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Pharmacognostic Standardisation of *Didymocarpus humboldtianus Gard.* (Gesneriaceae)

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ABSTRACT

Didymocarpus humboldtianus Gard. was collected from the hills of W. Ghats of Tirunelveli, Tamilnadu. It is a scapigerous shrub, inhabiting moist crevices of rocks. *D. humboldtianus* being a co-species of *D. pedicellata* was believed to have the medicinal properties of *D. pedicellata*, which has been investigated by many investigators earlier, studies on *D. humboldtianus* are meagre and so the present study was carried out. This study deals with the microscopic analysis of all parts of *Didymocarpus humboldtianus.* The investigation provides the structural profile of leaf, midrib, petiole, rhizome and root which are found to be specific for this plant. The results are discussed critically in this study for proposing anatomical protocol for taxonomic diagnosis of *D. humboldtianus.*

INTRODUCTION

Didymocarpus is one of the interesting elements pervading the Western Ghats of Tirunelveli area. The taxon is not only interesting for its floral and vegetative morphology but also for its pharmaceutical potentials. *D. pedicellatus* one of the species of the genus *Didymocarpus* which has been more intensively studied for its biochemical aspects. It has been established that this species is a promising plant for several human ailments, particularly for treating the kidney stones (Kapoor & Kapoor, 1976; Sharma *et al.*, 1979; Methela *et al.*, 1980; Jain, 1991; Samant & Palni, 2000). *D. humboldtianus* Gard. is one of the species widespread in the W. Ghats. Per view of literature showed that this taxon has not been given due accent for its pharmacognostical and pharmacological aspects. Being a Co-species of other medicinally well recognized members of Gesneriaceae, an attempt is made to study the pharmacognostic aspects of *D. humboldtianus* Gard. Pharmacognosy is the study of botanical and preliminary phytochemical studies which provides the protocol characters for botanical identity of a herbal drug. Since different species of *Didymocarpus* simulates each other in external features, microscopic features are believed to offer helping hand to differentiate and identify the species for further studies. Furthermore, it is aimed to scientifically validate the folklore claims of medicinal values of the species.

Review of Literature

There are about 100 papers published on different species of *Didymocarpus*, especially on *D. pedicellatus*. Virtually no work has been carried out on *D.humboldtianus*. This fact prompted us to proceed with the pharmacognostical, preliminary photochemical and antimicrobial studies of the species.

MATERIALS AND METHODS

Specimens for the present study were collected from Kidavettipari, a Kani settlement area in the Western Ghats. The plant samples were fixed in a mixture of Formalin + Acetic acid + 70% Ethanol (5ml + 5ml + 90ml). Dehydration of the samples and paraffin wax infiltration and embedding of the specimens in paraffin wax were carried out as per procedure of Sass (1940). Serial sections to the thickness of 10µm were prepared with the help of Rotary Microtome. Dewaxed sections were stained with 0.025% aqueous Toluidine blue metachromatic stain as proposed by O'Brien *et al.*, (1964).

For venation pattern of the lamina, small squarish fragments of the lamina were warmed in alcohol to remove the chlorophyll followed by immersion of the specimen in warm 5%

sodium hydroxide for one or two days. After total clearing, the specimens were washed thoroughly in distilled water and stained with 0.5% safranin. The stained samples were mounted in glycerin for microscopic study.

For the study stomatal morphology, fragments of lamina were embedded in paraffin wax and paradermal sections were prepared. Stained sections were observed under the microscope.

Microscopic photographs:

Prepared slides were photographed under Nikon trinocular microscope and Nikon digital camera in different magnification. Calcium oxalate crystals, starch grains, lignin content were studied under polarized light since these substances possess birefringent property. Magnifications of the figures were indicated by magnification scales.

Powder preparation of the plant was observed under microscope to identify different elements in the powder. Histochemical observations were carried to localize compounds such as lipids, (Neutral Red, Sudan III), Starch grains (IKI), phenol (Toluidine blue), flavonoids (Picric acid), Tannin (Ferric chloride), Lignin (Phloroglucinol) and protein (CBB). Physico – chemical analysis, fluorescence analysis and qualitative analysis of various compounds were subjected to study.

Observation

Didymocarpus humboldtianus Gard is a scapigerous shrub and it inhabits the moist crevices of rocks. The shoot is modified into short thick, erect succulent rhizomatous stock. The leaves arise from upper portion of rhizome and roots from the lower portion. The inflorescence originates from the axils of the leaves (Fig. 1; 2).

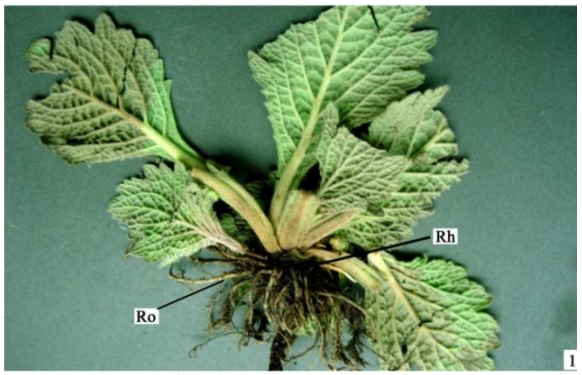

Fig. 1. An old shoot with dense bunch of adventitious roots arising from the rhizome and old leaves situated at the top of the rhizome. (Rh: Rhizome; Ro: Root)

Fig.2. A flowering shoot with central sessile young leaves and outer petiolate older leaves. The Inflorescence arises from the short reduced rhizome. (Fl: Flower; St: Stalk)

The leaves are ovate-lanceolate, thick, fleshy and soft. The petiole is long with decurrent wings. The leaf margins are deeply cut into elliptical serrate lobes. The adaxial surface of the leaf exhibits narrow blister like appearance. The abaxial surface exhibits thick prominently raised veins and deep furrows of inter coastal regions (Fig. 3; 4).

Fig.3. A leaf in abaxial view (left) and a leaf with axillary bud in adaxial view (right) (AB: Axillary Bud; Pe: Petiole)

Fig.4. Abaxial view of the lamina showing thick raised midrib, lateral veins and veinlets. (Vlt: Vein Islet; LV: Lateral Vein; MR: Midrib)

Inflorescence is a dichotomous cyme bearing fruits at the lower part and flowers at the apex (Fig.5). The flowers are pinkish purple. The corolla is gamopetalous and bilobed, with 2+3 lobes. The corolla is densely tomentose (Fig.6). The flower has two epipetalous stamens; the anthers are united with each other (Fig.7). The pollen grains are triangular in polar view and triporate. Exine is smooth. The pollen grains are 20μm in diameter (Fig. 8)

Fig.5. A flowering shoot with central sessile young leaves and outer petiolate older leaves. The Inflorescence arises from the short reduced rhizome. (Fl: Flower; St: Stalk)

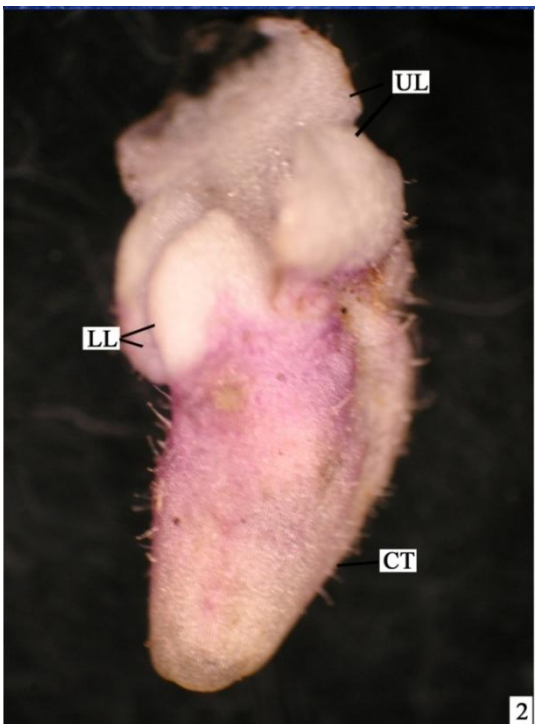

Fig.6. A two lipped corolla with corolla tube, two lateral lips and three upper lips. (CT: Corolla Tube; LL: Lower Lip; UL: Upper Lip)

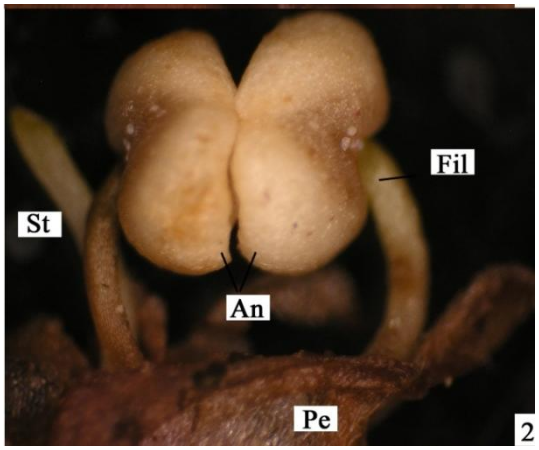

Fig. 7. Two anthers connate with each other forming syngenesious anthers. (An : Anther; Fil : Filament; Pe : Petiole; St : Stigma)

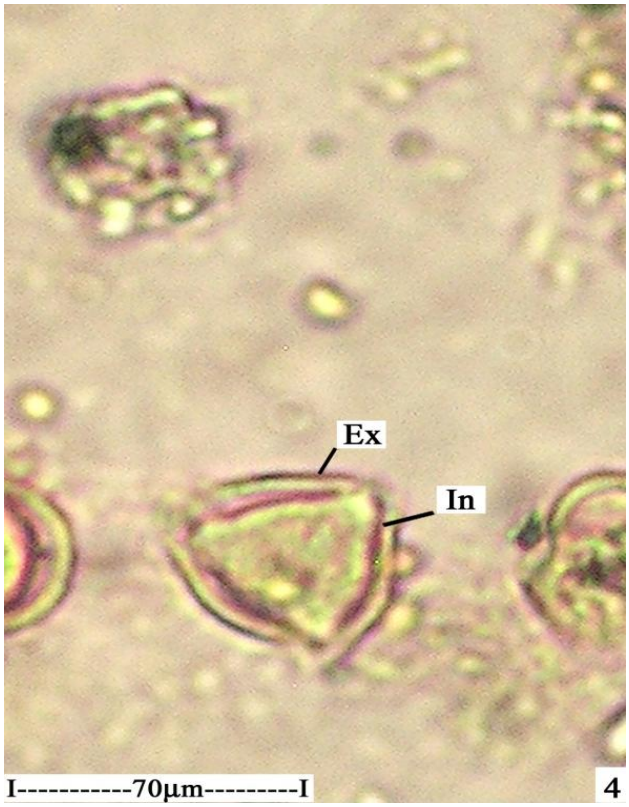

Fig. 8. Pollen grains acetolysed (Ex: Exine; In: Intine)

Fruit is a cylindrical capsule, dehiscing vertically along the sutures. The seeds are dark brown, ovoid with narrow micropylar end. The seed coat exhibits deeply reticulate ridges (Fig. 9).

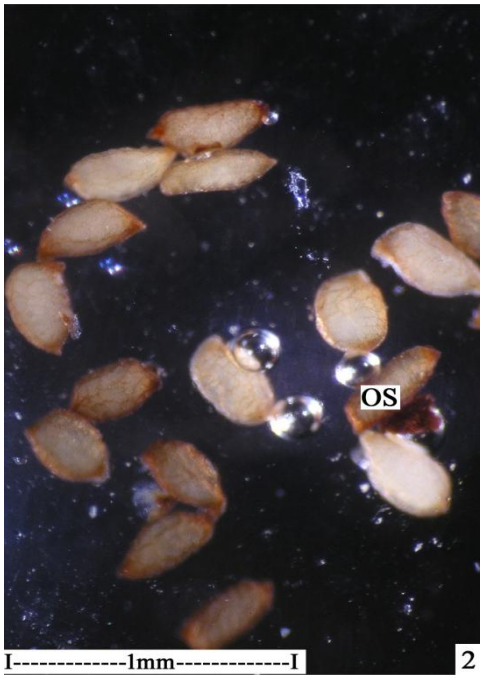

Fig.9. Seeds showing elliptical ovoid shape (OS: Ovoid Shape)

Microscopic features

Trichomes Both non-glandular and glandular trichomes are abundant on the abaxial surface of the lamina. The glands have spherical head and short stalk (Fig.10). The head is multicellular, compact and darkly stained. The stalk is one or two celled. Non-glandular trichomes are multicellular, uniseriate and unbranched. They have thick walls and wide lumen (Fig.11).

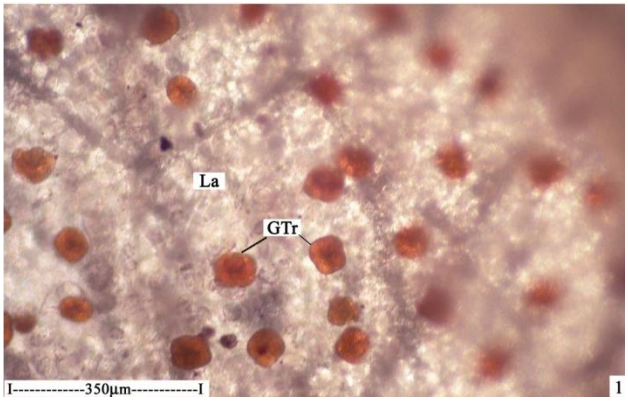

Fig.10. Abaxial surface view of the lamina showing distribution of the glandular trichome. (GTr: Glandular Trichome; La: Lamina)

Fig.11. Nonglandular epidermal trichomes from the lamina

Leaf in sectional view shows ridged–furrowed lamina and thick plano-convex midrib (Fig.12). The midrib is flat on the adaxial side and thick semicircular on the abaxial part. The midrib is 1.5mm thick and 1.4mm wide. The epidermal cells on the adaxial side of the midrib are thick and dilated and the cells are thin walled. The abaxial epidermis has narrow thin walled cells, the epidermis is stomatiferous. The stomata are of Cyclocytic type with one or two circles of subsidiary cells, each circle comprising seven or eight cells (Fig.13). The adaxial epidermal cells have prominent nodules on the anticlinal walls (Fig.14).

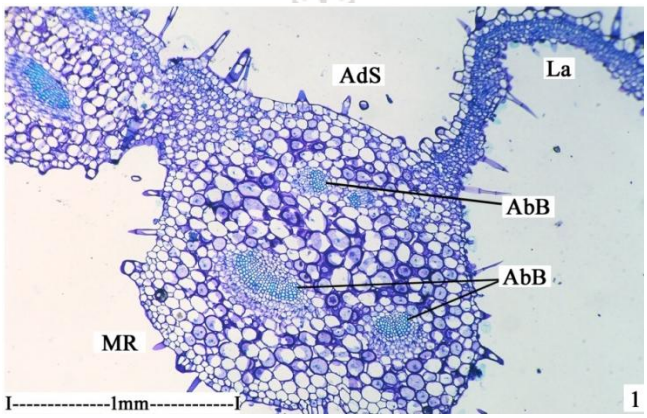

Fig.12. T.S. of leaf passing through lamina and midrib (AbB: Abaxial Bundle; Ads: Adaxial side; La: Lamina; MR: Midrib)

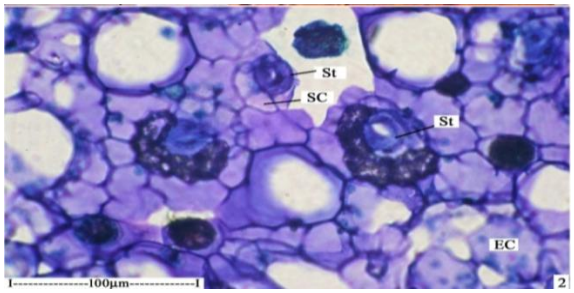

Fig.13. Paradermal section of the abaxial epidermis showing cyclocytic stomata. (EC: Epidermal Cell; SC; Subsidiary Cell; St: Stomata)

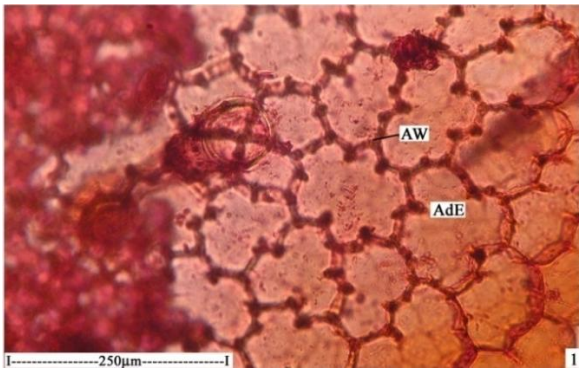

Fig.14. Paradermal section of the adaxial epidermis in surface view (AdE: Adaxial Epidermis; AW: Anticlinal Wall)

Venation pattern (Fig.15) The lamina has reticulate venation with wide vein – islets. The vein-terminations are less and occur only in certain islets. The terminations are simple or forked once.

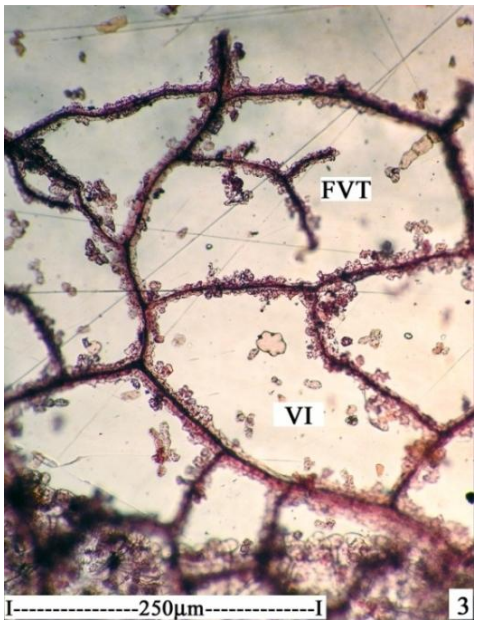

Fig.15. Bifid forked vein termination. (FVT: Forked Vein Termination; VI: Vein Islets)

Petiole (Fig.16): The petiole is flat on the adaxial side and broadly semi-circular on the abaxial side. It consists of dense epidermal trichomes, thin epidermis, wide homogeneous parenchymatous ground tissue and discrete vascular bundles (Fig.16). There are about nine, large and small collateral vascular bundles arranged in the form of wide hollow semicircular line.

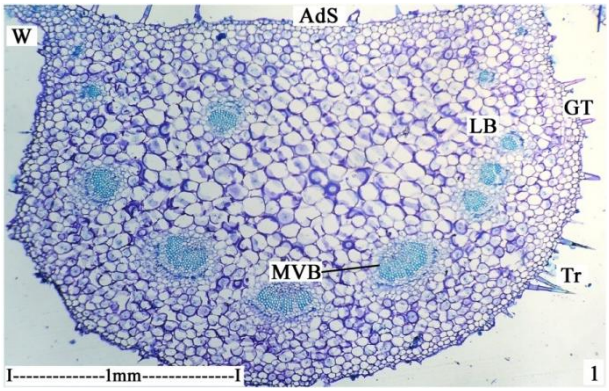

Fig.16. T.S. of petiole entire view (Ads: Adaxial Side; GT: Ground Tissue; LB: Lateral Bundle; MVB: Middle Vascular Bundle; Tr: Trichome; W: Wing)

Rhizome (Fig.17): The rhizome is thick and fleshy. The epidermis is replaced by incipient periderm. Major portions of the rhizome includes cortex and pith which are parenchymatous. The secondary xylem and secondary phloem form a cylinder enclosing central pith. The leaf traces originate from the upper portion of the rhizome while the root traces originate from the lower portion. The vascular cylinder is often broken and distorted due to the proliferation of the parenchyma cells of the rhizome. Calcium oxalate crystals are abundant in the parenchyma cells of the rhizome. The crystals are of rosette type. They are circular plates with central dark core of organic substance and outer zone of calcium oxalate crystals (Fig.18) These crystals are Rosette type and they are 50µm in diameter.

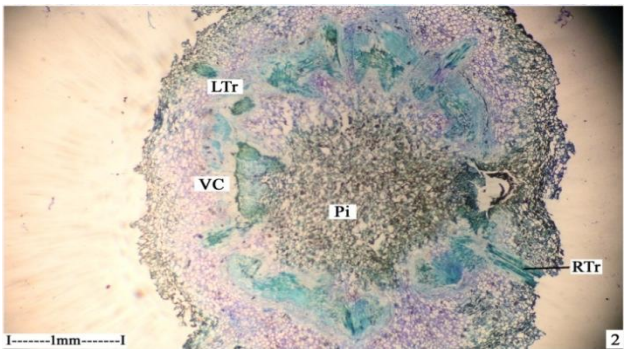

Fig.17. T.S. of upper portion of the rhizome showing leaf gaps and leaf traces. (LTr: Lateral root; Pi: Pith; RTr: Root Trace; VC: Vascular Cylinder)

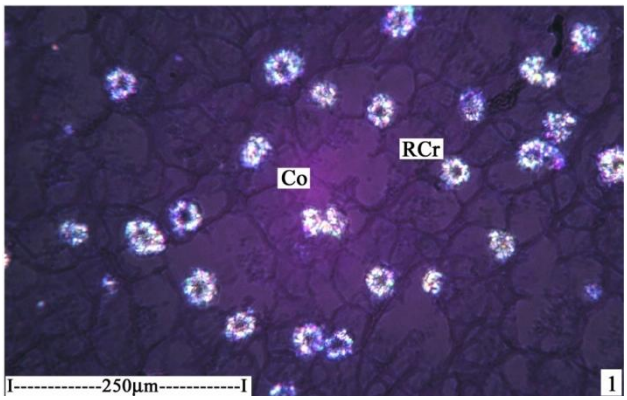

Fig.18. Rosette type of calcium oxalate crystals distributed in the cortical cells of the rhizome. (Co:Cortex; RCr : Rosette Crystal)

Root (Fig.19): Thick root measuring 750µm in diameter was studied. The epidermal layer of the root is disintegrated and is replaced by a thin periderm. The cortical zone is much thicker measuring 150 -250µm in diameter. The cortical cells are elliptical in shape and are arranged in regular concentric layers. The vascular cylinder is thick and dense and is 230µm thick. It includes a thin layer of secondary phloem ensheathing secondary xylem cylinder. Secondary xylem consists of angular, thick walled, solitary diffuse vessels and narrow lignified fibers. The vessels are 15-20µm in diameter.

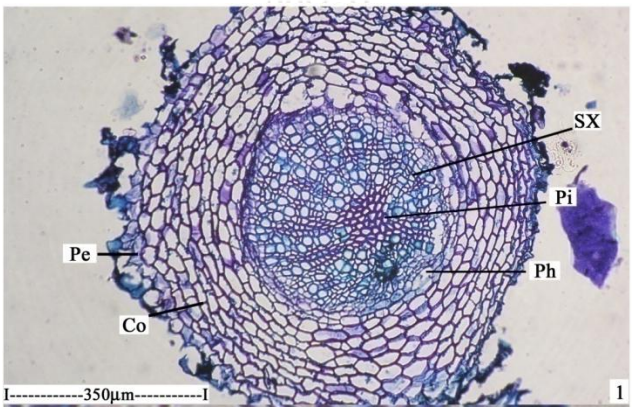

Fig.19. T.S. of root - entire. (Co: Cortex; Pe : Periderm; Ph : Phloem; Pi : Pith; SX : Secondary Xylem)

Powder Microscopic Observation

Powder preparation of the plant was examined under the microscope and the following major components were observed.

(i) Non-glandular epidermal trichomes (Fig.20):-

Covering type of epidermal trichomes are abundant in the powder. They are 4-8 celled, uniseriated and unbranched. The cell walls are thick and the cell lumen is wide. The trichomes are 200 - 750µm long.

(Fig. 20. Non-glandular epidermal trichomes from the lamina. (CL: Cell Lumen; Se: Septum)

(ii) Glandular trichomes (Fig. 21):-

Peltate type of glandular trichomes are common in the powder. They have four – celled, darkly stained secretory head and short, one or two celled stalk. The head measures 35-40µm in diameter.

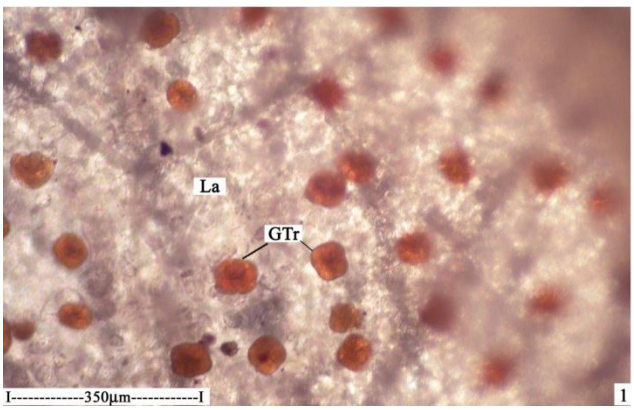

Fig. 21. Abaxial surface view of the lamina showing distribution of the glandular trichome. (GTr: Glandular Trichome; La: Lamina)

(ii) Fibres (Fig. 22 & 23):-

Narrow, long, thick walled fibers are frequently seen. Their walls are lignified and the cell lumen is narrow. Their ends are tapering. The fibers are 550µm long and 30µm wide.

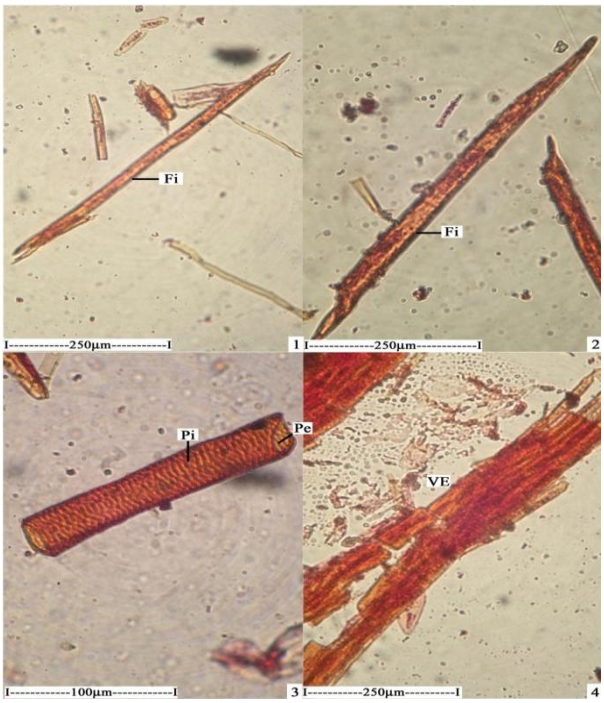

Fig.22.1 & 2. Xylem fibers were seen in the powder. 3. A single vessel element. 4. A cylindrical bundle of vessel elements. (Fi:Fibre; Pe:Perforation; Pi:Pits; VE:Vessel Elements)

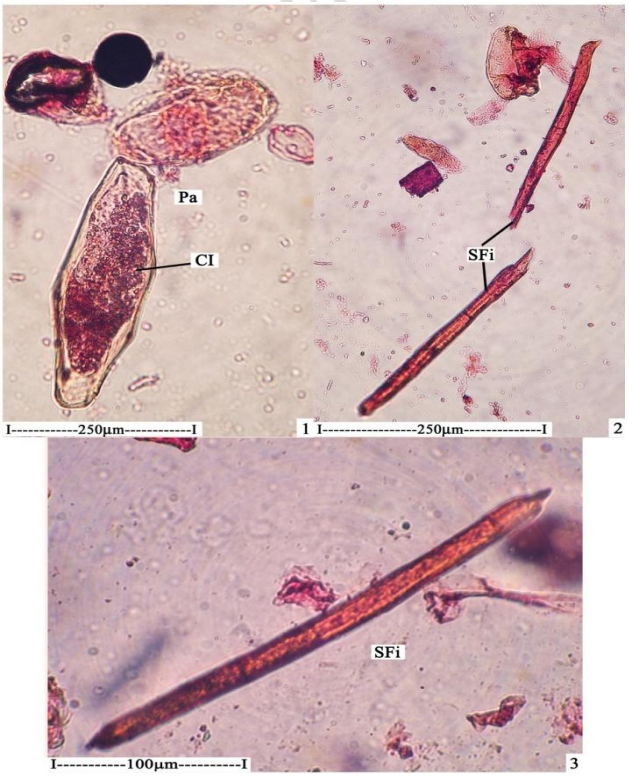

Fig.23.1. A single parenchyma cell showing starch grains in the cell. 2. Septate fibers 3. A septate fiber –Enlarged. (CI: Cell Inclusion; Pa: Parenchyma; SFi: SeptateFibre)

(iv) Vessel elements (Fig. 24):-

The vessel elements are narrow, long and cylindrical. They have wide, circular horizontal perforations at the end walls and transversely elongated, multiseriate bordered pits. The vessel elements are 100µm long.

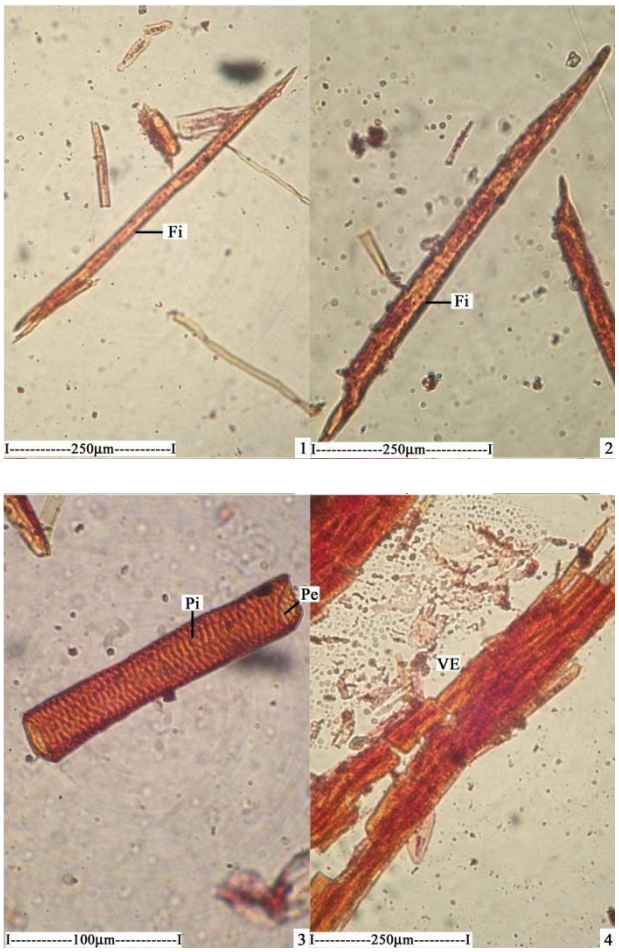

Fig.24.1 & 2. Xylem fibers were seen in the powder. 3. A single vessel element. 4. A cylindrical bundle of vessel elements. (Fi:Fibre; Pe:Perforation; Pi:Pits; VE:Vessel Elements)

(v) Parenchyma cells (Fig.25):-

Wide, elliptical elongated parenchyma cells are common in the powder. The ends are conical. The cells are septate or nonseptate. They have dense, darkly staining cell inclusions. The parenchyma cells are 250µm long and 30µm wide.

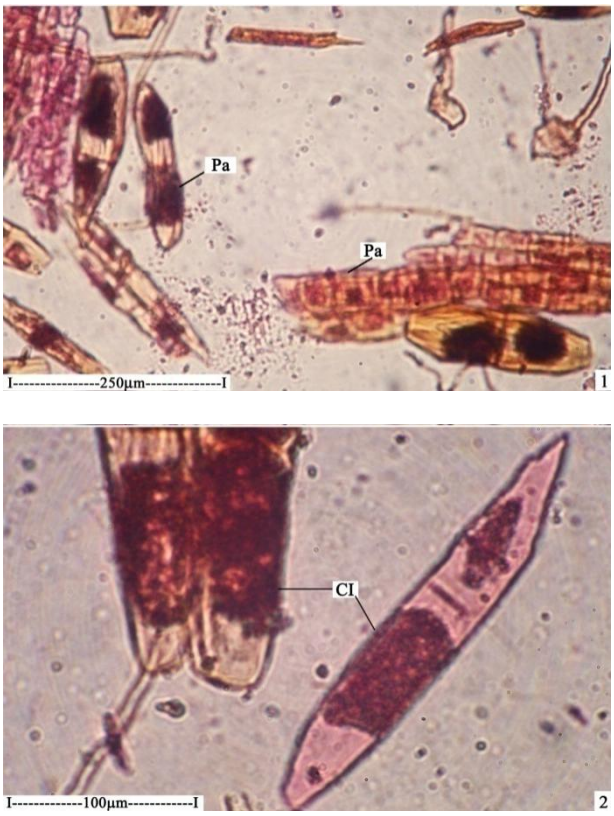

Fig.25.1. Isolated parenchymal cells of the root powder. 2. Parenchyma cells enlarged showing cell inclusions. (CI: Cell Inclusions; Pa: Parenchyma)

Histochemical studies:-

Localization of some ergastic substances and cell inclusions were carried out employing specific stains. The cell inclusions and the stains are given below:

(i) Lipids:-

Neutral Red and Sudan III were used to stain the lipid which turns red on staining. Lipid was found localized in the parenchyma cells of the midrib, petiole (Fig.26), cortical cells of the root and rhizome (Fig.27, Fig.28) glandular trichomes (Fig.29)

Fig.26. Palisade cells with lipids (Li: Lipid)

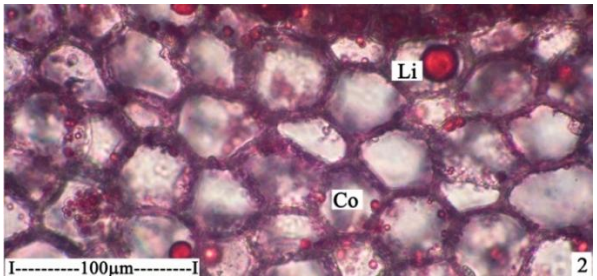

Fig.27. Lipids in the cortex of the root - enlarged. (Co:Cortex; Li:Lipid)

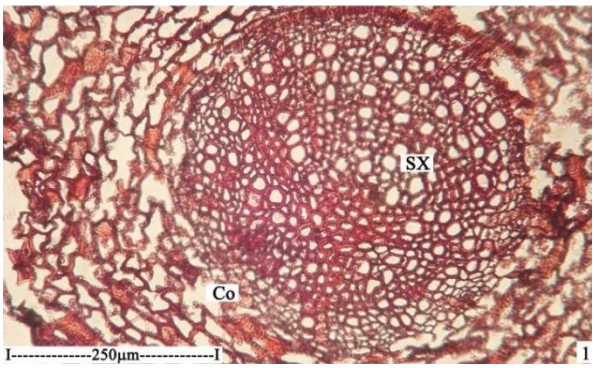

Fig.28. Neutral Red employed for the localization of lipids. 1. T.S. of root having lipid in the cortical parenchyma and some of the xylem elements. (Co: Cortex; SX: Secondary Xylem)

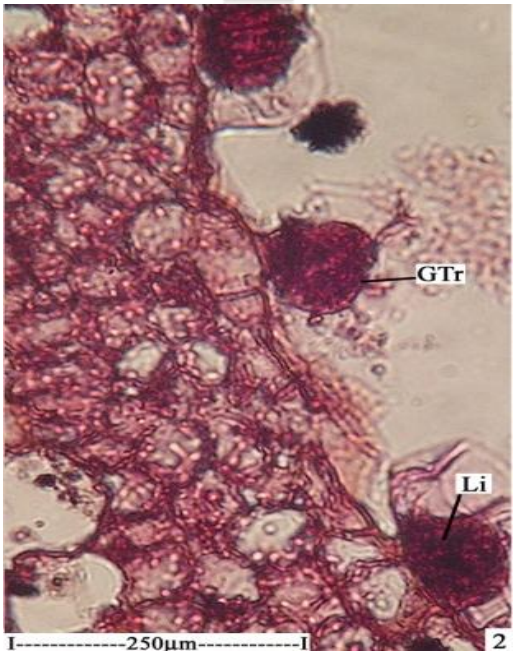

Fig.29. Neutral red employed for the localization of lipids 2. Glandular trichomes of the leaves having lipids. (GTr: Glandular Trichome; Li: Lipid)

(ii) Phenols :-

Toluidine blue was used to stain the phenol. The phenol contents turn blue on staining. Root hairs and per**iderm cells of the rhizome showed phenol accumulation (Fig.30).**

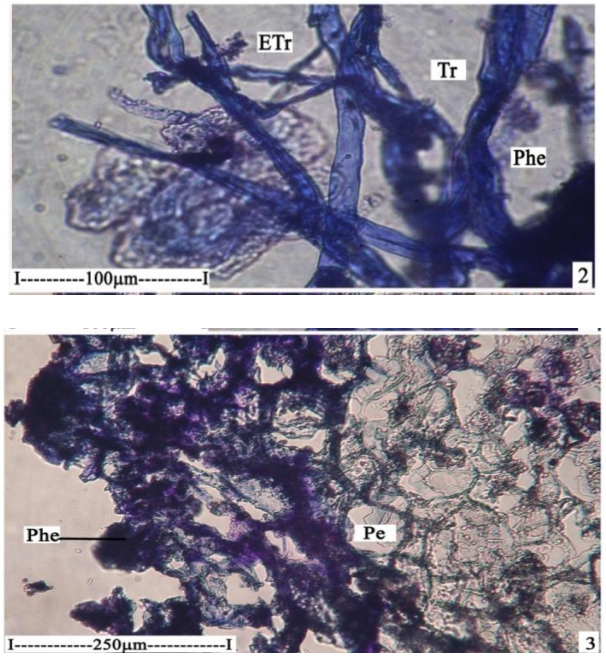

Fig.30. Toluidine Blue for phenol. 2. Root hairs with phenol- Enlarged. 3. In the rhizome, periderm cells are stained dark blue showing phenol content. (ETr: Epidermal Trichome; Pe: Periderm; Phe: Phenol; Tr: Trichome)

(iii) Flavonoids:-

Picric acid stains flavonoids yellow. Epidermal cells of the midrib, petiole and secondary phloem of root showed presence of flavonoids (Fig.31).

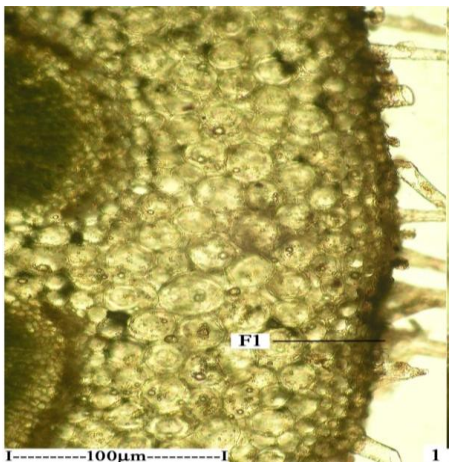

Fig. 31.Picric acid used for localizing flavonoids. 1. In the midrib of the leaf, the epidermal and subepidermal layers stain yellow showing presence of flavonoid. (Fl: Flavonoid)

(iv) Tannin :-

Ferric chloride is specific to localize tannin. Tannin turns black or dark brown. Outer cortex of the root and ground parenchyma of the rhizome possess tannin. (Fig.32)

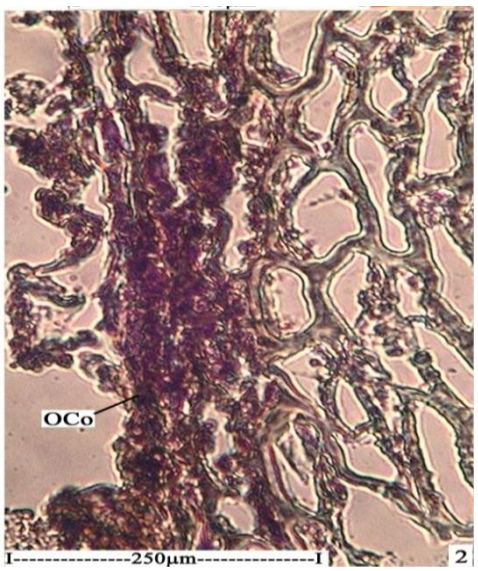

Fig.32. Ferric chloride employed for localization of Tannin. 2. In the root periderm, cells exhibit storage of tannin. (OCo: Outer Cortex)

(v) Lignin :-

Phloroglucinol + dilute HCl stain lignin bright red. Xylem elements, sclereids and fibers in all organs of the plant exhibit lignified cells (Fig.33).

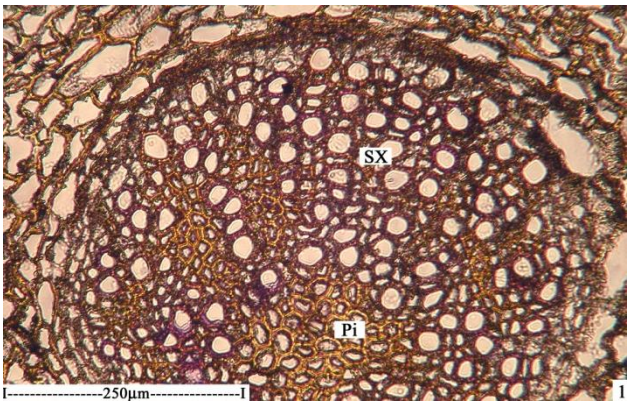

Fig.33.Phloroglucinol for lignin. 1. In the root, xylem elements possess reddish lignified walls. (Pi: Pith; SX: Secondary Xylem)

(vi) Starch

IKI stains starch grains black, starch grains are accumulated in the ground parenchyma of midrib and petiole (Fig.34).

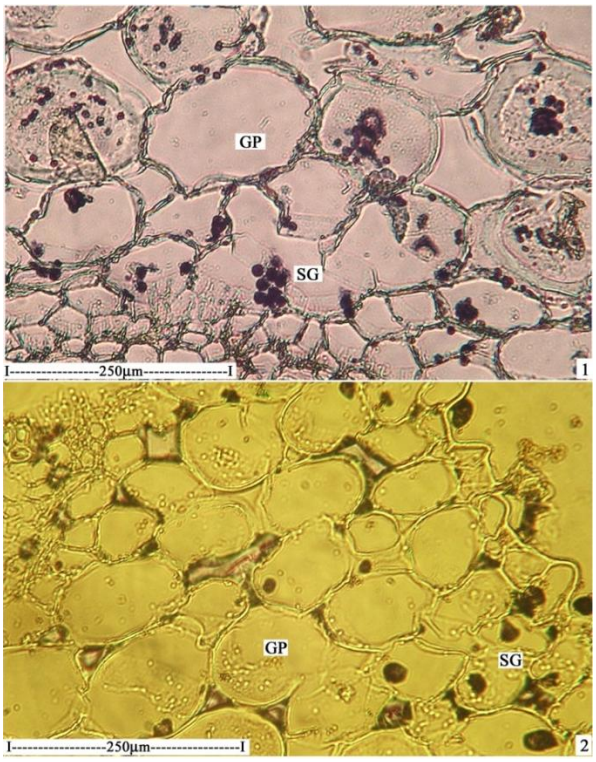

Fig.34.1 & 2. IKI used for staining the starch grains. 1. In the leaf, some of the cells of the midrib have prominent starch grains. 2. In the petiole, starch grains were found in the peripheral cells of the ground parenchyma. (GP: Ground Parenchyma; SG: Starch Grains)

(vii) Protein

Coomassie Brilliant Blue (CBB) stains protein blue. Protein occurs in the petiole, cortex of root and phloem parenchyma cells of the rhizome (Fig. 35).

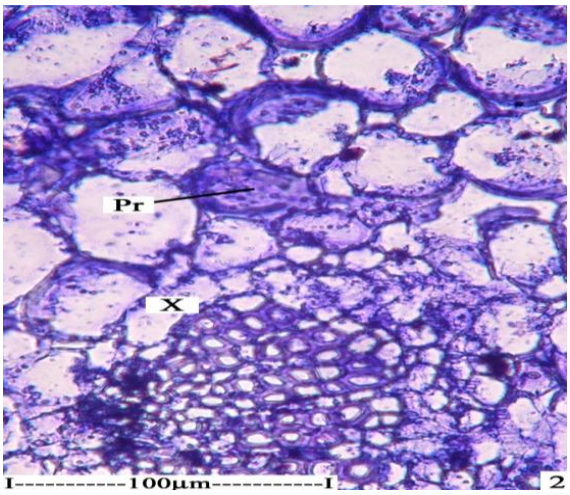

Fig.35. CBB for Protein. 2. In the rhizome, cortical parenchyma cells possess protein bodies. (Pr: Protein; X: Xylem)

DISCUSSION

Didymocarpus is much valued for its enchansing floral appearance and for its hidden pharmacological potentials. Albeit its importance as medicinal properties, the taxon remains unused by the herbal botanists. About 12 species of *Didymocarpus* have been reported to be widespread in the Western Ghats. *D.pedicellatus* has been studied fairly in detail exploring its biochemical compounds and their biological activities. Studies on other available species of *Didymocarpus* remain unexplored for its pharmacognostical and biological studies. Reliable folklore claims of Kani tribals of the Kedavetti parai of the W.Ghats of Tamil Nadu have attributed *Didymocarpus* spp. For certain human ailments.

Didymocarpus humboldtianus is found to be an important herbal used for certain human diseases and it warrants investigation of this taxon. Pharmacognostical studies were undertaken as a preliminary step for botanical identification of the plant.

The leaves of *D. humboldtianus* are ovate-lanceolate with deeply cut elliptical lobes of the margin. The leaf base is decurrent up to the base of the petiole. This foliar character can be considered as one of the reliable diagnostic clues for the identification of the species (Fig.3).

Palynology is one of the valid aspects of botany, especially in systematic study. In *D. humboldtianus*, the pollen grains are triangular in polar view and triporate (Fig.13) In other species studied, the pollen grains are circular and triporate.

Anatomy of the midrib and petiole offers highly reliable diagnostic clues and also solve some systematic and phylogenetic problems. Howard (1979) has shown that it is possible to use a combination characters to create a key to sterile materials of local flora. In the extensive study of petiolar anatomy, Howard (1979) has emphasized the anatomy of petiole is much reliable aspect of study in diagnostic purpose. In *D. humboldtianus*, the midrib has two larger vascular bundles in the abaxial part and two smaller bundles in the adaxial part (Fig.12). The petiole has 9 or 10 vascular bundles arranged in a wide arc (Fig 16). This vascular pattern of the midrib and petiole seem to be specific for this taxon not shared by other species. The midrib and petiolar vasculature may be of considerable help in the identification of crude drug.

The stomatal morphology and epidermal cell structure are yet another set of features of taxonomic value. The anticlinal walls of the epidermal cells of the lamina have prominent spherical nodular outgrowths (Fig.14).

Anatomical vistas is a time renowned field of botanical science. The structure of plants are closely correlated with ecological factors; the structure is also related to the functional (physiological) aspects. It is also equally related to the pharmacognostical, taxonomical and phytogenetical studies. The present study of *D.humboldtianus* provide microscopical protocol for botanical diagnostic and pharmacological parameters.

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