

RESEARCH ARTICLE

## Anatomical Studies on *Scilla indica* (Wt.) Baker (Liliaceae)

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### Abstract

Wild onion, *Scilla indica* Baker is a rare medicinal plant, a bulbous and scapigerous herb. It was considered to be synonymous with *Ledebouria hyacinthina* (Roth) and *Barnardia indica* (Wight) which is endemic to Tiruvannamalai of Tamil Nadu in India. Members of the Liliaceae are famous for their use as medicinal herbs. *Scilla indica* Baker bulb is used for the treatment of paralytic attacks, ailments of the heart, calculous affections, rheumatism and skin diseases, anthelmintic, cardiac stimulant, digestive, diuretic, emmenagogue and an expectorant. It is also used against the respiratory problems such as asthma, cough and bronchitis. The present study records the first report about anatomical parameters of leaf, bulb and root of *Scilla indica* Baker.

**Keywords:** Anatomical parameters, scapigerous herb, Liliaceae, *Scilla indica* Baker.

### Introduction

*Scilla* of Liliaceae includes 4075 species at global level. In India, there are 189 species (Singh and Jain, 2009). They have scaly bulbs. This plant grows wild in the forests of Tamil Nadu. Gamble (1935) described the species occurring in Madras Presidency as *Scilla indica* Baker in West Coast Districts, sea level to 4,000 ft. Bulb is ovoid or globose, 0.5-1.5 inch dia, leaves linear, oblong or lanceolate, obtuse or acute, narrowed into a sheathing petiole, rather fleshy, obtusely keeled on the back, 1.5-7 inch long, 0.3-1 inch wide, dark-green above, often blotched with black, paler and glaucous below; scape 2-6 inch long; flowers greenish-purple, usually dense; perianth-segments linear-oblong, 0.15-0.25 inch long; capsules subglobose, 0.2-0.3 inch dia. Light microscopic study of Sheeba and Vijayavalli (1998) revealed that the pollen grains are large sized in *Scilla indica*, boat shaped elongate monocolpate and exine shows finely reticulate ornamentation. At the time of dehiscence, all the pollen grains are in monads with binucleate condition. Foliar epidermal study of *Scilla indica* revealed that the shape of the epidermal cells is regular and rectangular with oblique cross walls. Stomata are amphistomatic and anomocytic. Guard cells are kidney shaped with chloroplast. Trichomes are totally absent and stomatal distribution pattern is parallel to the longer axis of the lamina. Kumavat *et al.* (2015) studied Indian squill *Urginea indica* Kunth which is one of the ancient medicinal plants and its bulb has several therapeutic uses. It is used as diuretic, emetic, emmenagogue, expectorant, cathartic, anticancer agent etc. According to the previous literature, the marketed sample of *Urginea indica* is often mixed with the bulbs of *Scilla indica* Baker and hence, to confirm the controversial status of any crude drug, there is a necessity involves determining various pharmacognostic

parameters such as macroscopy, microscopy, powder study, physio-chemical analysis and preliminary phytochemical screening. The previous report provides a preliminary anatomical work that has been carried out in *Scilla indica* Baker. So, the present work is aimed at an elaborate study on the anatomical features of leaf, bulb and root of the same. To analyze the therapeutic value of the selected ethnobotanical plant, the present study has been initiated with the identification using microscopic and anatomical parameter as the primary step for further pharmacognostic studies.

### Materials and methods

**Collection and identification of plant material:** Materials for the present study were collected from the hills near Tiruvannamalai district of Tamil Nadu, India during different seasons. The specimen was identified and authenticated by Prof. Dr. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai. 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 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule given by Sass (1940). Infiltration of the specimens was carried by gradual addition of paraffin (melting point 58-60°C) until TBA solution attained super saturation. 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 done according to customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method of O'Brien *et al.* (1964).

Since, toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the 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 safranin and Fast-green and Iodine-KI (for Starch). Photomicrographs were prepared with Nikon lab photo microscope and digital camera (Esau, 1964).

## Results

**External features:** *Scilla indica* is a scaly and bulbous herb. The bulb of this plant measures 1.3-3.8 cm dia, leaves linear, oblong to oblanceolate, obtuse, narrowed into a sheathing petiole, rather fleshy, obtusely keeled on the back, 3.8-17.8 cm long, 0.8-2.5 cm wide, dark-green above, often blotched with black, paler and glaucous below; scape 5.1-15.2 cm long; flowers greenish-purple, usually dense; perianth-segments linear-oblong, 0.4-0.6 cm long; capsules subglobose, 0.5-0.8 cm dia (Fig. 1a and b).

Fig. 1. a. *Scilla indica* Baker whole plant, b. With inflorescence.



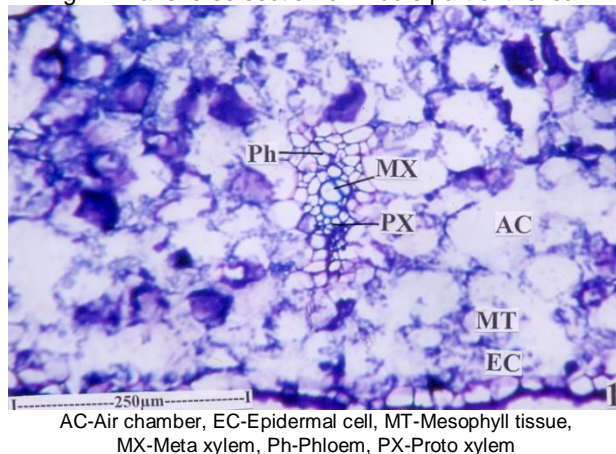
## Anatomical features

**Leaf:** In cross sectional view, the leaf exhibits isolateral symmetry. There is no distinction between adaxial and abaxial sides. The epidermal layers are fairly thick with squarish or rectangular thin walled cells with a thick cuticle (Fig. 2 and 3). The mesophyll tissue consists of small, spherical or lobed thin walled parenchyma cells which form dense reticulate system enclosing wide air chambers. Mucilagenous substance is abundantly found in the mesophyll cells. The vascular bundles occur in the median part of the leaf. The bundles are in a single horizontal row and the size of the bundles becomes gradually smaller towards the margin of the leaf. The median midrib bundle consists of 3 or 4 wider thick walled metaxylem elements and 4 to 6 small protoxylem elements. Phloem occurs in small cluster of 4 or 6 elements (Fig. 2). The vascular bundle in the lateral median part is slightly smaller and the metaxylem elements are 3 or 4 and the phloem elements occur in small cluster of 6 cells (Fig. 3). The lamina is 350-450  $\mu\text{m}$  thick. The marginal part of the lamina is thin and conical with highly dilated wide and thick walled

squarish epidermal cells with article. The median portion of the marginal part includes a few compact thick walled cells (Fig. 4). It has 100  $\mu\text{m}$  thick small vascular bundles occur in the median part of the leaf margin.

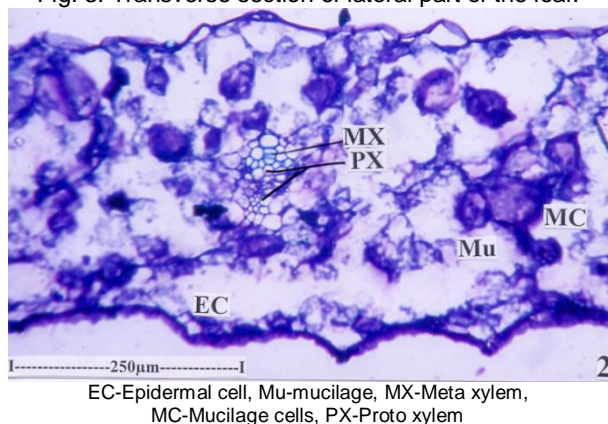
**Epidermal and Stomatal types:** The adaxial epidermal cells of the leaf were studied in surface view of the paradermal sections. The epidermal cells are vertically elongated and parallel to the lamina. The cells are wide, rectangular and thin walled.

Fig. 2. Transverse section of middle part of the leaf.



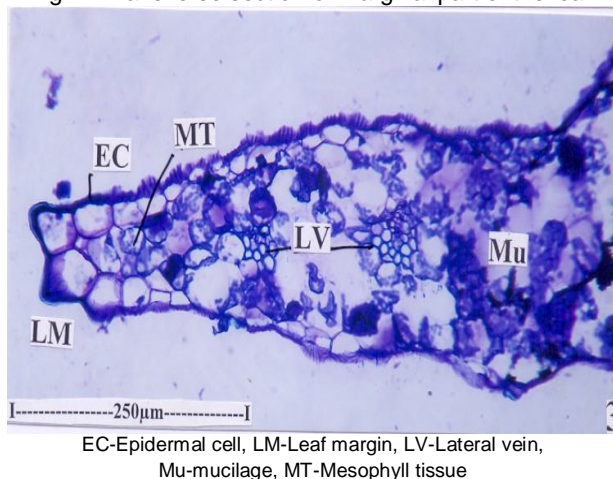
AC-Air chamber, EC-Epidermal cell, MT-Mesophyll tissue, MX-Meta xylem, Ph-Phloem, PX-Proto xylem

Fig. 3. Transverse section of lateral part of the leaf.



EC-Epidermal cell, Mu-mucilage, MX-Meta xylem, MC-Mucilage cells, PX-Proto xylem

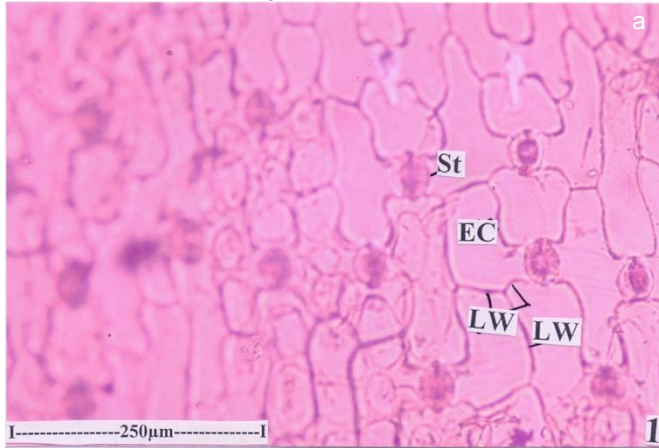
Fig. 4. Transverse section of marginal part of the leaf.



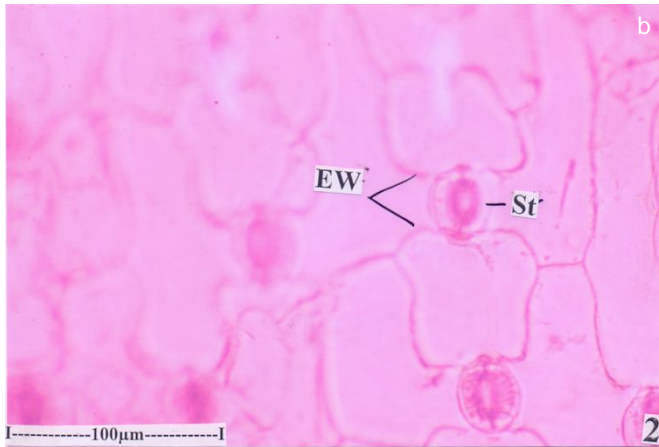
EC-Epidermal cell, LM-Leaf margin, LV-Lateral vein, Mu-mucilage, MT-Mesophyll tissue



Fig. 5a and b. Paradermal section of the adaxial epidermis of the leaf showing stomata and epidermal cells.

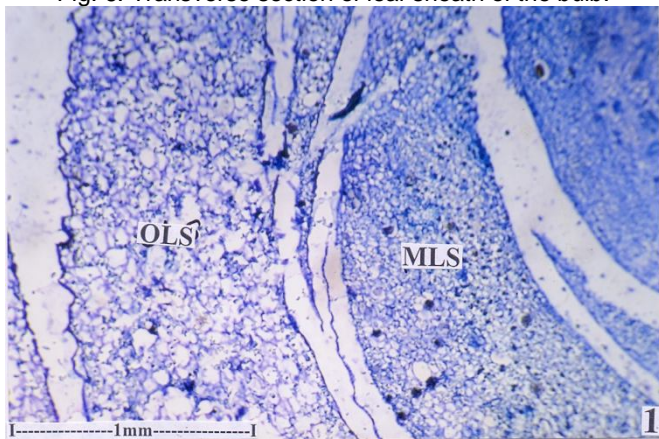


EC-Epidermal cell, LW-Lateral wall, St-Stomata



EW-End wall, St-Stomata

Fig. 6. Transverse section of leaf sheath of the bulb.



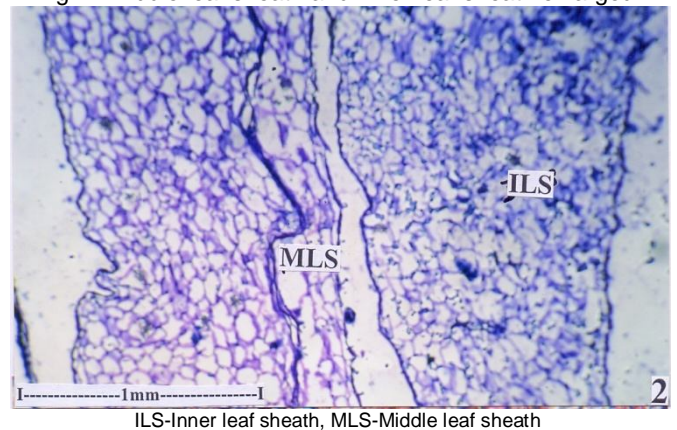
MLS-Middle leaf sheath, OLS-Outer leaf sheath

The cells are parallel to each other (Fig. 5a and b, 14a and b). The stomata are located at end walls of the upper and lower epidermal cells (Fig. 14a,b and 15) or at the mid-point of horizontally extended lateral walls of the two adjacent epidermal cells (Fig. 15). The guard cells are broadly elliptical, thick walled and have narrow slit like stomatal aperture (Fig. 15).

The stomata are  $90 \times 70 \mu\text{m}$  in size. The abaxial epidermal cells are different from the adaxial cells. The cells are narrow, very long and run parallel to each other. The stomata are diffuse in distribution (Fig. 16a). The stomata occur in between the end walls of vertically oblong epidermal cells (Fig. 16a and b). Occasionally the stomata may also occur in between two epidermal cells and located on the lateral wall extensions of epidermal cells (Fig. 17). The stomata are broadly elliptical and have narrow slit like stomatal pore (Fig. 18). The guard cells are  $35 \times 25 \mu\text{m}$  in size. The epidermal cells are thin walled and straight.

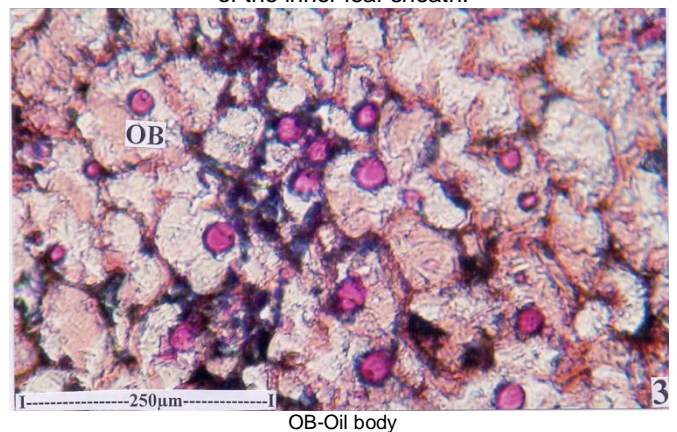
**Leaf sheath:** The leaf sheaths are compactly arranged forming an elliptical or spherical pseudo bulb (Fig. 6). The leaf sheath consists of thin epidermal layers on the inner and outer sides and the mesophyll tissue is homogeneous comprising small, polygonal, compact thin walled parenchyma cells (Fig. 6 and 7). In the inner leaf sheath are seen several prominent spherical oil bodies which stain red with Sudan red (Fig. 8). The leaf sheaths are  $800 \mu\text{m}$  to  $1 \text{mm}$  thick.

Fig. 7. Middle leaf sheath and inner leaf sheath enlarged.



ILS-Inner leaf sheath, MLS-Middle leaf sheath

Fig. 8. Oil bodies stained with Sudan red seen in the cells of the inner leaf sheath.



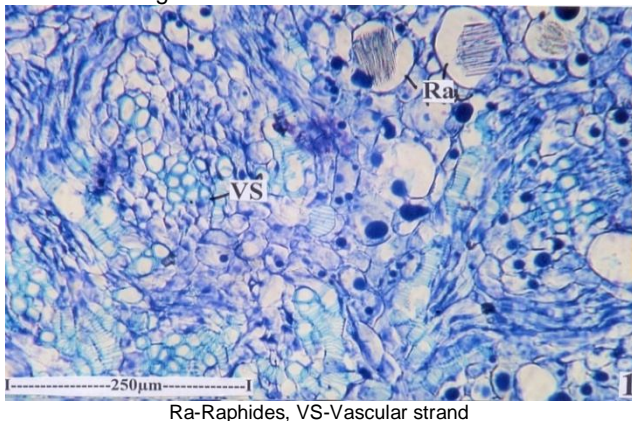
OB-Oil body



**Stem:** The stem occurs as inner most small cylindrical organ. In sectional view, it consists of several scattered, small vascular bundles which are of monocot type. The vascular bundles include a group of thin walled, less lignified xylem elements which are small and angular in sectional view (Fig. 9). Phloem occurs on the outer ends of metaxylem elements. The ground tissue of the stem consists of thin walled, compact, parenchyma cells and the cells are filled with large spherical tannin bodies (Fig. 10). Some of the ground parenchyma cells are dilated into wide circular idioblasts and each cavity is having a bundle of raphides (Fig. 11). The raphide bundles are 70  $\mu\text{m}$  in length (Fig. 11).

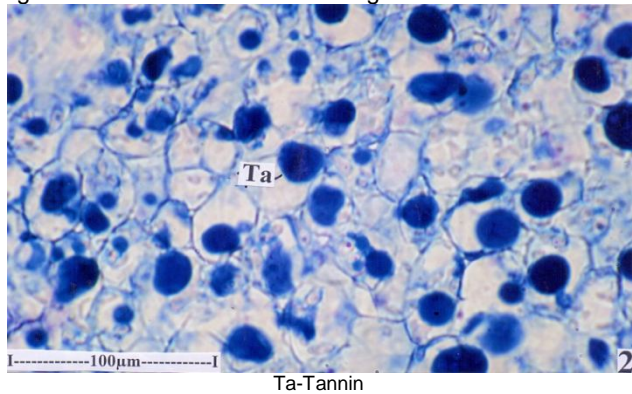
**Root:** Root exhibits typical monocot type of organization. The root is circular in cross sectional view and it is 800  $\mu\text{m}$  in dia. It consists of a thin epidermal layer, wide parenchymatous cortex and central stele. The cortical zone includes small air chambers formed by the cortical tissue (Fig. 12). The stele includes several protoxylem elements which are exarch and a few wide, circular thick-walled metaxylem elements. In between the protoxylem points, some small groups of phloem elements occur. The xylem elements are surrounded by small, thin walled fibres. The metaxylem elements are 40  $\mu\text{m}$  in dia.

Fig. 9. Transverse section of stem.



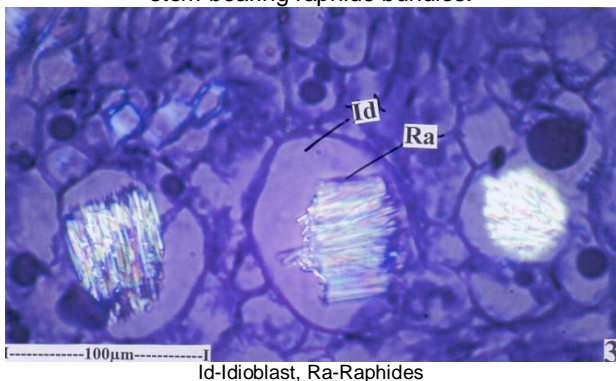
Ra-Raphides, VS-Vascular strand

Fig. 10. Tannin bodies found in the ground tissue of the stem.



Ta-Tannin

Fig. 11. Idioblasts in the ground tissue of the stem bearing raphide bundles.



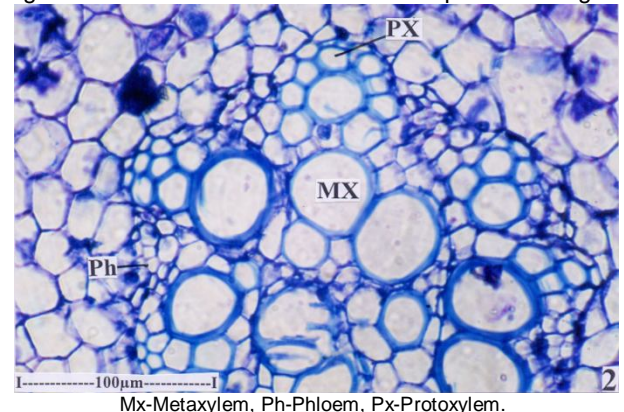
Id-Idioblast, Ra-Raphides

Fig. 12. Transverse section of root—Entire view.



Co-cortex, Ep-Epidermis, St-Stele.

Fig. 13. Transverse section of root stellar portion enlarged.



Mx-Metaxylem, Ph-Phloem, Px-Protoxylem.

## Discussion

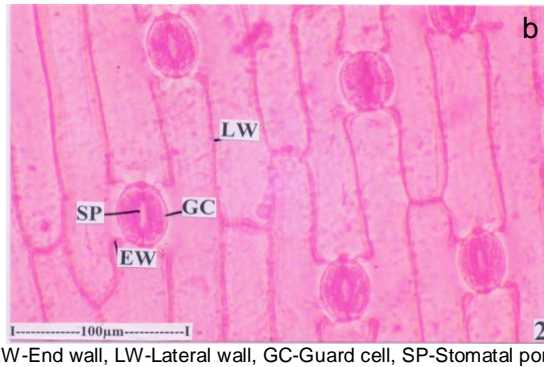
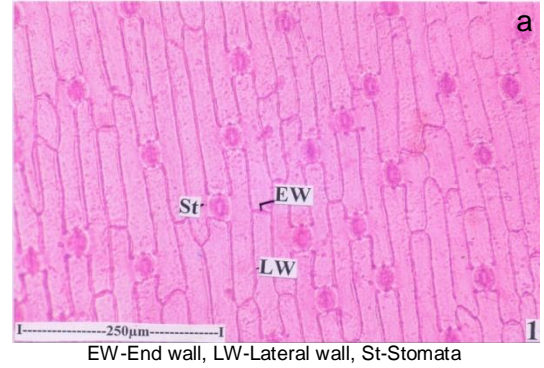
Kumavat *et al.* (2015) observed a very few stomata in *Scilla indica* which were nearly circular. But in the present study, the stomata are located at end walls of the upper and lower epidermal cells (Fig. 14a,b and 15) or at the midpoint of horizontally extended lateral walls of the two adjacent epidermal cells (Fig. 15). The guard cells are broadly elliptical, thick walled and have narrow slit like stomatal aperture (Fig. 15). Sheeba and Vijayavalli (1998) while examining the anatomical features of *Scilla indica* claimed that the stomata are found to be amphistomatic and anomocytic in distribution pattern is parallel to the longer axis of the lamina. Guard cells are kidney shaped with chloroplast.



In the present study, the stomata are diffused in distribution (Fig. 16a), the stomata occur in between the end walls of vertically oblong epidermal cells (Fig. 16a and b). The guard cells are 35x25 µm in size. Occasionally the stomata may also occur in between two epidermal cells and located on the lateral wall extensions of epidermal cells (Fig. 17). The stomata are broadly elliptical and with the narrow slit like stomatal pore (Fig. 18). Kumavat *et al.* (2015) observed that the vascular bundles were conjoint, collateral and closed, embedded in the ground tissue. Mesophyll tissue is made up of large polyhedral parenchymatous ground tissue. Mucilage ducts were few and scattered in mesophyll tissue.

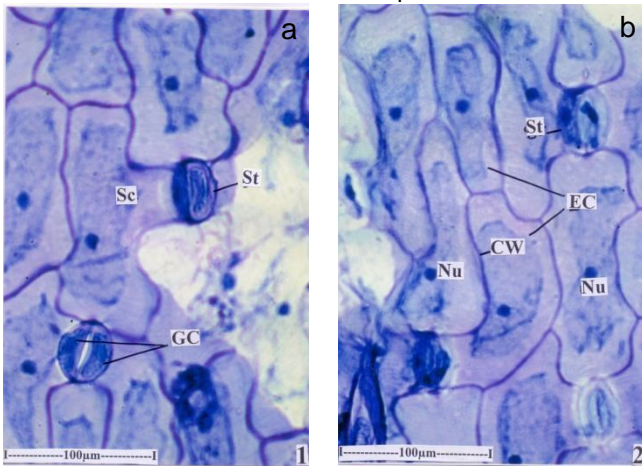
Some of the ground parenchyma cells are dilated into wide circular idioblasts and each cavity is having a bundle of raphides (Fig. 11).

Fig. 16a and b. Paradermal section of the abaxial epidermis showing the epidermal cells and stomatal distribution.



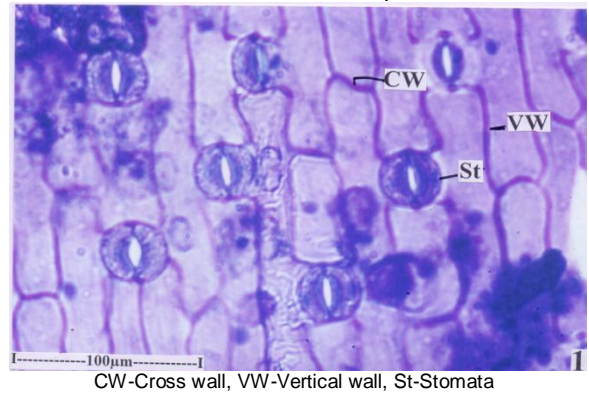
EW-End wall, LW-Lateral wall, GC-Guard cell, SP-Stomatal pore

Fig. 14a and b. Position of the stomata in the middle part of lateral extensions of two epidermal cells.



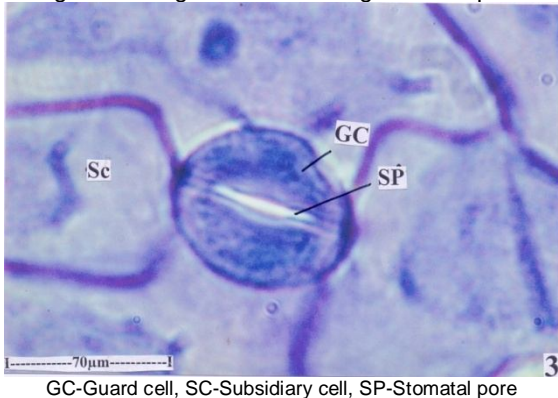
CW – Cell wall, EC-Epidermal cell, GC-Guard cell, Nu-Nucleus, SC-Subsidiary cell, St-stomata.

Fig. 17. Epidermal layer of cells with stomata located in between horizontal walls of epidermal cells.



CW-Cross wall, VW-Vertical wall, St-Stomata

Fig. 15. A single stoma showing stomatal pore.



GC-Guard cell, SC-Subsidiary cell, SP-Stomatal pore

Fig. 18. A single stoma enlarged.



GC-Guard cell, SP-Stomatal pore

Bundles of acicular calcium oxalate crystals were observed in many idioblast cells. Whereas, in the present study, the ground tissue of the stem consists of thin walled compact, parenchyma cells and the cells are filled with large and spherical tannin bodies (Fig. 10). The mesophyll tissue consists of small, spherical or lobed thin walled parenchyma cells which form a dense reticulate system enclosing with wide air chambers. Mucilagenous substance is abundantly found in the mesophyll cells.

## Conclusion

In this investigation of anatomical features of *Scilla indica* Baker, I observed that oil bodies are present in leaf sheaths. Ground tissue parenchyma cells are filled with large spherical tannin bodies and some of the ground parenchyma cells are dilated into wide circular idioblasts and each cavity is having a bundle of raphides. The present study has shown the microscopic features of different organs of *Scilla indica* Baker to expand the diagnostic dimensions of the taxon and to supplement the external characters with the internal features to confirm the identity.

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