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## Pharmacognostical Studies of *Dioscorea bulbifera* Linn. Tubers

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### ABSTRACT

The present study on *Dioscorea bulbifera* Linn. tuber deals with the macroscopical and microscopical details including powder microscopy. Some important and distinct diagnostic characters were observed with sections of matured tuber. The anatomy of tuber was studied by taking transverse sections. Narrow and indistinct epidermal layer, wider cortex with compact and dilated cell containing tannin, thin and less conspicuous periderm, central pith of circular and compact parenchyma cells and large cylindrical and elliptical starch grains were observed. Powder microscopy of the powder revealed the presence of bundles of xylem element and thick walled parenchyma cells. Physicochemical parameters and preliminary phytochemical screening was done on the tuber. The present Pharmacognostical study on the tubers of *Dioscorea bulbifera* Linn will provide the necessary informations regarding the identification of this drug, which will be further helpful in acceptability of this plant worldwide.

**Keywords:** *Dioscorea bulbifera*, tuber, microscopy

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## INTRODUCTION

Medicinal plants were used in India for centuries as an important therapeutic source for treating wide variety of ailments and has been found to be of immense global importance. The family *Dioscoreaceae* consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phyto chemical constituents. The species *Dioscorea bulbifera* is commonly found in India, Brazil, East Africa, Ceylon, Malaya peninsula, Latin America etc.<sup>1</sup>

The plant *Dioscorea bulbifera* Linn. (Family- *Dioscoreaceae*) was commonly known as Barahikhanda in Sanskrit in our ancient texts. The tuber, aerial bulbils and leaf part of the plant is claimed to have medicinal uses such as antitumor, diuretic, aphrodisiac, used in boils, swelling of thyroids, sores, as fattening tonic, pain in the abdomen, treatment of piles, anthelmintic, improving appetite, leucoderma, treatment of bronchitis, jaundice etc.<sup>2,3</sup>.

It is a small annual climber 3-5 m long with tubers and bulbils. Tubers are variable large, solitary globose to pear shaped, bitter and pungent. Stem is terrate and bulbiferous in the leaf axils twinning towards left. Leaves are usually alternate about 10-15 cm by 7.5-10 cm, often much larger or smaller, ovate, acuminate, base more or less deeply cordate, lobe rounded with 7-11 nerves.<sup>4</sup>

## MATERIALS AND METHODS

### Collection of Specimen

The plant species for the proposed study was collected from Nandgaon and Atgaon hamlets of Shapur Taluk of Maharashtra in the month of November. It was identified and authenticated by Dr. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai. The collected tubers were first dried in shade and then using hot air oven at a temperature not exceeded 70<sup>0</sup> C. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5 ml.+ Acetic acid-5 ml. + 70% Ethyl alcohol-90 ml.). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass.<sup>5</sup> Infiltration of the specimens were carried by gradual addition of paraffin wax (melting point 50-60°C) until tertiary-Butyl alcohol solution attained supersaturation. The specimens were cast into paraffin blocks.

### Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was made by customary

procedure Johanson.<sup>6</sup> The section was stained with toluidine blue as per the method published by O'Brien *et al.*,<sup>7</sup>. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with saffranin and Fast-green and Iodine in potassium iodide (for Starch).

### **Photomicrograph**

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photograph of different magnifications were taken with Nikon Lab Phot 2 Microscopic unit. For normal observation bright field was used. For the study of crystal, starch grains and lignified cells polarized light were employed. Since these structures have birefringent property under polarized light they appear bright against dark background. Magnifications of the figures is indicated by Scale-bars.<sup>8</sup>

### **Preliminary Phytochemical screening**

Preliminary phytochemical screening was done on the different extracts and it revealed the presence of different chemical constituents.<sup>9</sup>

### **Physicochemical Parameters**

Various physicochemical parameters,<sup>10</sup> like total ash, sulphated ash, acid insoluble ash, water soluble ash, water soluble extractive value extractive and alcohol soluble extractive values were done on the tuber powder. The results were tabulated in Table 1

## **RESULTS AND DISCUSSION**

### **Macroscopy of tuber**

The tubers are remiferous or hemispherical with a sear in a depression with which it is attached on the plant. The tubers are reddish brown when fresh and when it is dried it is clay coloured. The inner part of the tuber is pale yellow in colour. The surface of the tuber is warty, with reticulate depression and small tuberculate ridges; the ridges have small pimple like out growths. The tubers are slightly bitter in taste with characteristic odour. The tuber is shown in Figure.1

### **MICROSCOPY OF THE TUBER**

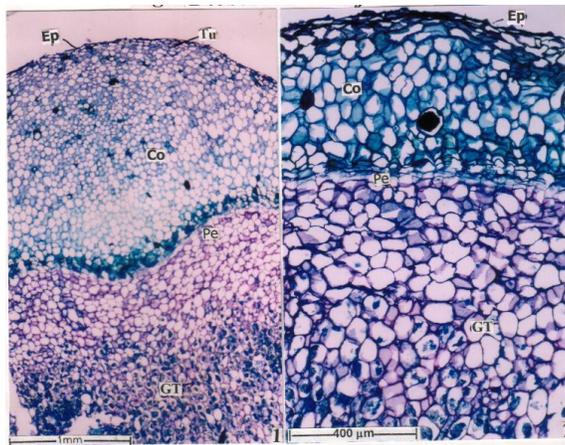
In cross sectional view the tubers showing the following structures.

#### **Epidermal layer:**

It is narrow and not very distinct (Figure.2). It consists of narrow, tangentially, rectangular thick walled cells.



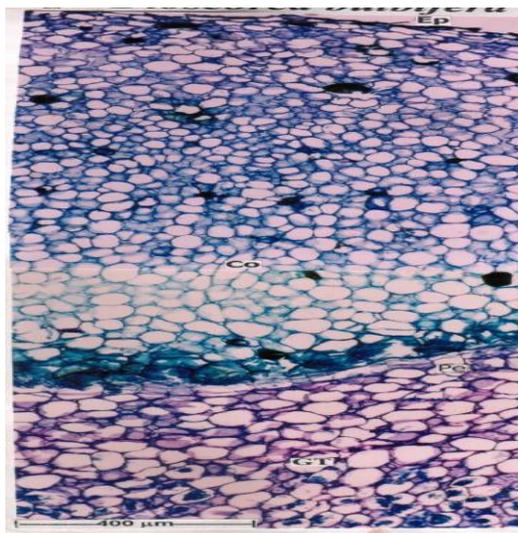
**Figure.1** *Dioscorea bulbifera* linn. tuber



**Figure.2** Anatomy of the Tuber

**Cortex:**

The cortex is wider with small, compact, thin walled cells in the region of tubercle (Figure.3.). In other regions the cortex is narrow with larger thick walled parenchyma cells. Some of the cortical cells are dilated and contains dark tannin content.



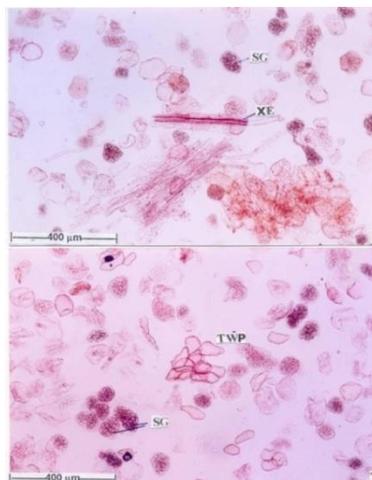
**Figure 3** Structure of tubers from periphery towards the center.

**Periderm:**

Inner to the cortex is the thin less conspicuous periderm.(Figure 3). It is wavy in outline and consists of 3 or 4 layers of tubular cells. Tannin is accumulated in the inner boundary of periderm.

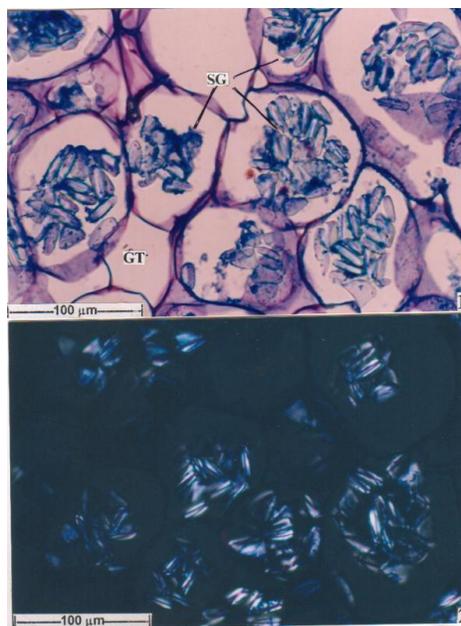
**The central pith:**

The central pith ground tissue consists of circular compact parenchyma cells. The cell walls are thin and several small vascular strands are seen scattered in the central ground tissue. The vascular strands have a small cluster of Xylem elements and a few Phloem elements. (Figure.4.)



**Figure.4 Xylem elements in powder**

- 1.Cylindrical starch grains in the ground cells. (Under bright field microscope)
- 2.Under polarized microscope showing bright starch grains.



**Figure.5 Distribution of starch grains in the ground cells.**

**Starch grains:**

Large cylindrical or elliptical starch grains are heavily loaded in the central ground cells. (Figure.5.). The starch grains are 30 micrometer long and 20-25 micrometer in thick. When the starch grains are viewed under polarized light microscope, the starch grains appear bright with median longitudinal hilum.

**POWDER MICROSCOPY**

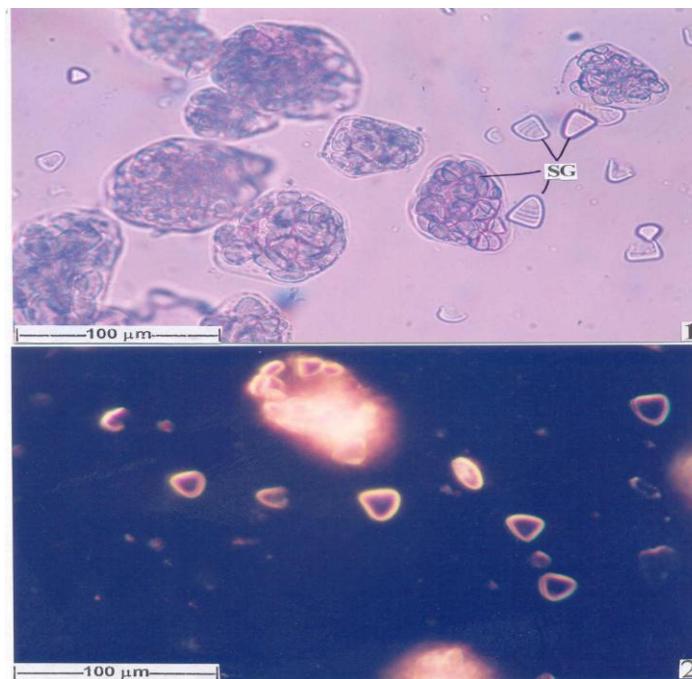
The powder of the tuber when examined under the microscope, exhibits the following cell components.

**Xylem elements:**

Thin bundles of Xylem elements are sparsely seen in the powder; the element are narrow, long and has spiral or reticulate lateral wall thickness. (Figure.6.)

**Thick walled parenchyma cells:**

These cells are fairly thick walled, polyhedral empty cells. These cells are fairly common in the powder. (Figure.6).



**Figure.6 Thick walled parenchyma cells in powder**

**Starch containing parenchyma cells:**

Large, thin walled parenchyma cells are densely packed with starch grains. These cells were abundant in the powder. The starch grains are either ellipsoidal or triangular. The starch grains have centric striations. (Figure.7). Free starch grains are seen freed from the cells. The ellipsoidal starch grains are 15-30 micrometer long. The triangular grains are 20 micrometer long.

### Physicochemical Parameters

*Dioscorea bulbifera* Linn powder showed the presence of Total ash-8.13% w/w, Sulphated ash - 3.88%w/w, water soluble ash - 1.14%w/w, acid insoluble ash - 2.75% w/w, water soluble extractive - 6.83% w/w and alcohol soluble extractive - 8.62% w/w. The results were tabulated in Table. 1

**Table. 1: Various physicochemical parameters of dioscorea bulbifera Linn.**

Sl. No.	Test	Result % w/w
1.	Total ash	8.13
2.	Sulphated Ash	3.88
3.	Water soluble Ash	1.14
4.	Acid insoluble Ash	2.75
5.	Water soluble Extractive value	6.83
6.	Alcohol Soluble Extractive Value	8.62

### Preliminary Phytochemical screening

Preliminary phytochemical screening of the tuber powder after extracting with various solvents showed the presence of glycosides, flavonoids, saponins, tannins, phenolic compounds, proteins and fixed oils in different extracts.

### CONCLUSION

The macroscopic study of the tuber indicated that it is reniform or hemispherical with a scar in a depression with which it is attached on the plant. This is an important characteristic feature for the identification of this tuber. The anatomy of the tuber was studied by taking transverse sections. Transverse section of the tuber showed narrow and not very distinct epidermal layer, wider cortex with small compact thin walled cells in the tubercle region, less conspicuous periderm, central pith with circular and compact parenchyma cells. Large cylindrical or elliptical starch grains are loaded in the central ground cells. Powder microscopy of the tuber revealed the presence of xylem elements which are sparsely seen. It also shown thick walled parenchyma cells and starch containing parenchyma cells. Physicochemical and preliminary phytochemical studies will also be useful tools along with macroscopical and microscopical characters of *Dioscorea bulbifera* Linn tubers.

The present Pharmacognostical studies of *Dioscorea bulbifera* Linn tuber will be useful in providing information in the identification of this drug which helps to improve a way of acceptability of herbal drugs in the current scenario which lacks regulatory laws in the quality control of herbal drugs.

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