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## Macro and microscopic profiling of different parts of a folk medicinal plant - *Dioscorea oppositifolia* L.

According to the literatures of Ayurveda all drugs in this universe are having medicinal values. So it is the duty of a physician to know the use of such plants as food and medicine. They have also advised us to use such drug after a detailed examination. The standardization includes Macroscopic study which gives the description of a crude drug. The description of a crude drug includes the size, shape, nature of outer and inner surface, type of fracture and organoleptic characters like color, taste etc. and the Microscopic studies is a technique of qualitative evaluation, it is a part of pharmacognosy where drug is studied in entire or powder form and it includes study of features like stomata, trichomes, calcium oxalate crystals, starch grains, and vessels are important anatomical characteristics of the organized drug which varies to individual drugs and serves as a main identification tool of a particular drug.

Sagri et al.



# Detailed Macro-microscopic Atlas of Different Parts of a Folk Medicinal Plant *Dioscorea oppositifolia* L.

Ravikrishna Sagri<sup>\*</sup>, Shrikanth Padigar, Tantrady Shridhara Bairy, Sunil Kumar Koppala Narayana<sup>1</sup>

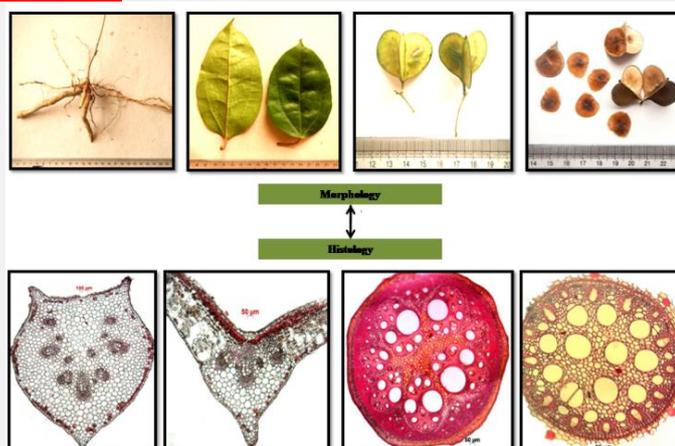
Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, <sup>1</sup>Siddha Central Research Institute (CCRS, Ministry of AYUSH), Anna Hospital Campus, Arumbakkam, Chennai 600106, India.

## ABSTRACT

**Introduction:** Human being depends mainly on plants for his survival in all the aspect particularly for the food. From the beginning of civilization his interest to find new plant and its utility is a continuous process. One such plant is *Dioscorea oppositifolia* L. (Dioscoreaceae) found frequently in and around Udupi locally known as *Kaadu genasu*. The plant is large climber with numerous cylindrical tubers which are consumed as food as it is very nutritive and used as food during famine. On account of these utilities of this less explored plant material, a detailed pharmacognostical study including macro and microscopy was conducted. **Methods:** *D. oppositifolia* L. was subjected to macro and microscopic characterization employing standard methodology mentioned in pharmacopoeia and other herbal analysis protocols. **Results:** The Macro and microscopic studies of different parts of the plant viz. leaf petiole, lamina, stem, root and tubers were performed. **Conclusion:** The finding of the study will be helpful for the identification of the plant.

**KEYWORDS** Dioscoreaceae, Folklore, *Kaadu genasu*, Pharmacognosy.

## PICTORIAL ABSTRACT



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**CORRESPONDENCE** Dr Ravikrishna S, PhD Scholar, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Kuthpady, Udupi 574118, India. Email: drrkaithal@gmail.com

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## 1. INTRODUCTION

According to the literatures of Ayurveda there exists no single drug in this universe which does not have medicinal values.<sup>[1]</sup> So it is the duty of a physician to know the use of such plants as food and medicine. They have also advised us to collect such knowledge about a new drug from the folklore informers and that has to be practiced after a detailed examination.<sup>[2]</sup> *D. oppositifolia* L. is one such plant which is a large climber, it contains many tubers and grows easily and these tubers are nutritive and it is used as a tuberous vegetable. The standardization includes Macroscopic study which gives the description of crude drug includes the size, shape, nature of outer and inner surface, type of fracture and organoleptic characters like color, taste etc. and the Microscopic studies is a technique of qualitative evaluation and used to confirm the structural details of drugs, it is a part of pharmacognosy where drug is studied in entire or powder form and it includes study of features like stomata, trichomes, calcium oxalate crystals, starch

grains, and vessels are important anatomical characteristics of the organized drug which varies to individual drugs and serves as a main identification tool of a particular drug. Hence an attempt was made to study the macro and microscopical structure of the plant *D. oppositifolia* L in this article.

## 2. MATERIALS AND METHODS

### 2.1 Collection of sample

Tubers of *D. oppositifolia* were collected from Hebri, Hiriyaadka and Belman region of Udupi district during July and August 2016. The authenticity of plant was confirmed by consulting a botanist followed by comparison with flora of Udupi.<sup>[3]</sup>

### 2.2 Preservation of sample

Tubers were air dried and preserved in airtight containers at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi for phytochemical studies. For microscopic examination sample was preserved in fixative solution FAA

(Formalin 5 ml + Acetic acid – 5 ml + 70% Ethyl Ethanol – 90 ml) for more than 48 h.

### 2.3 Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.<sup>[4]</sup>

### 2.4 Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl Ethanol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were

photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.<sup>[5]</sup>

## 3. RESULTS AND DISCUSSION

The botanical source of the plant material is authenticated as *Dioscorea oppositifolia* L. Macro-microscopic examinations of leaf, petiole, stem root and tuber have been performed.

### 3.1 Macroscopy

Tubers many, cylindrical; stems twining to the right; leaves up to 12 x 8.5 cm, opposite and alternate, lanceolate to elliptic- oblong, acuminate or obtuse at apex, acute or rounded at base, glabrous; capsules pendulous, broader than long; seeds winged ( Fig 1).

Figure 1. Macroscopic features of *Dioscorea oppositifolia*



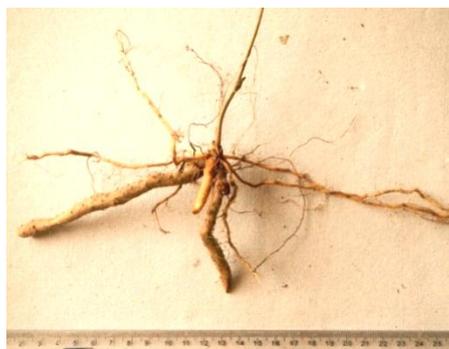
1.1 Whole plant



1.2 Leaf



1.3 Seed



1.4 Roots



1.5 Fruits - Lower surface and lateral view

### 3.2 Microscopy

TS of petiole is oval shaped with three acutely winged projections; two at upper lateral sides and the third at the base. There is an epidermis formed by cells with outer thick wall having hypodermal cells, few with contents. The ground tissue is formed of thick-walled parenchyma cells without intercellular spaces, mostly without any prominent contents except for those in the hypodermal regions, many of the parenchyma of the ground tissue are pitted on their surface. The three winged regions of the section show few layers of collenchymas cells, the other features being similar to the non winged regions. There are about 7 vascular bundles arranged like 'U' with small trace bundles between every prominent bundle. The vascular bundles show fibres at the basal region, vessels towards upper side and small patch of phloem in between them (Fig 2).

TS of lamina passing through midrib show a prominent midrib with a prominent winged projection at the lower side and concave elevation at the upper side. Lamina extension with mesophyll is attached to the lateral sides of midrib. The lamina region has an upper and a lower epidermis, the upper ones with 2 to 3 layers of hypodermal cells with contents instead of well marked palisade cells. Ten to 12 layers of spongy parenchyma with lot air space are seen as the prominent tissue of the mesophyll. The midrib region has tissue similar to the petiole, the lower side being projected as a prominent wing with few collenchyma cells (Fig 3).

TS of matured stem is circular in outline having narrow cortex and phloem, majority of the central portion are composed of 6 to 8 oval shaped vascular bundles with pith parenchyma congesting between the vascular bundles. Detailed transverse section shows an outermost thick-walled epidermis having about 3 layers of cortex formed by collenchyma beneath it; the cortex is not very prominent, after the collenchyma region there is a narrow strip of phloem cells having some contents; a prominent endodermis seen underlying phloem which encloses lot of secondary xylem and some pith parenchyma; xylem is majorly composed of thick walled fibres, in between the vascular bundles 2 to 3 seriate pitted ray cells are

seen; each vascular bundle has about 4 to 6 large vessels and few of less diameter while all the vessels are covered with tracheids, in turn, they are densely packed in thick-walled fibres; few small vascular bundles are also visible towards centre; the force from the developing vascular bundles exerts pressure on the pith parenchyma in the centre resulting their constriction in between the vascular bundles; there are few thick-walled fibres in the pith region; the pith parenchyma also contains few simple starch grains (Fig. 4).

T.S of root is almost circular with uneven outline consisting of single layered epidermis with thick wall; cortex 14 to 18 celled broad, formed with thin-walled parenchyma, few with mucilage; distinct endodermis is present with thickening in the inner wall; central zone is occupied by xylem and consists of vessels, fibres and xylem parenchyma, few being pitted (Fig. 5).

T.S of tuber consists of 3 to 5 layers of thin-walled cork cells, followed by cortex formed from thin-walled parenchyma having lot of starch grains; ground tissue consists of parenchyma cells consisting of cells with contents, starch grains and acicular crystals; endodermis layer which is distinct is present following cortex; groups of scattered vascular bundles, comprising of vessels with crown of phloem patches occur towards the inner regions (Fig. 6).

Various studies have been reported on pharmacognostic features of different *Dioscorea* species to distinguish from one another<sup>[6]</sup>. Glandular trichomes were present in *D. bulbifera* species<sup>[7]</sup> but not observed in *D. oppositifolia*. The genus *Dioscorea* is characterized by the presence anamocytic stomata in leaf<sup>[8]</sup> while both anamocytic and anisocytic stomata were reported in *D. bulbifera*<sup>[7]</sup>. In *D. hispida* glandular trichomes and rosette crystals in petiole and raphides in lamina were reported by Salmah, et al. (2013)<sup>[8]</sup>, whereas these characters were not recorded in the current study. *D. oppositifolia* showed acicular crystals in tuber while *D. polystachya* possessed druses, *D. villosa* and *D. bulbifera* had prismatic crystals in their rhizome and styloids were reported only in *D. bulbifera* rhizome<sup>[9]</sup>.

**Figure 2. Microscopy of Petiole of *Dioscorea oppositifolia***

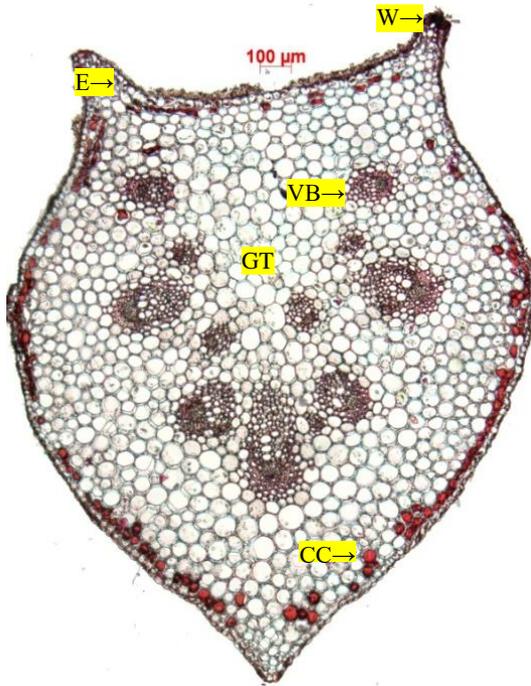


Fig 2.1 TS of entire petiole

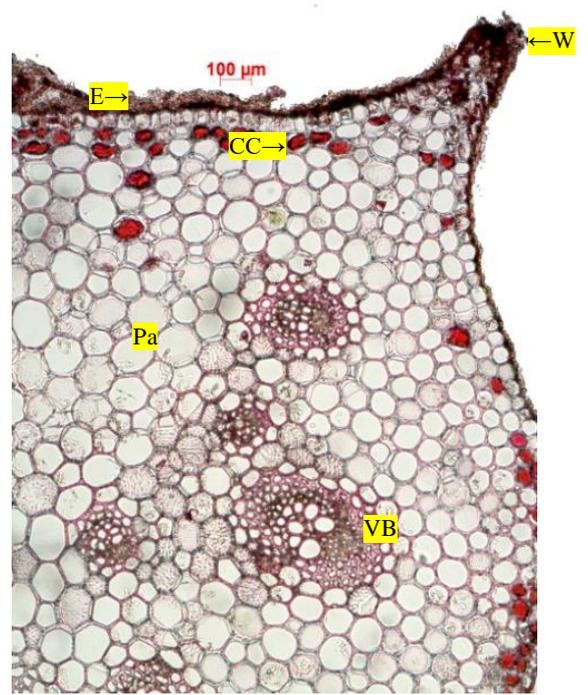


Fig 2.4 Winged portion

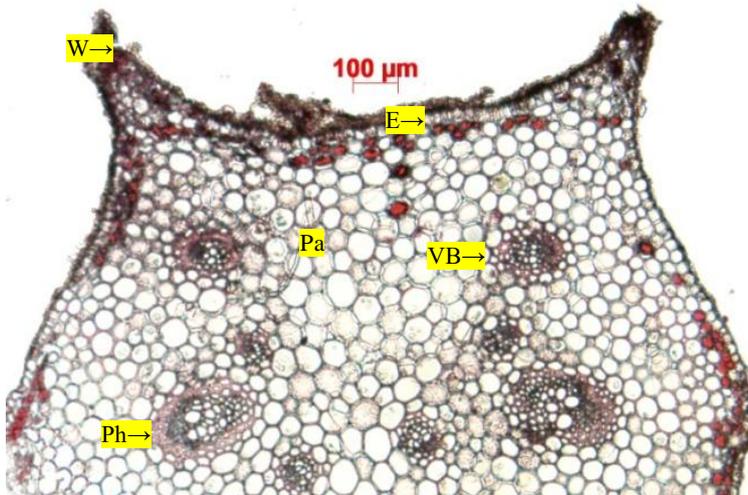


Fig 2.2 Upper region enlarged

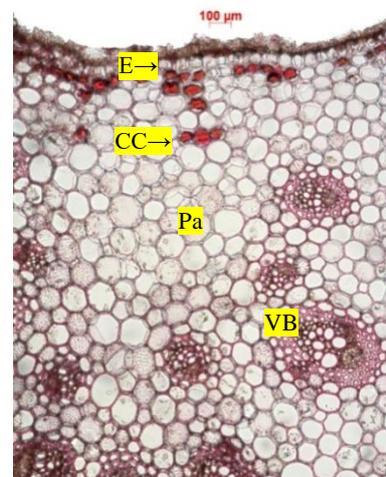


Fig 2.5 A portion of upper region enlarged

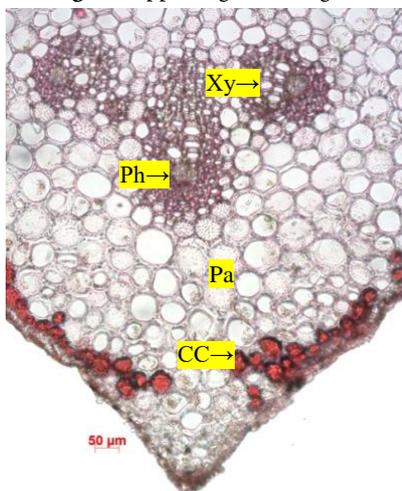


Fig 2.3 Lower region enlarged

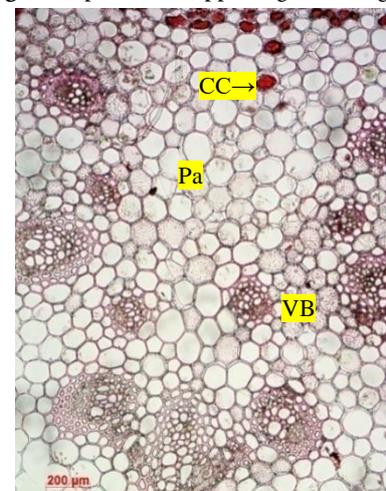


Fig 2.6 A portion of inner region enlarged

CC – content cell; E – epidermis; GT – ground tissue; Pa – parenchyma; Ph – phloem; VB – vascular bundle; W – wing; Xy – xylem.

**Figure 3. Microscopy of leaf of *Dioscorea oppositifolia***

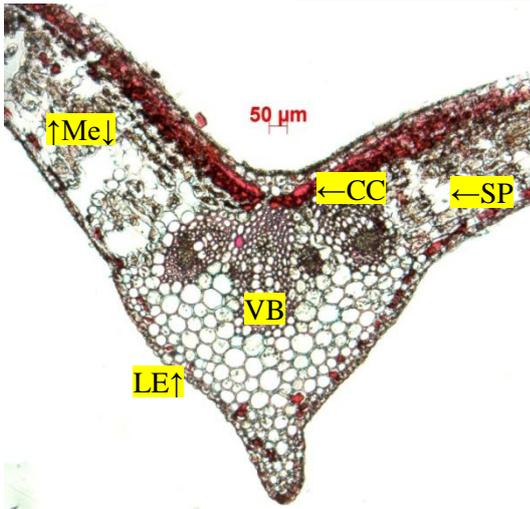


Fig 3.1. TS of lamina passing through midrib

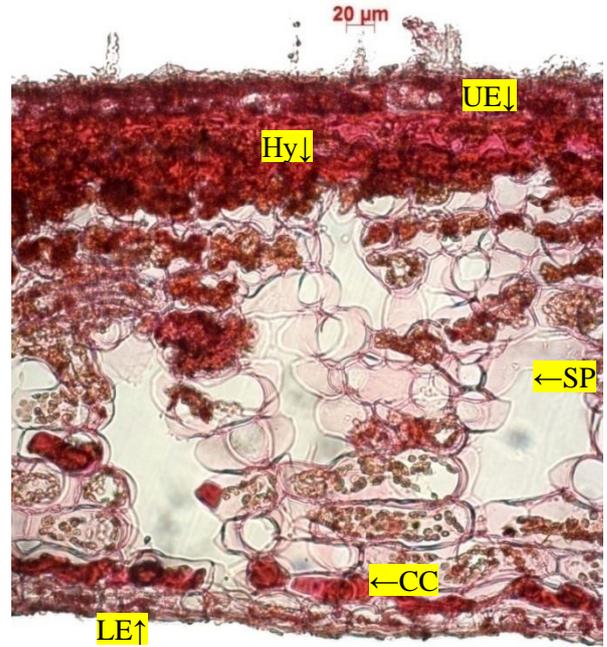


Fig 3.4. A portion of lamina enlarged

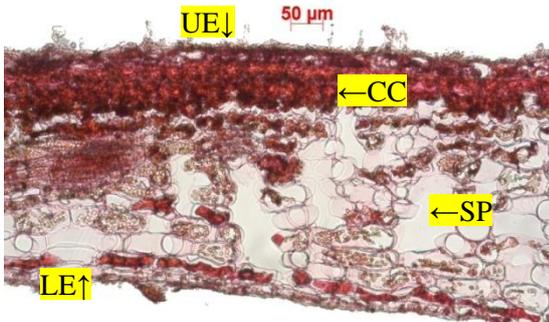


Fig 3.2. TS of lamina

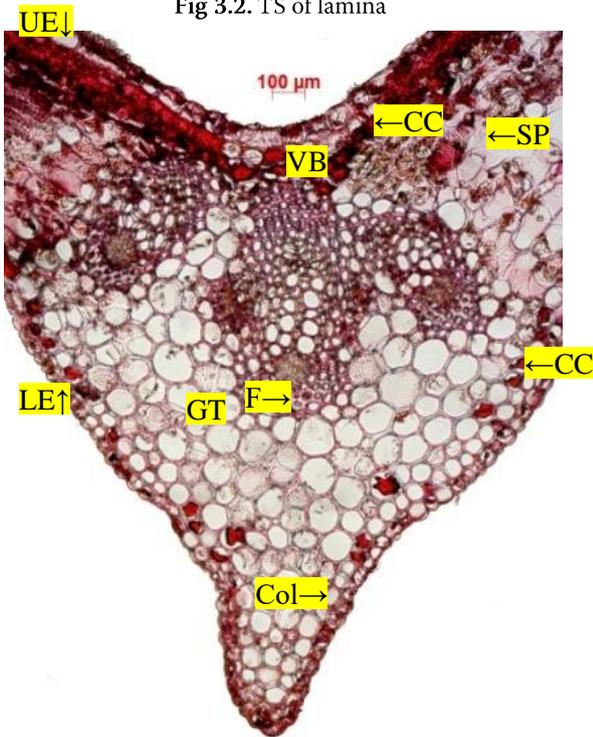


Fig 3.3. TS of midrib

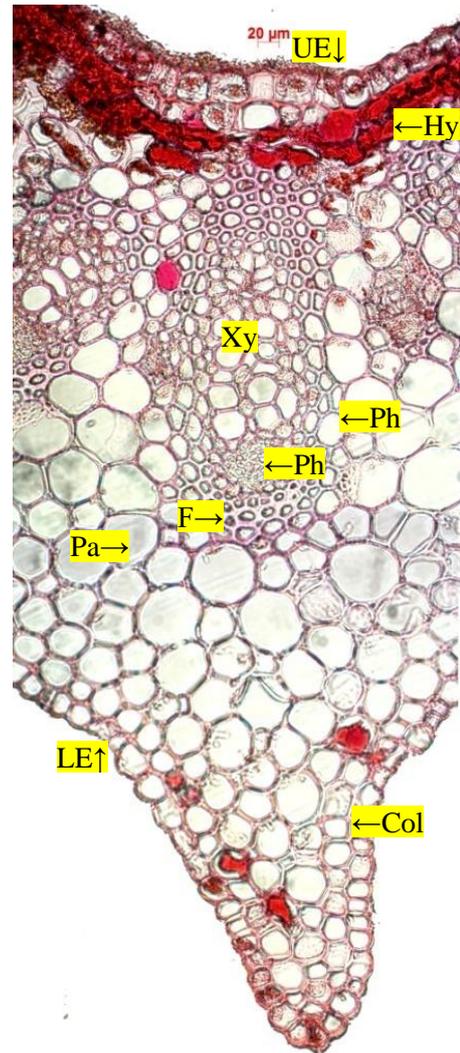


Fig 3.5. A portion of midrib enlarged

CC – content cell; Col – collenchyma; F – fibre; GT – ground tissue; Hy – hypodermal cells; LE – lower epidermis; Me – mesophyll; Ph – phloem; SP – spongy parenchyma; UE – upper epidermis; VB – vascular bundle; VB – vascular bundle; Xy – xylem.

**Figure 4. Microscopy of stem of *Dioscorea oppositifolia***

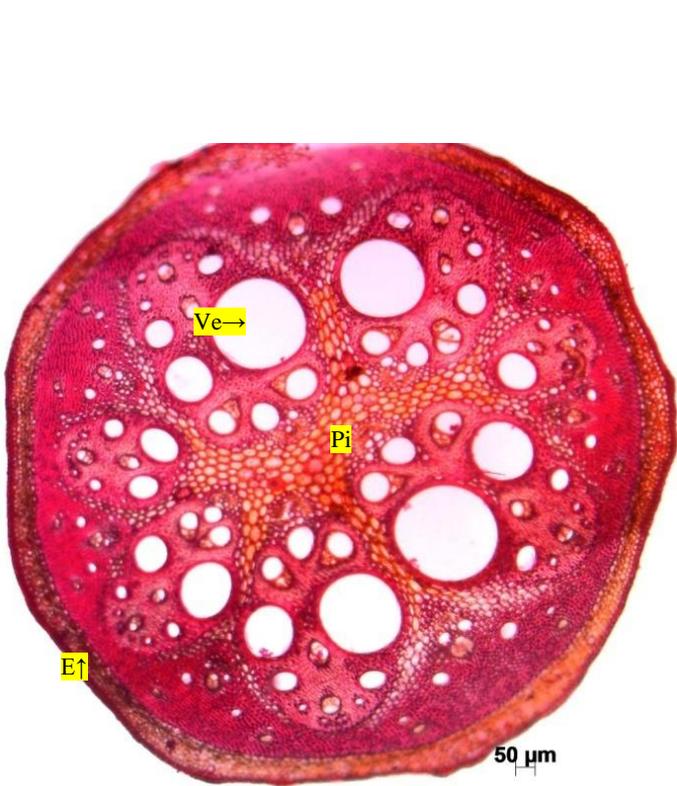


Fig 4.1 TS of stem



Fig 4.2 A portion of stem TS enlarged

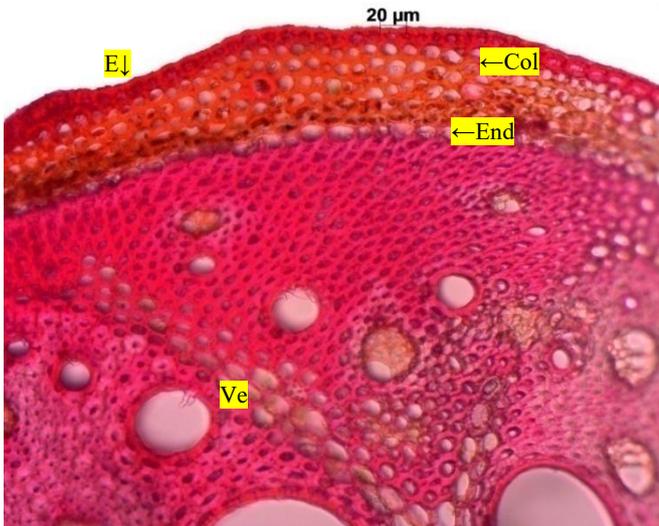


Fig 4.3 Outer region

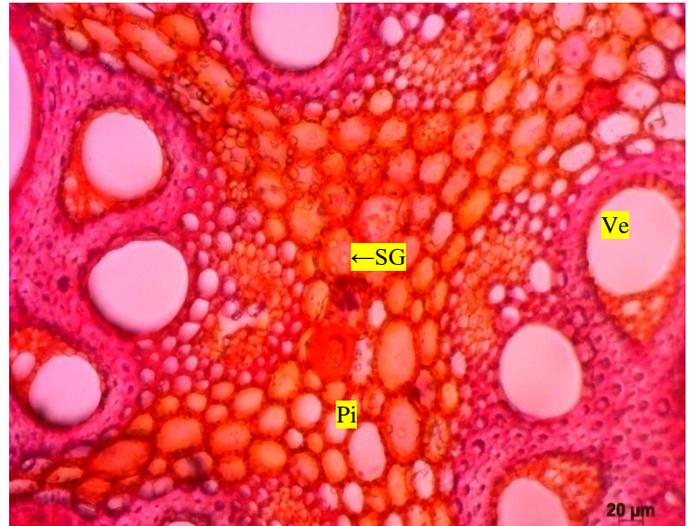


Fig 4.4 Pith region enlarged

Col – collenchyma; E – epidermis; End – endodermis; F – fibre; Pa – parenchyma; Ph – phloem; Pi – pith; SG – starch grains; VB – vascular bundle; Ve – vessel; XF – xylem fibre.

**Figure 5. Microscopy of root of *Dioscorea oppositifolia***

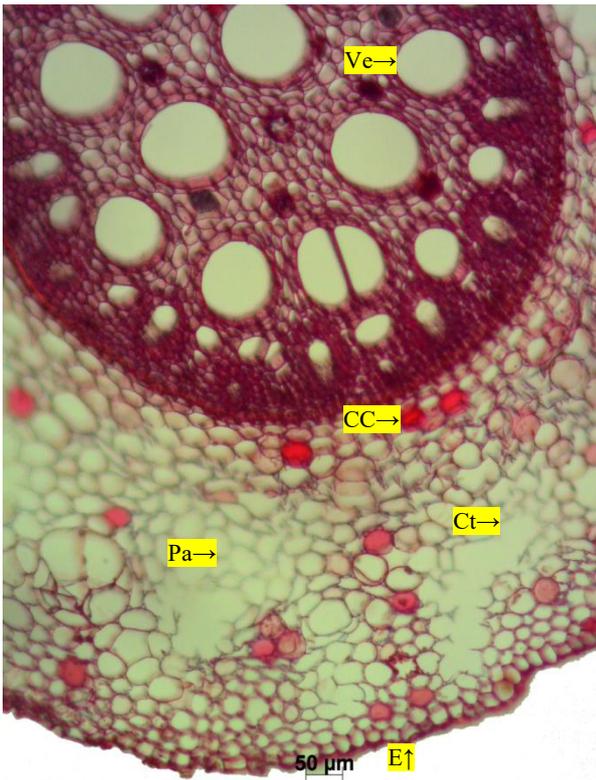


Fig 5.1 TS of root

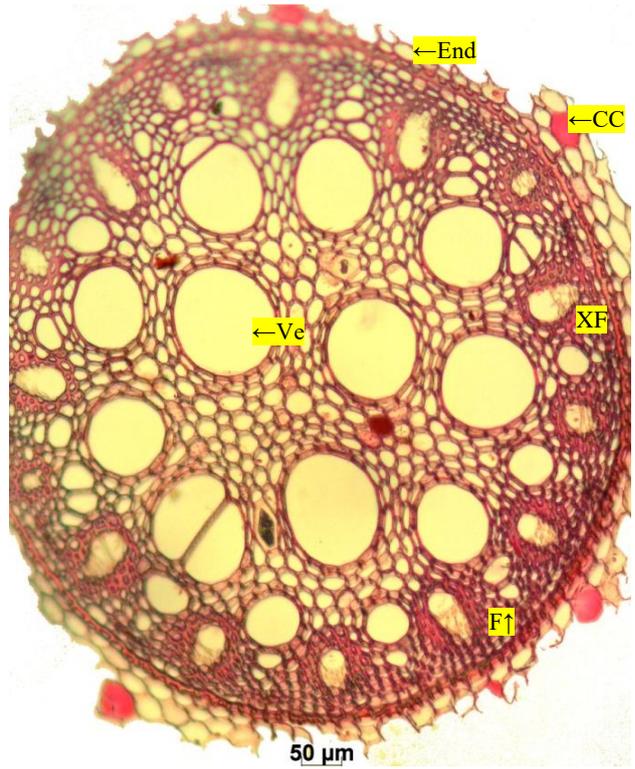


Fig 5.2 A portion of root enlarged

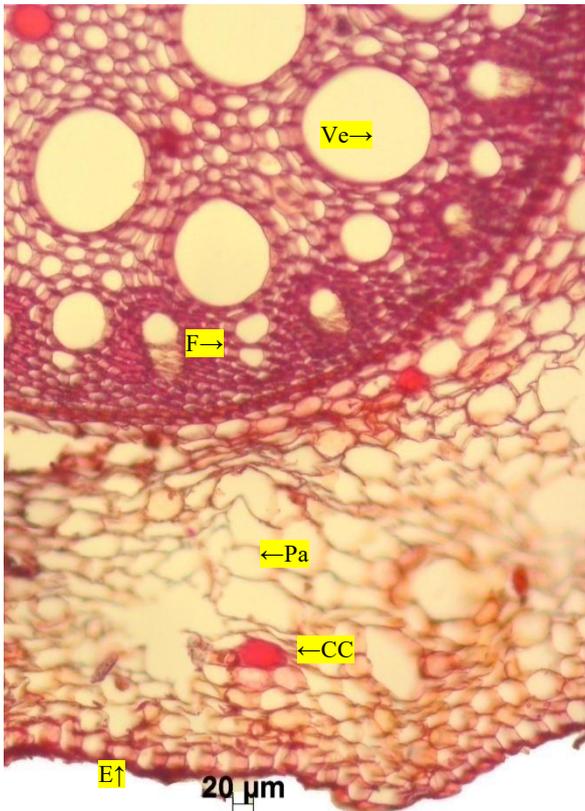


Fig 5.3 TS of root

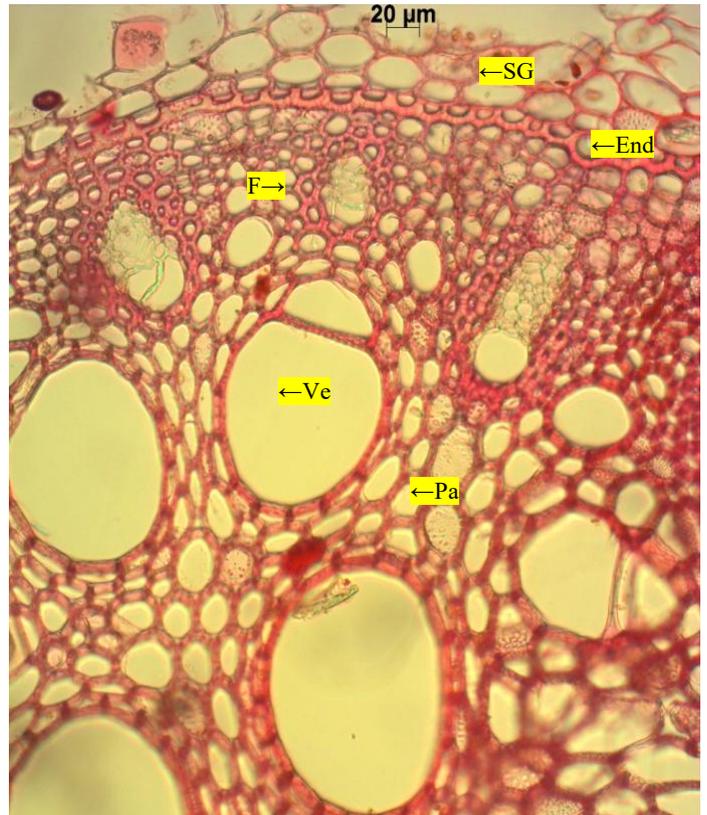


Fig 5.4 A portion of root enlarged

CC – cell content; Ct – cortex; E – epidermis; End – endodermis; F – fibre; Pa – parenchyma; SG – starch grains; Ve – vessel; XF – xylem fibre.

Figure 6. Microscopy of tuber of *Dioscorea oppositifolia*

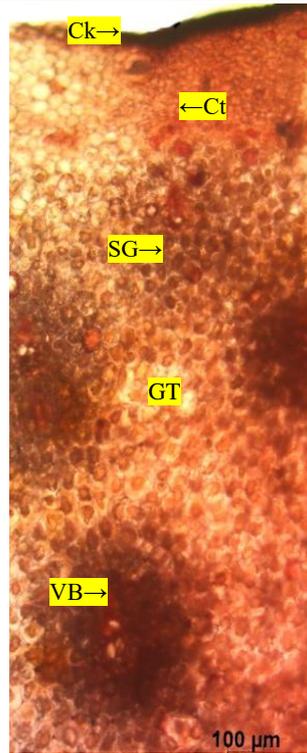


Fig 6.1 TS of tuber

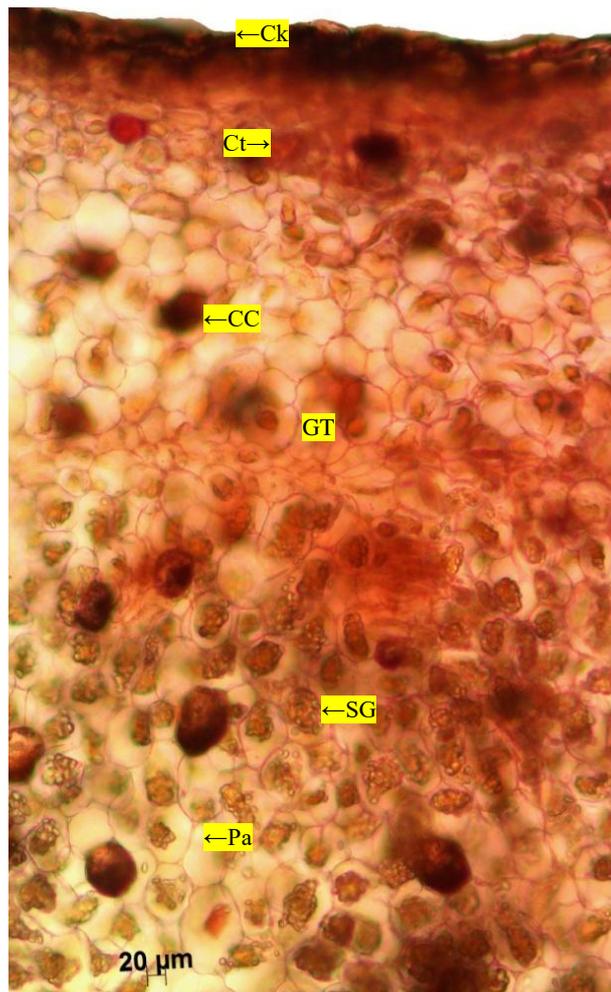


Fig 6.2 A portion of stem TS enlarged

CC – cell content; Ck – cork; Ct – cortex; GT – ground tissue; Pa – parenchyma; SG – starch grains; VB – vascular bundle.

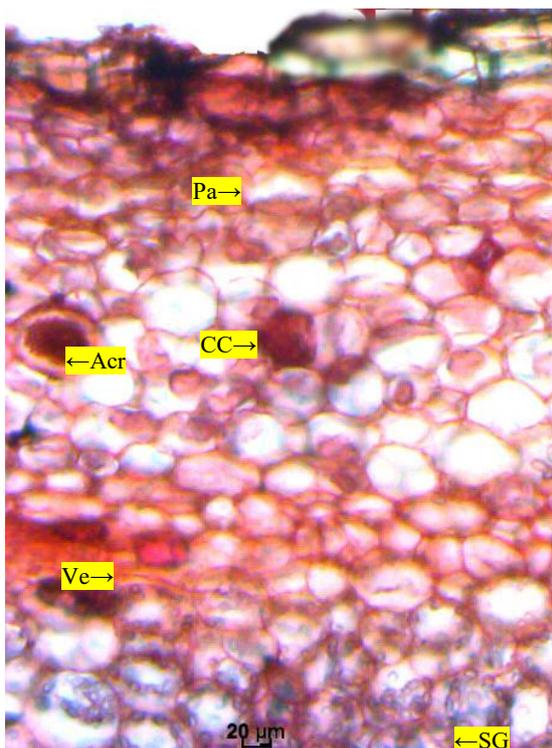


Fig 6.3 Outer region of TS enlarged

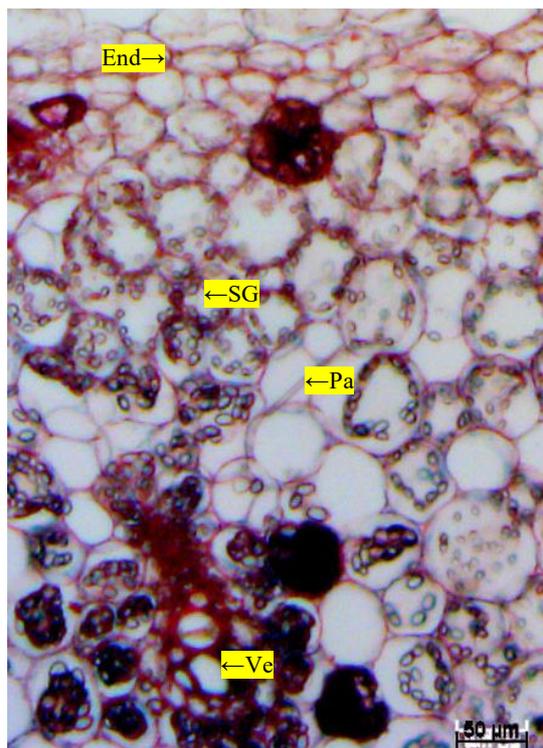


Fig 6.4 Endodermis and vascular bundle

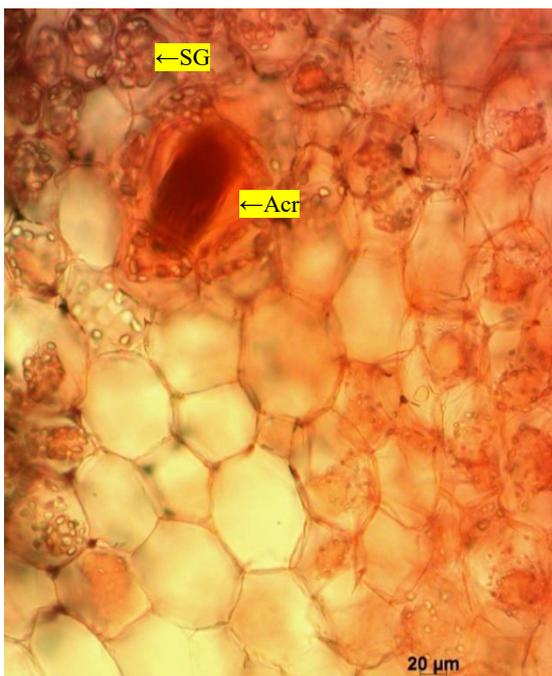


Fig 6.5 Raphide bundle

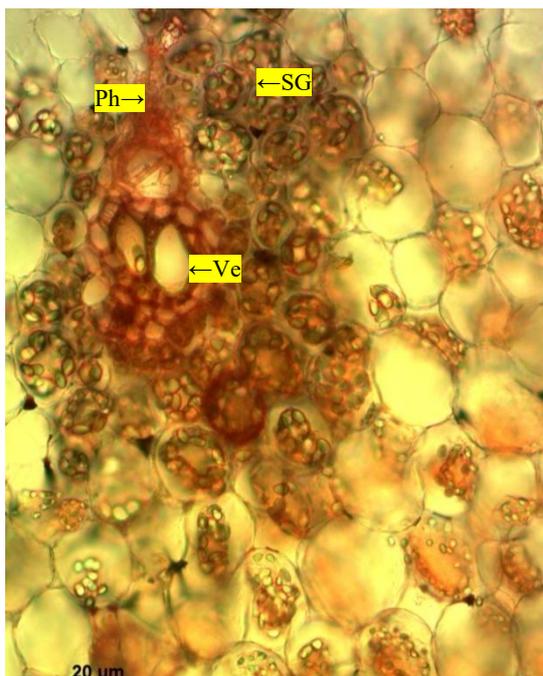


Fig 6.6 Parenchyma with starch

Acr – acicular crystals; CC – cell content; End – endodermis; Pa – parenchyma; Ph – phloem; SG – starch grains; VB – vascular bundle; Ve – vessel.

#### 4. CONCLUSION

Macroscopic features of whole plant and the microscopic features of petioles, lamina, stem, root and tuber of *D. oppositifolia* L. was recorded with its description. These diagnostic features can be used as a fingerprint for the identification of the plant. This can be considered as the standard parameters of the study drug.

#### CONFLICT OF INTEREST Nil

#### SOURCES OF SUPOORT Nil

**CONTRIBUTORS** Dr. Ravikrishna S contributed to collection of plant material and macro and microscopical study; Dr. Shrikanth P contributed to conceptualization of the topic, Dr. Shridhara Bairy contributed to intellectual content, manuscript

editing; Dr. Sunil Kumar provided proper guidance and supervision for the study until the finalization of the paper.

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