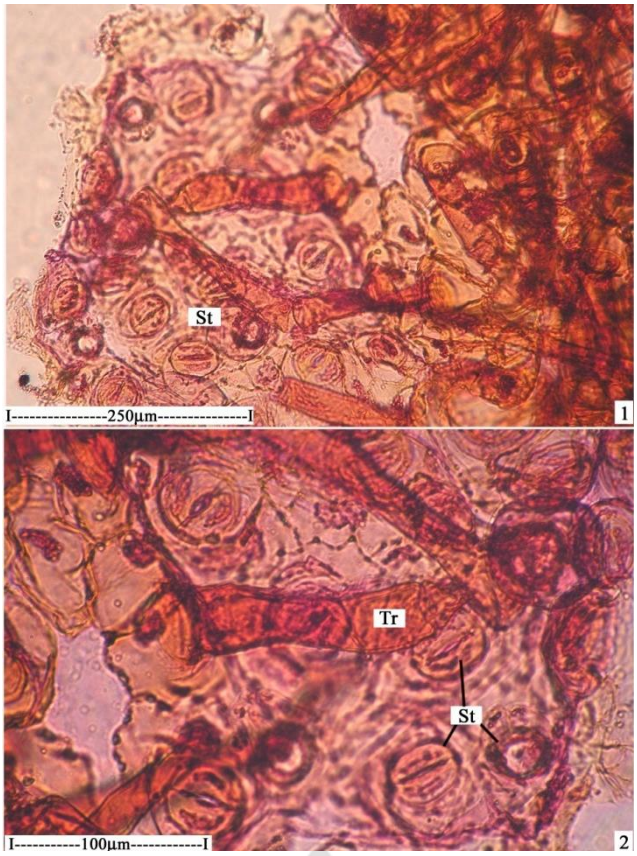


Fig. 8.1 & 8.2

1. Paradermal view of the abaxial side of the lamina showing stomata, trichome and epidermal cells.

2. A few stomata enlarged.

(St: Stomata; Tr: Trichome).

The epidermal cells are circular with thin wavy anticlinal wall. The venation is dense and reticulate. The vein islets are wide with simple or branched thin slightly curved vein terminations.

(ii) PETIOLE

The petiole is planoconvex along the distal part and it is prominently winged (Fig.9.1 & 2). The petiole has thin less prominent layer of epidermis and angular thin walled parenchymatous ground tissue. The vascular system of the petiole is multistranded. There is a horizontal abaxial row of vascular bundles. The vascular bundles in the central part of the row are larger and the bundles become gradually smaller along the lateral part.

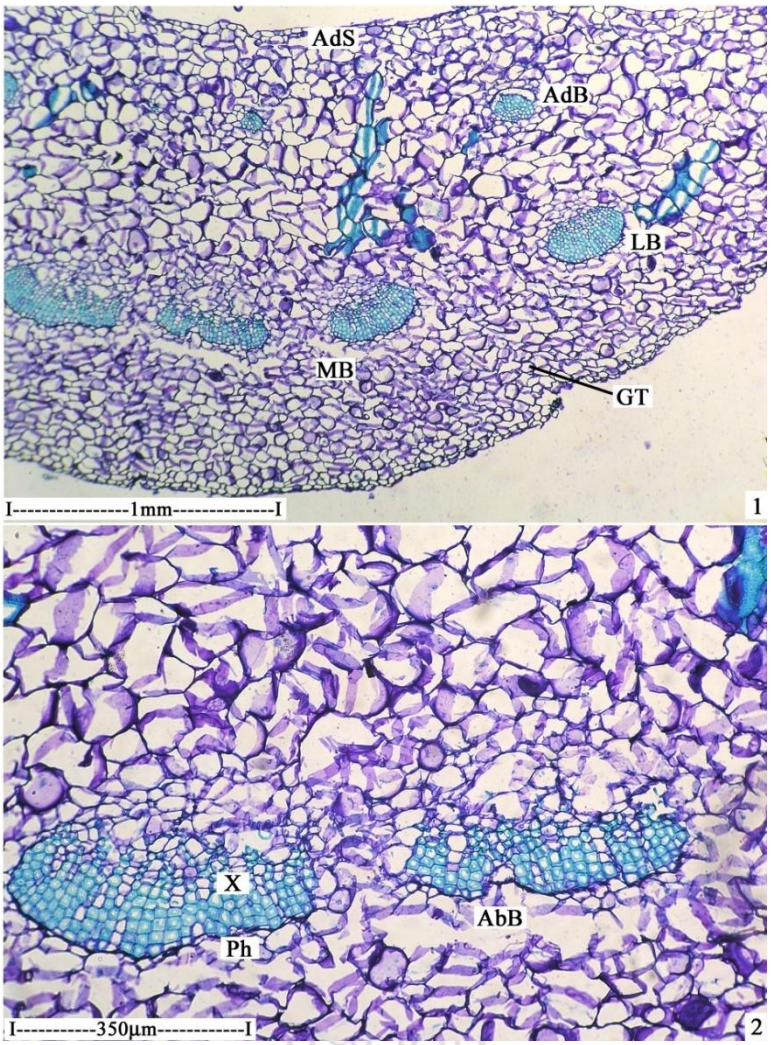


Fig.9.1 & 9.2

1. T.S.of distal part of the petiole.

2. Abaxial vascular bundles of distal part of the petiole

(AbB: Abaxial Bundle; AdB:Adaxial Bundle; AdS: Adaxial Side; GT: Ground Tissue; LB: Lateral Bundle; MB: Middle Bundle; Ph: Phloem; X: Xylem).

The bundles have long compact vertical rows of xylem elements which possess thick walls and narrow lumen. The phloem occurs in thin abaxial layer beneath the xylem strands. The wing bundles are smaller, circular and they possess prominent xylem elements and a few phloem elements (Fig.9.2; Fig.10.1 & 2). Some of the ground tissue of the petiole have thick lignified wall and dense circular wide pits (Fig.11.1).

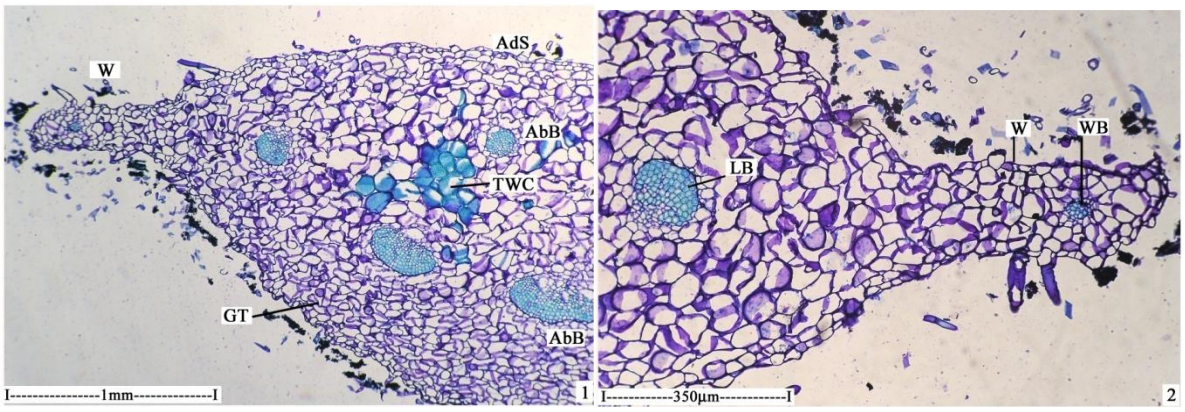


Fig.10.1 & 10.2

1, 2. T. S. of distal petiole showing short thick wings with wing bundles.

(AbB: Abaxial Bundle; AdS: Adaxial Side; GT: Ground Tissue; LB: Lateral Bundle; TWC: Thick Walled Cells; W: Wing; WB: Wing Bundle).

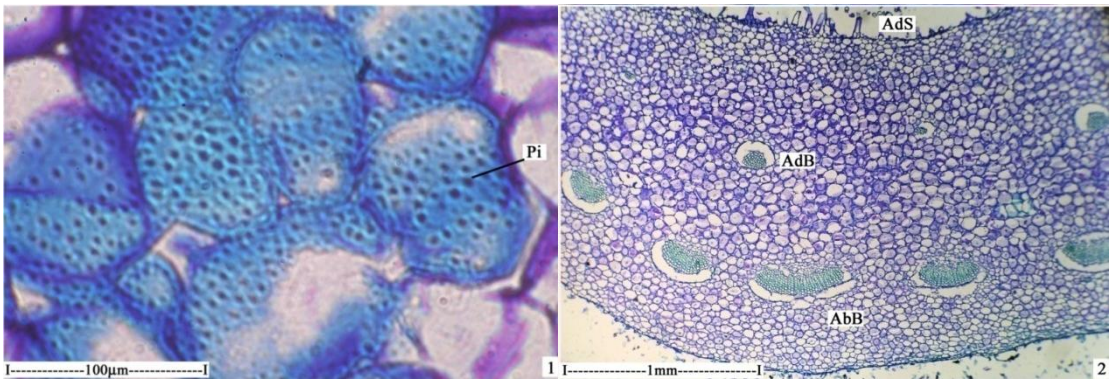


Fig.11.1 **Fig.11.2**

1. Thick walled parenchyma cells with dense reticulate pits.

2. T. S. of proximal part of the petiole.

(AbB: Abaxial Bundle; AdB: Adaxial Bundle; AdS: Adaxial Side; Pi: Pith).

The proximal part of the petiole is flat with slight adaxial concavity. The petiole has several vascular bundles located in horizontal row. The vascular bundles at the lower part of the petiole are circular and those in the upper part are smaller and circular (Fig.11.2; Fig.12).

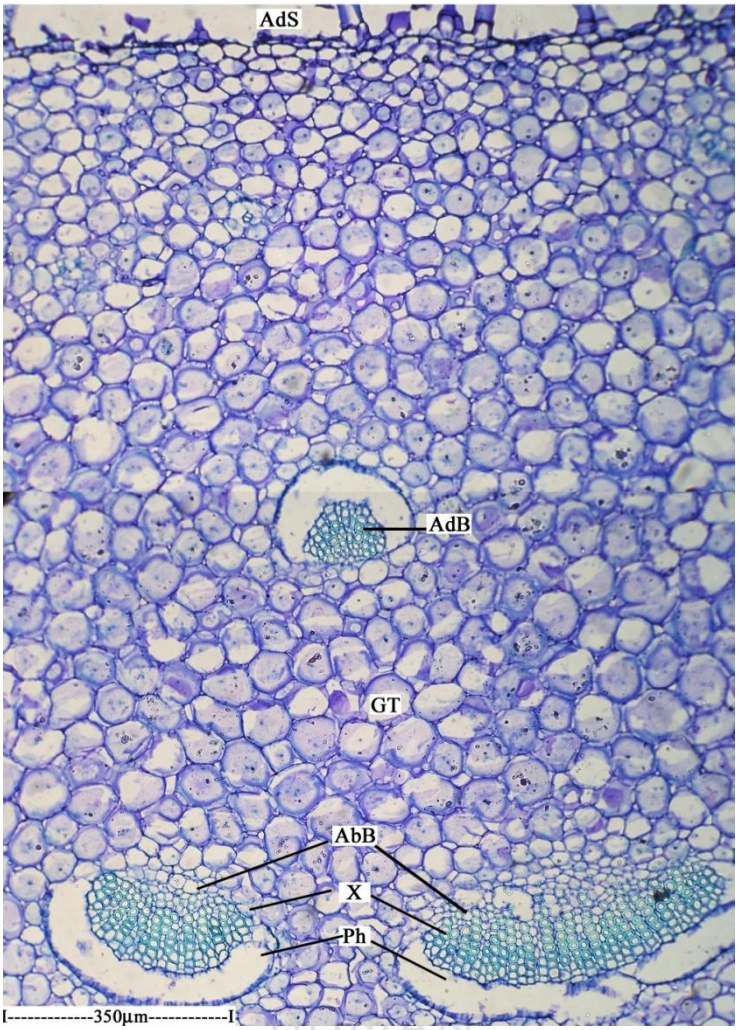


Fig.12: Proximal part of the petiole showing ground parenchyma and adaxial and abaxial vascular bundles

(AbB: Abaxial Bundle; AdB: Adaxial Bundle; AdS: Adaxial Side; GT: Ground Tissue; Ph: Phloem; X: Xylem).

(iii) ROOT

The root consists of broken, discontinuous layer of epidermis. The cortex is homogeneous and parenchymatous measuring 250 µm thick. The vascular cylinder consists of four exarch xylem strands alternating with four small clusters of phloem elements. The central core of the vascular strand has thick walled, wide, lignified fibres. The root is 650 µm in diameter and the vascular cylinder is 230 µm thick. The xylem elements are 70 µm wide (Fig.13.2 & 3).

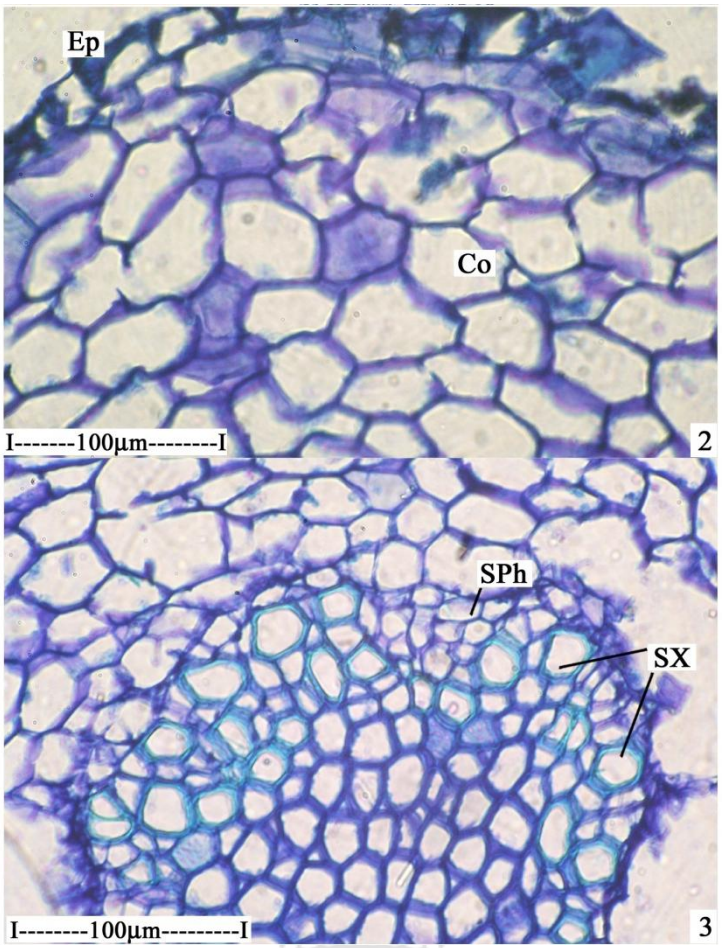


Fig.13.2 & 3

2. Cortical tissues of the root.

3. Xylem and phloem elements and central fibres of the vascular strand.

(Co: Cortex; Ep: Epidermis; SPh: Secondary Phloem; SX: Secondary Xylem; VC: Vascular Cylinder).

(iv) RHIZOME

The rhizome is short, erect, unbranched and thick. It is circular in cross sectional view. It consists of parenchymatous cortex and pith. The vascular tissues occur in thick hollow cylinder. The cylinder includes radial, conical segments of xylem and phloem bundles. In between the vascular bundles are seen wide parenchymatous rays. The root traces and leaf traces are seen emerging from the vascular cylinder (Fig.14.1 & 2). The vascular segments of the vascular cylinder possess compact radial files of vessels and fibres

(Fig.15.2). The vessels are narrow, angular and thin walled. The xylem fibres are wide thin walled and radially elongated (Fig.15.3).

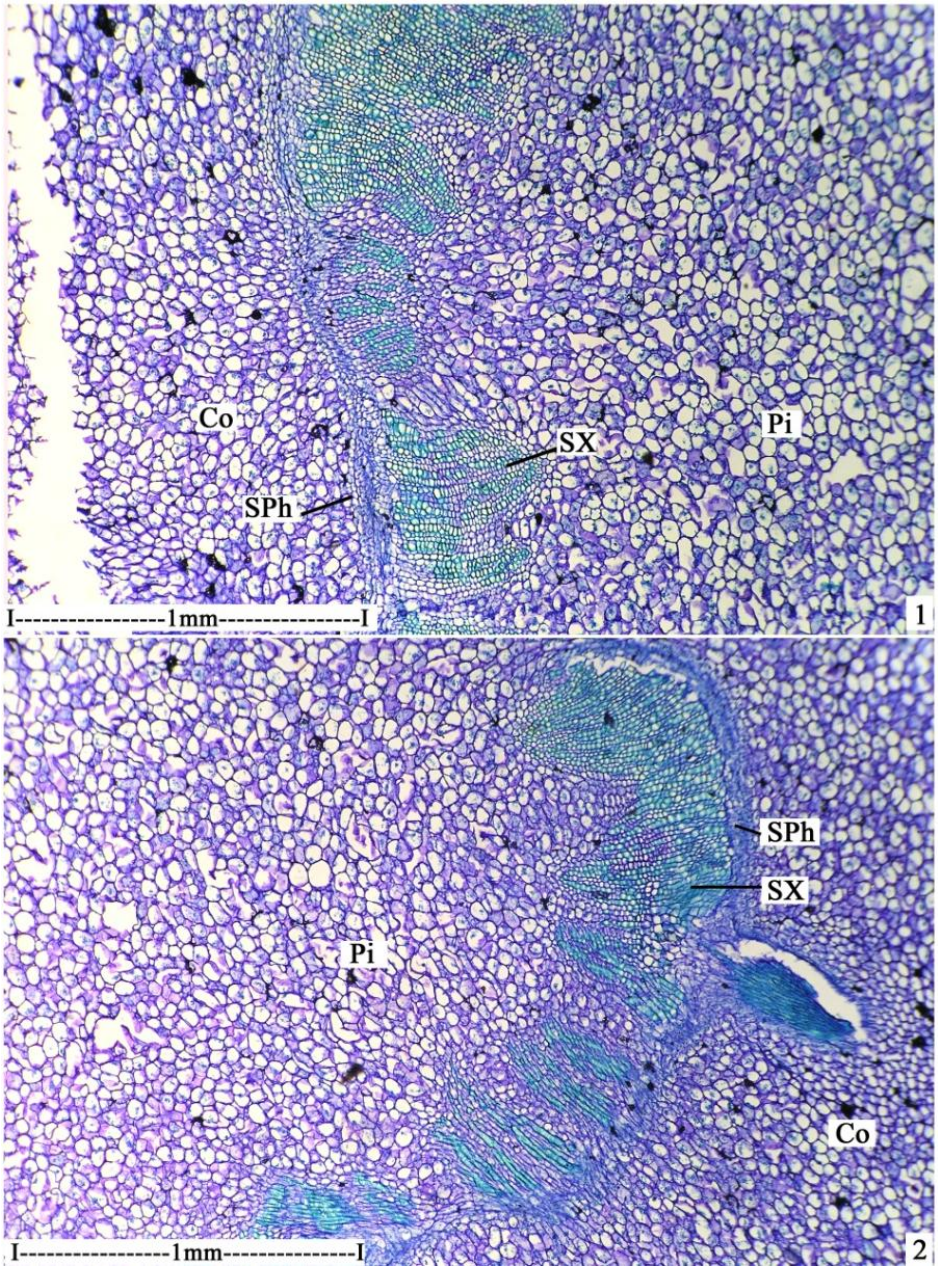


Fig.14.1 & 14. 2

1. T.S.of rhizome -one sector of the vascular cylinder.

2. Another sector of the vascular cylinder of the rhizome.

(Co: Cortex; Pi: Pith; SPh: Secondary Phloem; SX: Secondary Xylem).

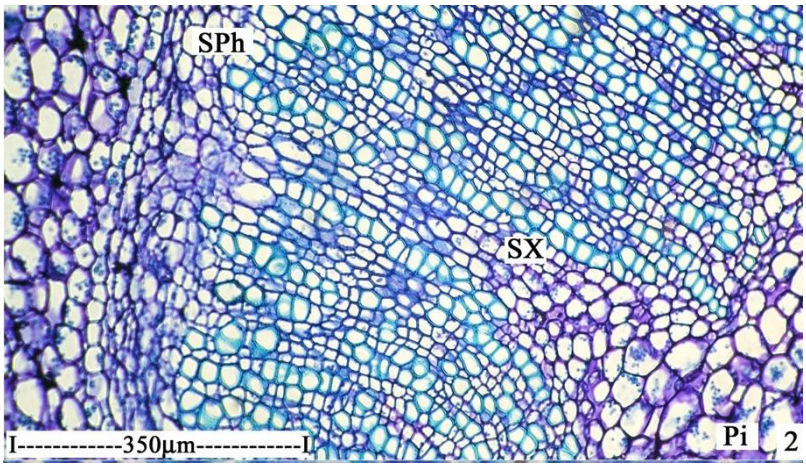


Fig.15.2

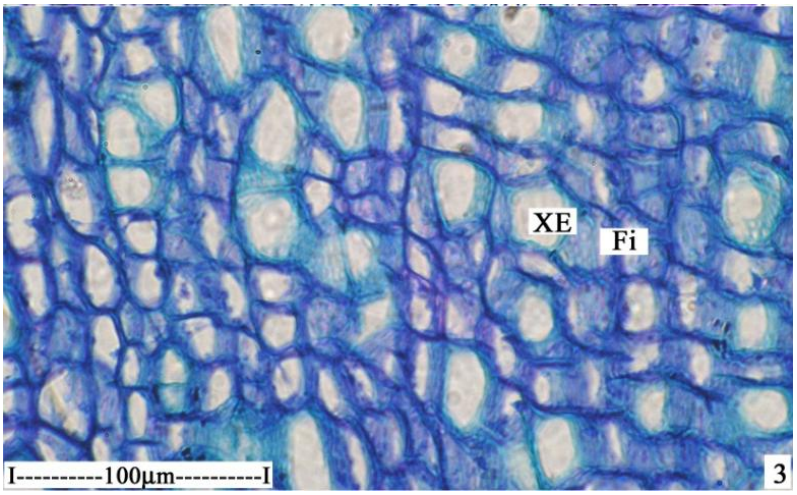


Fig.15.3

2. Secondary xylem cylinder showing radial files of xylem elements and fibres.

3. A portion of the xylem cylinder enlarged showing xylem elements (vessels) and fibres.

(Fi: Fibre; Pi: Pith; SPh: Secondary Phloem; SX: Secondary Xylem; XE: Xylem Element).

(v). POWDER MICROSCOPY

The powder includes dense epidermal trichomes. The trichomes are of two types. Some are long, filiform trichomes (Fig.16.3). These trichomes are of unlimited in length, thin walled and intertwined. Some cell inclusions are seen in the cells of the trichome. The trichome is 25 µm thick. The second type of trichomes is non glandular covering type (Fig.17.2 & 3). These

trichomes are six to eight celled, uniseriate and unbranched. The cells of the trichome are vertically elongated thick walled and rectangular in shape. The trichomes are broad at the base and become gradually tapering at the tip. The cell lumen is wide and when the trichomes are viewed under polarised light the cell wall appears bright indicating the lignification of the cell wall (Fig.17.3). The trichomes are 700 μm long, 600μm thick at the base and 10 μm at the tip.

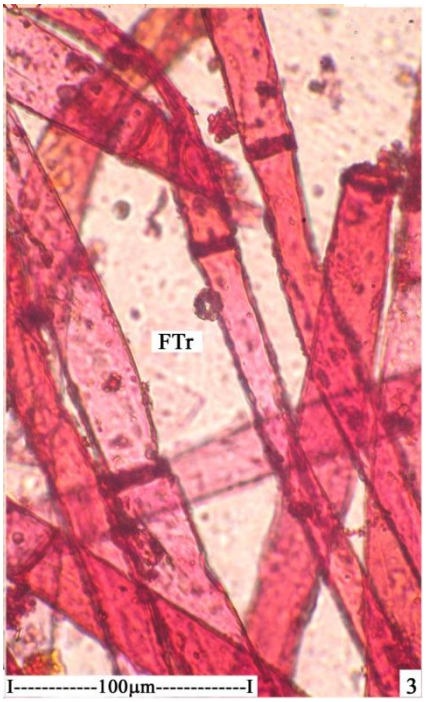


Fig.16.3: Trichome enlarged showing cell walls and cell lumen.

(FTr :Filiform Trichome).

Fig.17.2 & 3

2. A single trichome enlarged.

3. Two trichomes as seen under polarised light

(NGT: Non Glandular Trichome)

The powder preparation also shows elongated, cylindrical or rectangular parenchyma cells. The parenchyma cells may be solitary or in groups of two or more cells. The cells are thick walled and possess some amorphous cell inclusions (Fig.18.4). The cells are 100 µm to 200 µm long and 40 µm wide.

Fig.18.4: Three parenchyma cells with dense amorphous cell inclusions.

(PC: Parenchyma Cell).

Fibres:

Libriform fibres are abundant in the powder. They are long, narrow, cylindrical cells with lignified walls and tapering ends. The cell lumen of the fibres contains starch grains (Fig.19.2). The fibres are 300µm long and 20µm wide (Fig.20.1).

Fig.19.2: A fibre possessing starch grains.

(Fi: Fibre)

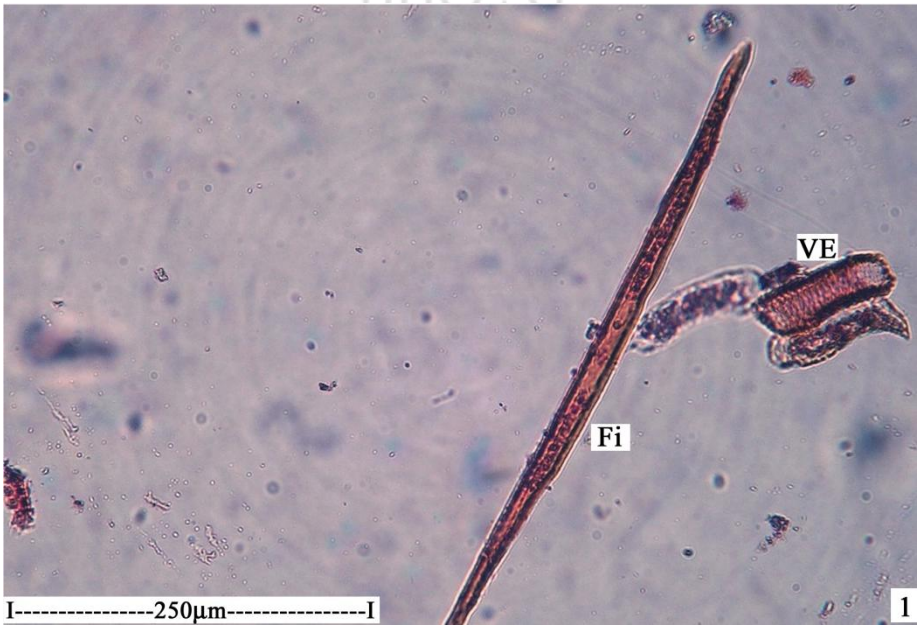


Fig.20.1: A fibre and a vessel element

(Fi: Fibre; VE: Vessel Element)

Vessel elements are short, wide and cylindrical with elliptical, multiseriate lateral wall bordered pits. The end wall perforations are circular either horizontal or oblique (Fig.21.1 & 2). The vessel elements are 100 µm to 120 µm long and 30 µm wide.

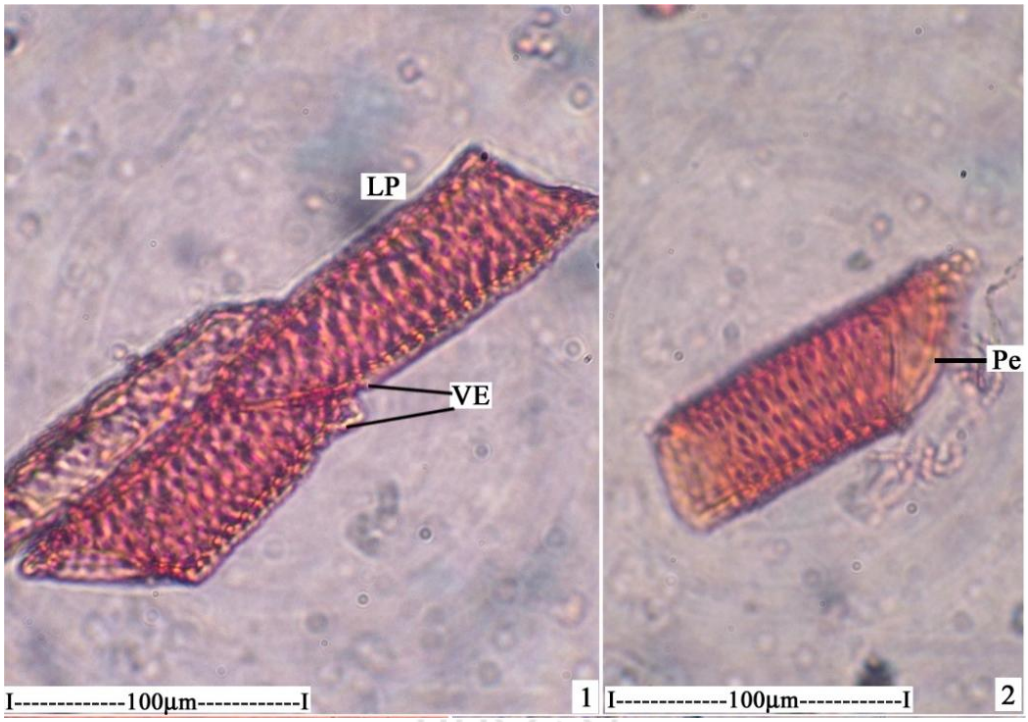


Fig.21.1 & 21.2

1. Two vessel elements attached to their end walls

2. A single vessel element.

(LP: Lateral wall Pits; Pe: Perforation; VE: Vessel Element).

3. HISTOCHEMISTRY

(i) LIPIDS

Lipids were identified by neutral red stain. Lipids were found to be localized in the ground parenchyma and phloem parenchyma cells of the petiole. In rhizome, lipids occur in the cortical parenchyma cells (Fig.22.1, 2 & 4). Nile blue also stains lipids. Nile blue stained lipids were found in the parenchymatous cells of the petiole and rhizome.

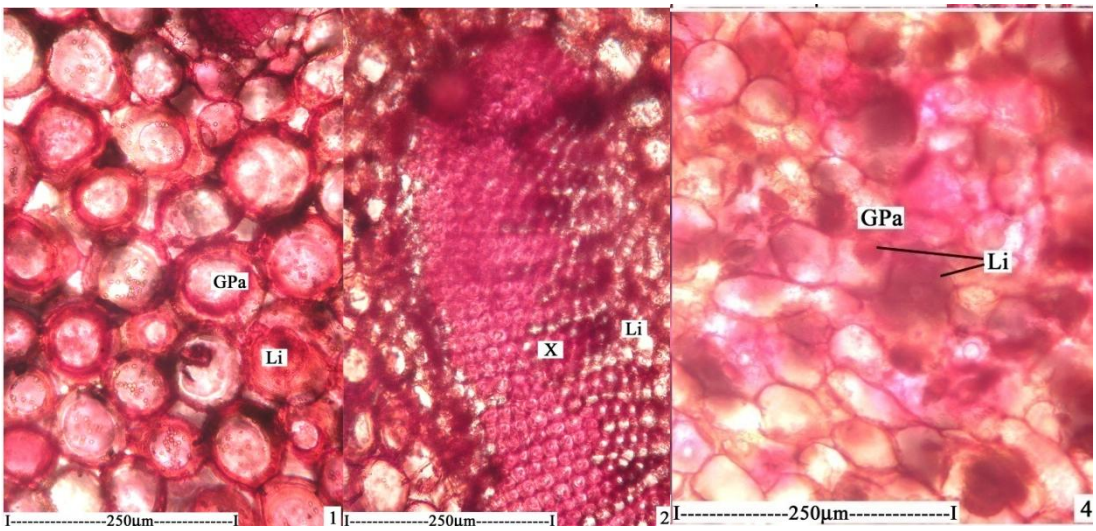


Fig.22.1 & 2, 4

1. Ground parenchyma cells of the petiole having lipids.

2. Some of the cells of the vascular bundle with lipids.

4. Ground parenchyma cells of the rhizome with lipids

(GPa: Ground Parenchyma; Li: Lipid; X: Xylem)

(ii) PHENOL

Toluidine blue turns phenol dark blue. Phenols were found in the ground tissue of the petiole, xylem parenchyma and phloem parenchyma of rhizome. (Fig. 23.1 & 2).

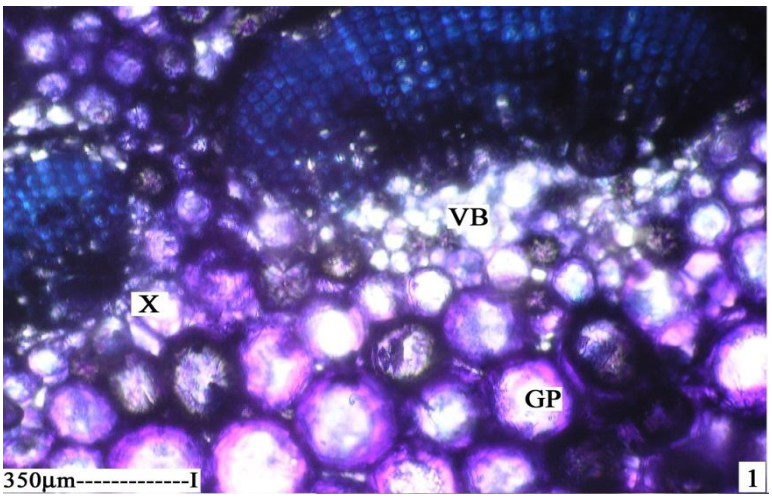


Fig.23.1: Petiole with phenol deposition in the ground tissue and xylem elements.

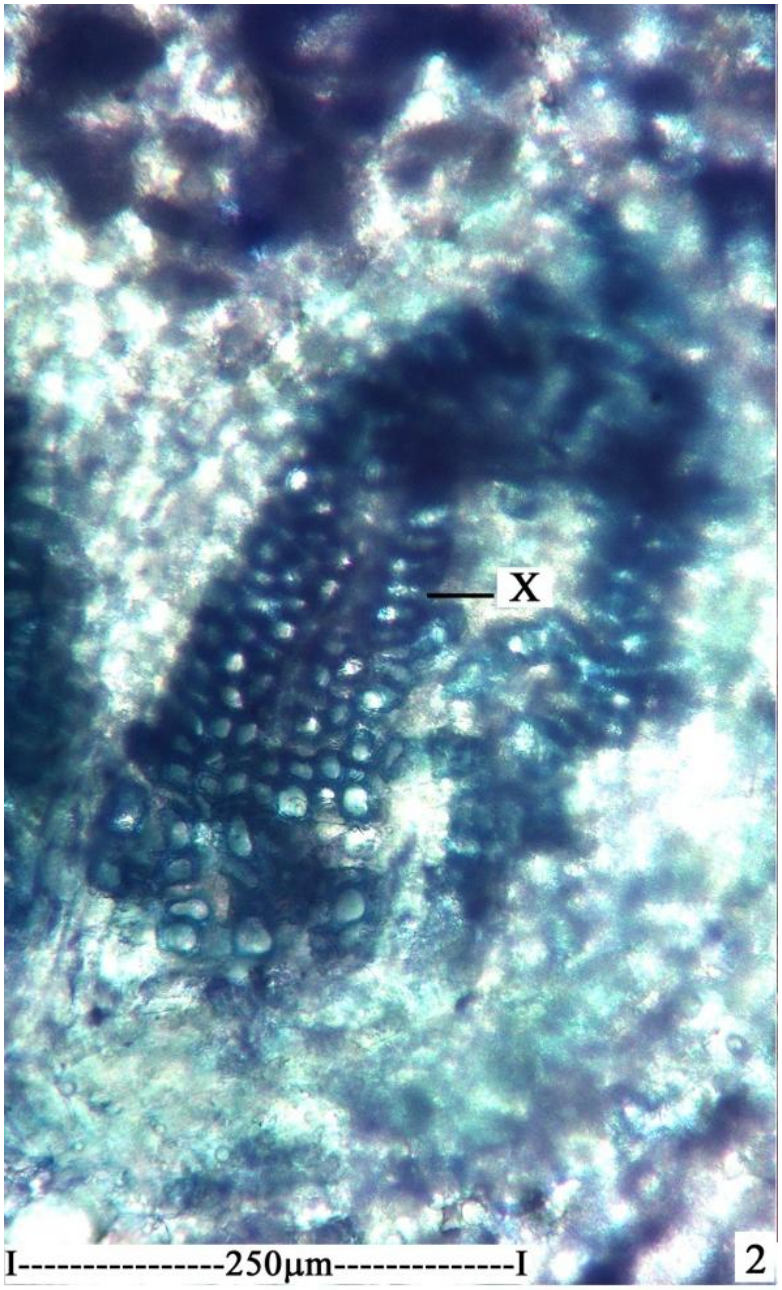


Fig.23.2: Phenol located in the xylem elements and cortical cells.

(GP: Ground Parenchyma; VB: Vascular Bundle; X: Xylem)

(iii) FLAVONOIDS

Picric acid indicates the presence of flavonoids by turning flavonoid into yellow. Yellow substances of flavonoids are found in the pith cells of the rhizome (Fig.24.3).

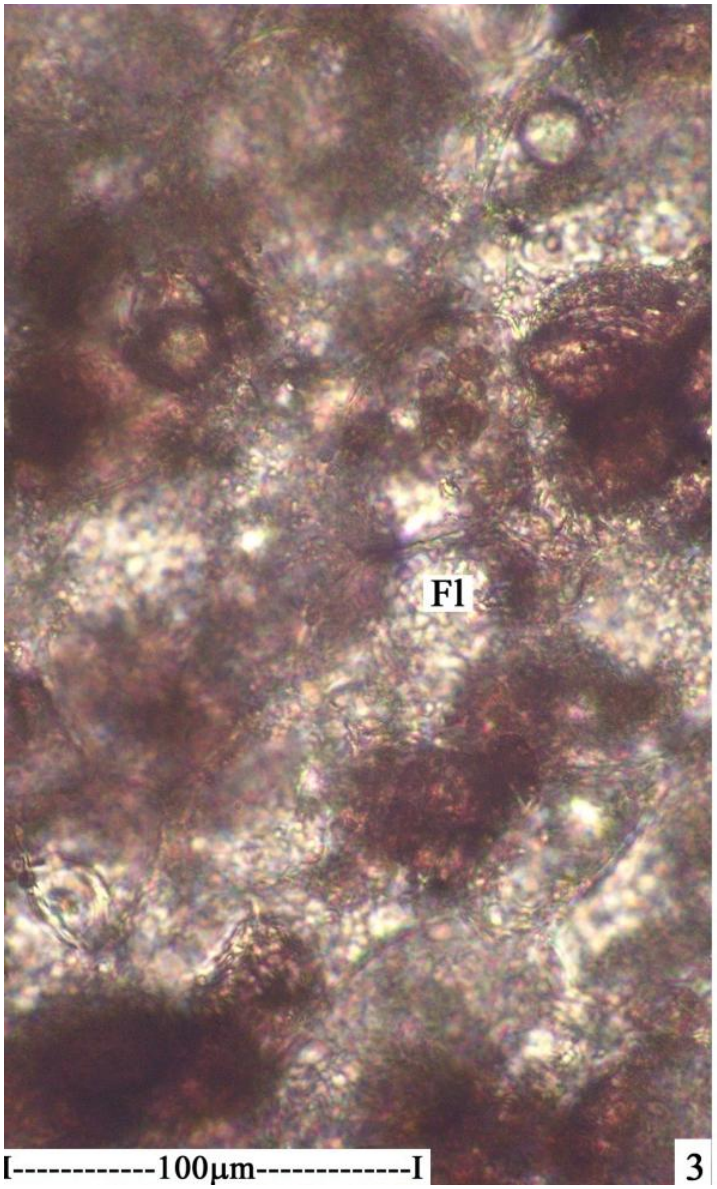


Fig.24.3: Picric acid stained for localization of flavonoids. Rhizome, pith, ground parenchyma cells having flavonoids.

(Fl: Flavonoid)

(iv) LIGNIN

Phloroglucinol stains lignified cells which turn into red. In the petiole and in the rhizome the xylem elements and xylem fibres become red when stained with phloroglucinol (Fig.25.2).

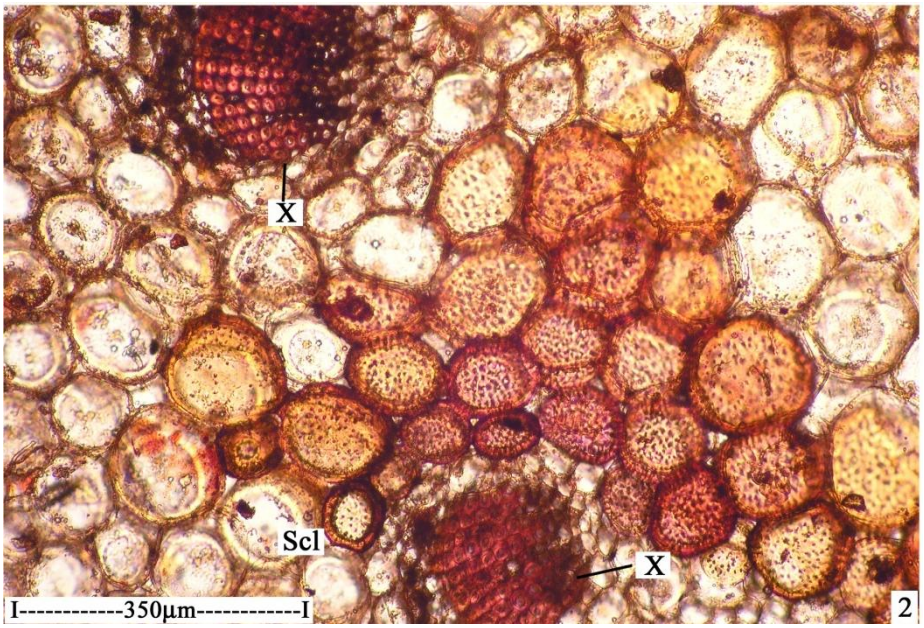


Fig.25.2: Petiole with xylem strand and pitted sclerenchyma cells in the ground tissue having lignified walls.

(Scl: Sclerenchyma; X: Xylem)

(v) STARCH

IKI (Iodine Potassium Iodide) stains starch grains either black or blue. Starch grains were localized in the parenchyma cells of the rhizome (Fig.26.1).

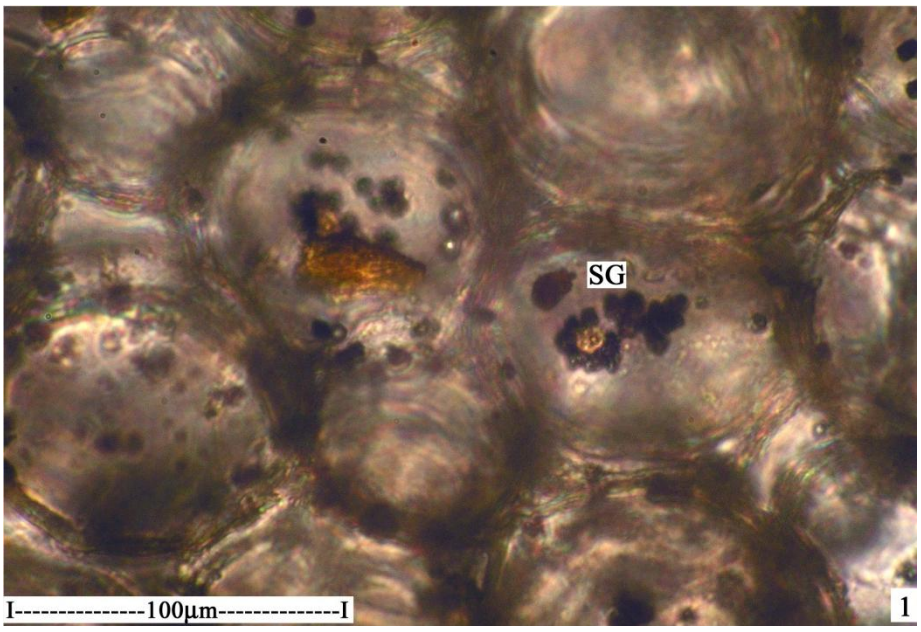


Fig.26.1: Spherical starch grain bodies located in the ground parenchyma cells.

(SG: Starch Grain)

(iv) PROTEIN

CBB (Coomassie Brilliant Blue) stains protein dark blue. Proteins have accumulated in the parenchyma cells of the petiole (Fig.27.1).

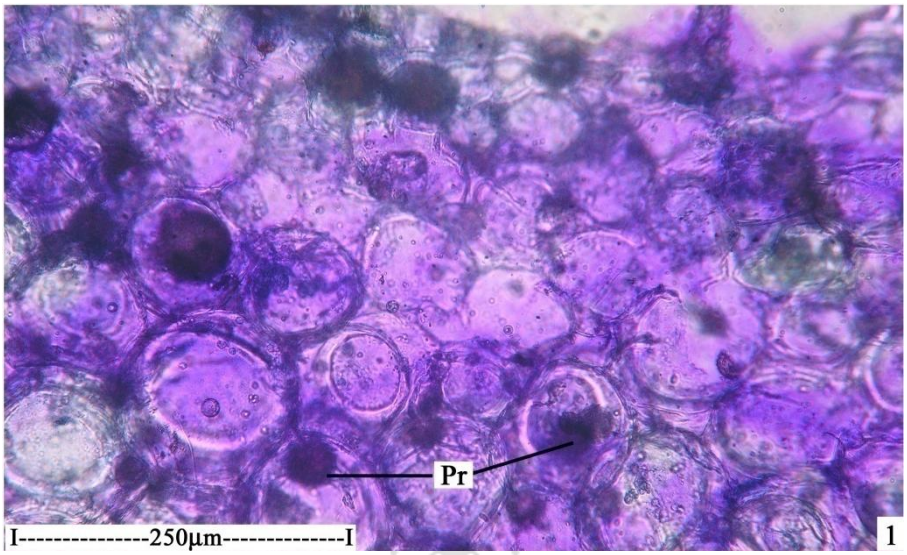


Fig.27.1: T.S. of petiole stained with CBB to shows presence of protein in the ground parenchyma.

(Pr: Protein)

DISCUSSION

Didymocarpus gambleanus is a scapigerous herb as in the other species of *Didymocarpus*. The petioles of the leaf are either winged or wingless. The adaxial surface of the lamina has blister like raised spots. The abaxial surface of the leaf is densely tomentose. The anatomical feature of the leaf includes planoconvex midrib with abaxial fan shaped layer of discrete vascular bundles and some smaller bundles on the adaxial surface. The stomata are cyclocytic type and lamina is amphistomatic. The petiole is planoconvex and multistranded, the vascular bundle being distributed in distinct pattern. The leaf trichomes are of two types. Some of them being thin very long thread like. The other types of trichomes are multicellular, uniseriate and branched, non glandular and covering type. The rhizome is thick and erect with hollow cylinder of vascular tissues and central and cortical parenchyma. The vessel elements

are short, wide and cylindrical with elliptical, multiseriate, dense bordered lateral wall pits. The end wall perforations are circular, wide either horizontal or oblique.

The relevance of anatomical parameters of plants especially medicinal plants have been given importance in diagnosis of samples without reproductive parts. Anatomy also plays a crucial role in taxonomy, phylogeny and interrelationships of plants (Metcalfe & Chalk, 1957). The vascular system of the midrib is one of the reliable criteria for diagnosis of different species of the genus. In ***Didymocarpus gambleanus*** the midrib has a larger, lower vascular bundle and a smaller, upper vascular bundle. In the other species of ***Didymocarpus,*** the midrib has multistranded vascular system. The size of the midrib of ***Didymocarpus gambleanus*** is 700µm x 800µm. In other species where the midrib has multistranded vascular system, the size of the midrib is very low. This fact tends to believe that size of the midrib is directly related to the reduction in the number of vascular bundle. The epidermal cells walls of the leaf in ***Didymocarpus gambleanus*** are thin and straight; no outgrowths are seen on the cell wall. In ***Didymocarpus humboldtianus*** the epidermal cells of the leaf possess peg like outgrowths. The stomatal type is said to be anisocytic type (Metcalfe & Chalk 1957) and actinocytic in ***D.pedicellatus***. But in ***Didymocarpus gambleanus*** we observed cyclocytic type of stomata. Petiolar vasculature has considerable taxonomic importance because the structure of petiole is little affected by the environmental stresses. Howard (1979) has shown that the petiolar anatomy may complement other anatomical characters of the plants to propose diagnostic key of genera and families of dicotyledons. Solereder (1908) and Metcalfe & Chalk (1957) have given brief account of vascular patterns of the petiole in some members of Gesneriaceae. They classified the pattern of petiolar vasculature into seven categories. In these categories, there is an abaxial single vascular strand and many scattered adaxial vascular strands. In ***D.gambleanus*** the petiole has multistranded vascular system and the vascular bundles are concentric and amphivasal type. The parenchymatous ground tissue of the petiole consists of circular sclerides with dense simple pits. So the petiolar anatomy of ***D.gambleanus*** differs in certain aspects from other taxa of ***Didymocarpus***. In the rhizome, the vessel elements of the vascular cylinder are narrow, not differing much from the neighbouring fibres. In other species, the xylem consists of wide vessels which are easily distinguishable from the fibres. The thin narrow epidermal trichomes of ***D.gambleanus*** is specific for this taxon. Such types of trichomes are not recorded in other species of ***Didymocarpus***. Histochemical study of ***D. gambleanus*** has shown the localization of lipids,

phenol, flavonoids, tannin, starch, protein and lignin mostly in the petiole and sometimes in the rhizome.

The results of the present study have provided certain anatomical features specific for and different from other species of ***Didymocarpus***. These specific features are reliable for diagnosis of ***D.gambleanus*** and to segregate the sample from its adulterants.

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