



Powder microscopic observation of leaf epidermal appendages and anatomical features of leaf and petiole of some cucurbitaceous members: A comparative analysis

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Abstract

A comparative analysis of five species of Cucurbitaceae members such as *Lagenaria vulgaris*, *Benincasa hispida*, *Cucurbita maxima*, *Luffa acutangula* and *Cucumis sativus* was carried out on the powder microscopic observation, petiole and leaf anatomy. Similarities were observed in the distribution, differentiation and number of layers of cells and tissues in the petiole and leaf anatomical sections. However, variations were observed in the numbers of vascular bundles in the petiole and leaf and they were bicollateral in all the species. Numbers of petiolar vascular bundles were 20 in *Lagenaria vulgaris*, 10 in *Benincasa hispida*, 12 in *Cucurbita maxima* and 9 in *Cucumis sativus* and *Luffa acutangula* respectively. Presences of stomata are commonly seen in all five species. The Trichomes were multicellular, glandular or non-glandular, unbranched and uniseriate. Non-glandular trichomes were predominantly seen in the genera studied, whereas *Lagenaria vulgaris* and *Luffa acutangula* have glandular trichomes. The variations in the number of tiers within these trichomes were taxonomically significance.

Keywords: cucurbitaceae, anatomy, glandular trichome, non-glandular trichome

1. Introduction

According to Hutchinson this is the thirteenth order of the phylum Angiospermae, subphylum Dicotyledones and division Lignosae. The order comprises of four families, i.e., Cucurbitaceae, Begoniaceae, Datisceae, and Caricaceae. The Cucurbitaceae is primitive most and the Caricaceae is most evolved within the order. These two families have been described in this text in detail. Bentham and Hooker have placed the families Cucurbitaceae and Caricaceae (under the name Passifloreae) along with other five families in the thirteenth order-Passiflorales of class Dicotyledones, division Polypetalae the series Thalamiflorae. Engler and Prantl have included the family Caricaceae along with other twenty eight families including Violaceae in their twenty ninth order- the Parietales of class Dicotyledonae and subclass Archichlamydeae. They have placed the single family Cucurbitaceae in their ninth order- Cucurbitales of class Dicotyledoneae and subclass sympetalae^[4].

The family Cucurbitaceae includes about 100 genera and 850 species. Constantly unisexual; ovary inferior with parietal placentation; endosperm scanty or often present; corolla sometimes sympetalous; stipules present or absent. The members belonging to this family are mainly tropical and subtropical in distribution. Only few genera are found in temperate regions. In our country the family is represented by a large number of cultivated plants especially for their edible fruits, e.g., *Cucumis sativus*, *Cucurbita maxima*, etc.

The study of morphology with a compound microscope is known as anatomy (histology). According to some anatomists the anatomical features are more conservative than those of gross morphology and are, therefore, of greater use in tracing phylogeny or organogenesis. The following anatomical

characters are of taxonomic significance are epidermal appendages, trichomes and emergences of all kinds, structure of stomata, the wood anatomy including the elements of the secondary xylem; this is very useful in modern taxonomic studies, particularly in establishing the phylogenetic relationships among taxa, the nodal anatomy of the axis, the leaf trace nature, and floral anatomy, etc.

All unicellular and multicellular appendages are known as trichomes; more massive structure, e.g., warts, spinous bodies both epidermal and subepidermal is called emergences. The uses of trichomes in taxonomic studies are of much value, and have been known for many years. Simple unicellular or multicellular uniseriate trichomes occur in many taxa, e.g., Cucurbitaceae. Similarly stomata have been classified according to the position of subsidiary cells, guard cells in relation to the aperture. Anatomy of leaf and petiole has also been used for taxonomic delimitation of medicinal plants.

The objective of this investigation is to describe the petiole, leaf anatomical characters and powder microscopic studies of the *Lagenaria vulgaris*, *Benincasa hispida*, *Cucurbita maxima*, *Luffa acutangula* and *Cucumis sativus* and the assessment relevance and the extent to which anatomical features could be utilized in the biosystematics delineation of the Cucurbit species in view of their perceived similarities in structural and reproductive biology.

2. Materials and Methods

In order to study the variation of internal structural in the leaves, stems and roots of some cucurbits, microtome study was undertaken and microscopic anatomical observations were carried out following the method suggested by^[18, 23],^[9] and^[5].

2.1 Collection of specimens

The five plant species namely *Lagenaria vulgaris* Ser., *Benincasa hispida* (Thunb.) Cogn., *Cucurbita maxima* Duch., *Luffa acutangula* (L.) Roxb. and *Cucumis sativus* L of the family Cucurbitaceae were collected from field in Allithurai, Somarasampettai area of Tiruchirappalli District. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – Butyl alcohol (TBA) as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58°-60° C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.2 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 by customary procedure (Johansen, 1940) [15]. The sections were stained with Toluidine blue as per the method published by O'Brien *et al.* (1964) [21]. 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 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 IKI (for Starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) [25] were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

2.3 Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964).

3. Results and Discussion

The anatomical study features of the petiole and leaves of the *Lagenaria vulgaris*, *Benincasa hispida*, *Cucurbita maxima*, *Luffa acutangula* and *Cucumis sativus* have been presented in the form of photomicrographs of the transverse sections of these parts. Trichome differences in the taxa were remarkable. Using the most comprehensive trichome terminology [10, 22] there were two basic hair types (glandular and non – glandular) and many variations among the species under study. All species showed multicellular and non-glandular trichomes with various shapes. Glandular trichomes with various tiers were observed in *Lagenaria vulgaris* and *Luffa acutangula* [21]. The glandular trichomes have been implicated for storage [10]. The non-glandular trichomes were useful as defense organs for the plants that possess them [22]. Stomata are actinocytic in which the guard cells are surrounded by four or more subsidiary cells that elongated radially to the guard cells cyclocytic in which the 4 or more subsidiary cells are arranged in the form of a ring around the stomata.

Various investigations have been made on the anatomy of Cucurbits, most of them prior to 1940 [27]. Although differences exist between Cucurbit species, it had shown that, in general, there was considerable similarity among them [13]. There are some reports on epidermal and vegetative characteristics of the five species *Lagenaria vulgaris*, *Cucurbita maxima*, *Benincasa hispida*, *Luffa acutangula* and *Cucumis sativus* which agree with the results of this study [1, 2].

Table 1: Anatomical features of leaf in selected five species of Cucurbitaceae

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
Leaf	The leaf consists of a thick midrib and thin lamina. The midrib has a thick flat Adaxial hump and prominent and large adaxial part (fig 1.A).	The leaf consists of convex-conical midrib and thin tomentose lamina (fig.2.A).	The leaf is characterized by thick midrib and thin lamina, both of which have dense epidermal trichomes.	The leaf has prominent characteristic midrib and thin lamina (fig4.A).	The leaf consist prominent midrib; the midrib has small semicircular adaxial hump (fig.6.A) and thick semicircular abaxial part.
Midrib	The mid rib is 2mm thick and 1.6mm wide.(4X)	The midrib is 1.4mm thick and abaxial part is 1.3mm wide; the adaxial part is 450µm wide (4X).	The midrib is 1.7 mm thick and 1.9 mm wide (4X).	The midrib is 1.35 mm thick 2mm wide (4X).	The midrib is 2mm thick and 1.8mm wide (4X).
Vascular system	The vascular system includes two units of vascular bundles, one unit is adaxial in position and the other is abaxially placed (fig1.B).	The vascular system consists of a larger abaxial median layer bundle and adaxial smaller and another further smaller bundles (fig.2.C).	The vascular system of the midrib is multi stranded. There is a large median abaxial bundle, a pair of small lateral bundles, one on either side of the midrib and abaxial median bundle, and a pair of small bundle situate at the top of the midrib. Along the abaxial end of the midrib, these are four smallest, less prominent bundles arranged in horizontal row (fig.3.A)	The vascular system of the midrib consists of a large cup shaped vascular strand which is cleaved into a median large bundle and a small lateral bundle (fig 4.B).	The vascular system includes a vertical row of three vascular bundles of which the bundle in the adaxial hump is the smallest; the bundle below is slightly large and the median bundle is the largest (fig.6.B).
Vascular bundle	The vascular bundles are bicollateral.	The vascular bundles are bicollateral with phloem located on the both outer and inner portions of the vascular bundle (fig.2.C).	All vascular bundles are either circular or broadly conical and are bicollateral with phloem located both on the outer and inner parts of the vascular bundles (fig.3.A).	Both larger and smaller bundles are bicollateral, having phloem tissue both on the outer and inner sides if the xylem (fig4.B).	The median bundle is elliptical in outline.it is bicollateral with phloem located on both ends of the xylem strand (fig.6.B).
Xylem and Phloem	The Meta xylem vessels are 50µm wide. The phloem elements include small, angular, thick walled darkly stained sieve elements and parenchyma cells.	The xylem elements of the vascular bundles are thick walled, wide, circular and cluster of vessels. The metaxylem vessels are 40 µm wide. The vessels are bedded in xylem fibres. Phloem includes small angular, darkly stained sieve elements and phloem parenchyma (fig2.C).	Bundles have large cluster of circular/elliptic vessels which have wide, thick walls. Thick segments of phloem occur on both outer and inner part of the xylem and includes small sieve elements and parenchyma cells the adaxial row of four smallest bundles also have central small cluster of vessels surrounded by phloem element.	Xylem comprises about eight vertical rows of vessels, each row having four cells. Protoxylem is facing adaxially, the metaxylem elements are 70µm wide, and The adaxial phloem is in the form of cup. The phloem elements are small, square shaped and darkly stained.	Xylem consists of four or five vertical line of wide, circular thick walled vessels. The Protoxylem elements are directed towards upper side. Phloem units consist of densely packed small, thick walled sieve elements and phloem parenchyma.
Lamina	The lamina is 120µm thick. It is dorsiventral with district adaxial and abaxial sides. The adaxial and abaxial side has fairly thick epidermal layer of thin walled rectangular cells.	The lamina is 200 µm thick. The adaxial epidermis of the lamina consists of the thick and wide, rectangular and squarish cells. The Mesophyll tissue consists of adaxial single layer of columnar palisade	The lamina is 110µm thick. The lamina is dorsiventral with adaxial palisade layer and abaxial spongy Mesophyll cells (fig.3.C). The adaxial epidermis is thin and the cells are small elliptical and thick walled. The adaxial epidermis is also thin with small cells and thick cuticle.	The lamina is 80µm thick. The lamina is doriventral with adaxial palisade layer and abaxial spongy parenchyma. The adaxial and abaxial epidermal cells are circular wide and thin walled. The palisade zone is consists of	The lamina is 220µm thick. The lamina is uniformly thick and even on both upper and lower surface. The adaxial epidermis consists of broadly elliptical thin walled cells. The thickest epidermal cell is

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
	<p>The Mesophyll tissue is differentiated into adaxial horizontal two rows of palisade cells.</p> <p>The palisade cells are columnar in shaper and are loosely arranged.</p>	<p>cells and abaxial zone of about six layers of small lobed spongy parenchyma cells which form wide air-chambers.</p>	<p>The palisade parenchyma cells are in two horizontal layer, they are columnar cells and compactly arranged.</p>	<p>single horizontal layer of columnar, contact cells.</p>	<p>30µm thick.</p> <p>The Mesophyll tissue is differentiated into adaxial single layer of columnar palisade cells which are 80µm in height.</p>
Leaf-Margin	<p>The marginal part of the lamina is semicircular and 80µm thick.</p> <p>The epidermal cells are well developed and are circular and thick walled.</p> <p>There is a sub epidermal layer of circular cells; the Mesophyll tissue is not differentiated distinctly.</p> <p>The abaxial portion has spongy Mesophyll cells which are spherical, loosely arranged and arencymatous</p>	<p>The epidermis is 30 µm thick.</p> <p>The abaxial epidermis is thin and the cells are spindle shaped with thick cuticle.</p>	<p>The marginal and submarginal part of lamina is swollen and measures 229µm in the marginal and 300µm in the submarginal parts.</p> <p>The Mesophyll differentiation is not well expressed in the marginal tissue.</p> <p>The spongy parenchyma cells are small, spherical or lobed and are compact.</p>	<p>The marginal part of the lamina is slightly bentdown the leaf margin is 130µm thick.</p> <p>The adaxial epidermal cells are highly dilated and the cells are enlarged both in tangential and radial planes.</p> <p>The spongy parenchyma cells are about six layered, the cells being lobed and inter linked with other with wide air-chambers.</p>	<p>The abaxial epidermal cells are comparatively thin and the cells are rectangular in shape.</p> <p>They are also thin walled.</p> <p>The spongy Mesophyll tissue includes about six layers of spherical or lobed cells which are loosely arranged forming air spaces.</p>

Table 2: Anatomical features of petiole in selected five species of Cucurbitaceae

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
Petiole	<p>The petiole is broadly elliptical/ circular; it is a hollow cylinder with wide central cavity (fig1.C).</p> <p>The petiole is 1.5mm thick.</p>	<p>The petiole is broadly elliptical in sectional view (fig.2.D).</p> <p>The adaxial end of the petiole has a wide shallow groove.</p>	<p>The petiole is circular in cross seen oval outline with shallow ridges and furrows (fig3.D).</p> <p>The petiole is hollow cylinder with wide central canal.</p> <p>The circular segment of the petiole is 550µm.</p>	<p>The petiole is elliptic – oblong with two prominent lateral wings and central deep groove.</p> <p>With in the groove is a small hump on the adaxial part (fig 4.D).</p> <p>The petiole is 3.1mm in vertical plane and 2.5mm in horizontal plane.</p>	<p>The petiole is obliquely elliptical in outline with wide and deep adaxial groove (fig.6.C).</p> <p>The petiole is 3mm in vertical plane and 2mm in horizontal plane.</p>
Epidermal layer	<p>The epidermis is thick epidermal layer of thin walled rectangular cells.</p> <p>The ground tissue is</p>	<p>The epidermal layer of the petiole consists of radially oblong fairly wide cells with prominent cuticle.</p>	<p>The petiole includes epidermal layer, outer cortical collenchyma, inner parenchymatous ground tissue and 12 vascular bundles distributed all along the circular petiole (fig 3.E).</p>	<p>The petiole has thin epidermal layer of small squarish thick walled cells.</p> <p>The ground tissue within the adaxial wings includes</p>	<p>The petiole has thin epidermal cells.</p> <p>Inner to the epidermis is narrow zone of three layer of chlorenchyma.</p>

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
	parenchymatous. Some three or four outmost layers are collenchymatous			collenchyma cells (fig 4.E). The entire petiole has thin walled rectangular epidermal cells.	Remaining part of the petiole has homogeneous parenchymatous compact ground tissue.
Vascular system	About 20 vascular bundles are of different sizes are located all around within the petiole. The vascular bundles are bicollateral. The bicollateral vascular bundles have central horizontal band of xylem elements, the elements being wide, circular, thick walled vessels. The metaxylem vessels are 35µm wide. Xylem also includes xylem fibers. On the inner part of the vascular bundles these is a thick and massive inner phloem. On the outer part of the vascular bundle also occurs thick unity phloem. The phloem elements are fairly wide, thick walled and darkly stained. Phloem parenchyma cells are also occurs in the phloem (Fig.1.D).	All along the circular portion of the petiole, these are several vascular bundles arranged with in the petiole (fig.2.D). The vascular bundles are variable in size. They are broadly elliptical and bicollateral. The bundle consists of middle xylem elements and phloem elements on outer as well as in inner portions. The central xylem includes wide, circular thick walled cluster of vessels. The vessels are up to 60 µm wide. The phloem elements include wide angular, darkly stained sieve elements and phloem parenchyma.	The vascular bundles towards the adaxial groove are smaller than those in lower end of the petiole (fig.3.E). The vascular bundles are bicollateral with phloem located both on the outer and inner ends of the vascular bundles. The phloem elements are sieve elements which are wide, thick walled and compact phloem also includes phloem parenchyma cells which are smaller and thin walled (fig.3.E). The vessels are wide cluster; they are circular and thick walled. The meta xylem elements are 40µm in diameter.	The vascular system of the petiole includes nine collateral vascular bundle which are disposed all along middle-peripheral part of the petiole. The vascular bundles are radially oblong and bicollateral. The wing bundle is circular in outline with central clustering xylem elements and phloem elements located outer and inner part of the xylem. The bundles have three or more parallel radial lines of wide circular thick walled vessels. The metaxylem vessels are 50µm in diameter. Phloem elements are angular, wide lumened and thick walled (fig4.F).	The vascular system of the petiole consists of about nine vascular bundles which are independent and arranged in a wide area. The bundles are bicollateral with phloem located on the outer and inner sides of the Xylem strand (fig.6.D) of the several vascular bundles; one which is located at the wing portion is the smallest. Other abaxial and lateral bundles are large and broadly elliptical. The xylem element namely vessels are wide, circular to elliptical in out line, thick walled and apparently arranged in vertical lines. The metaxylem elements are 50µm in diameter. Phloem occurs in thick masses at the Protoxylem and metaxylem ends (fig.6.E). The phloem elements include wide, thick walled and compact sieve elements and phloem parenchyma cells.

Table 3: Powder Microscopic Observation selective five plants of Cucurbitaceae

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
Epidermal trichomes	Trichomes borne on the epidermal cells are common on all parts of the plants. Two types of trichomes are seen in powder. They are Glandular trichome and Non-glandular trichome.	Non glandular covering types of trichomes are abundant in the powder (fig.2.E). The trichomes are two to five celled, uniseriate and unbranched. The lower most cell is	The epidermal cells which bear the trichomes are visible. The structure consists of a central group of two to five cells which are called basal cells; from the basal cells arise the trichome. The trichome bearing basal cells are surrounded by a ring of semicircular	Multicellular, uniseriate, unbrached, nonglandular, epidermal trichomes are abundant in the powder. Some of the trichomes are seen marginal part of the lamina (fig 4.G). The trichomes are also seen isolated from the leaf. The isolated trichome has have bulged basal cell with which the	Some of the trichomes are two celled and are short with wide basal cell and sharp and pointed terminal cell. These trichomes are 200µm long (fig.6.F). The two celled trichome may all be 350µm long.

Characters	<i>Lagenaria vulgaris</i>	<i>Benincasa hispida</i>	<i>Cucurbita maxima</i>	<i>Luffa acutangula</i>	<i>Cucumis sativus</i>
	<p>The glandular trichomes are long with about five, vertical, uniseriate and unbranched structure.</p> <p>The cells are vertically elongated and thin walled.</p> <p>The terminal cell is swollen and modified into spherical, four or more celled, secretory head with deuse cytoplasm (Fig1.E).</p> <p>In most of the glandular trichomes, the glandular head or the basal cell possesses solid calcium carbonate cystolith crystal (Fig.5.B).</p> <p>The cells of the trichomes have deused darkly stained cell contents (fig1.E).</p> <p>The glandular trichomes are up to 750µm long and 150µm thick.</p> <p>The Non_glandular_trichomes are covering type without any secretory structure.</p> <p>They are multicellular, uniseriate and unbranched structure. They are broad at the base and gradually tapering with pointed ends.</p> <p>The trichomes are 250-450µm long and the basal cells are up to 40µm thick.</p> <p>The trichomes have dense protoplast and cystolith bodies at the basal cells (Fig5.B).</p>	<p>broad and the upper cells are gradually thin and the tip is pointed.</p> <p>The cell walls are thin and the cell lumen is wide.</p> <p>In some of the trichomes, large spherical calcium carbonate cystolith is present (fig.4.B).</p> <p>The epidermal cells around the epidermis form a circle of subsidiary cells.</p> <p>The subsidiary cells may also possess cystoliths (fig.5.B).</p> <p>The trichomes are 200-260 µm long.</p> <p>The basal cells are bigly dilated and form a whorl of subsidiary cells.</p>	<p>epidermal cells which are called the subsidiary cells.</p> <p>The subsidiary cells and basal cells are thick walled. Some of the basal cells possess solid crystal of calcium carbonate (fig.4.B).</p> <p>Non glandular, covering types of epidermal trichome are abundant in the power.</p> <p>The trichome are Multicellular uniseriate unbranched and broad at the base, gradually tapering into pointed tips (fig3.F).</p> <p>The trichome are straight or slightly curved (fig 3.G), the basal cell is slightly bulged and the cells are both vertically elongated and rectangular.</p> <p>The cells may shortly rectangular and wide; the cells become much elongated towards upper portion.</p> <p>The trichomes are surrounded by bulged epidermal cell at the base (fig.3.G).</p> <p>The trichomes are up to 650µm long and 80µm wide.</p> <p>Calcium carbonate crystal bodies are seen in some of the trichomes (fig.5.B).</p>	<p>trichomes are attached epidermal layer of the leaf.</p> <p>The trichomes may be straight or curved (fig 4.I).</p> <p>Some of the trichomes thick walls, wide lumen and prominent nuclei (fig 4.I).</p> <p>The straight trichome without cell inclusions are 500µm long and the basal part is 50µm wide.</p> <p>The trichome with prominent protoplasmic inclusions is 220µm long and 40µm thick.</p> <p>Glandular trichome (fig 4.H, I) is occasionally seen in the powder. They club shaped and short.</p> <p>They have a stalk cell, a neck cell and Multicellular spherical glandular head.</p> <p>The trichome is 150µm long and the Multicellular glandular head is 60µm thick.</p>	<p>These are trichomes which are four celled, long and tapering into pointed top (fig.6.F).</p> <p>These trichomes are 430-600µm long. The basal cells are 40-70µm wide.</p> <p>In many of the trichomes, basal cell possesses a thick spherical calcium carbonate cystolith (fig 5.B).</p> <p>In surface view of the epidermis, the trichome bearing epidermal cell is circular, wide and thick walled.</p> <p>This cell is surrounded by one or two rings of elliptical subsidiary cells; each ring has three or four subsidiary cells.</p> <p>The epidermal cells are angular thin Wally and highly way.</p>
Stomata	Stomata are common.(fig.5.A)	Stomata are common.	<p>Stomata are common.</p> <p>They are widely elliptic or circular in outline.</p> <p>The stomatal pore narrow and straight. The guard cells are 40x50µm in size.</p>	Stomata are common.	<p>The leaf powder shows epidermal peeling where stomata are visible.</p> <p>The stomata are narrowly elliptical measuring 50x150µm in size (fig.5.A).</p>

4. Conclusions

Aspects of leaf and petiole anatomy of these species *Lagenaria vulgaris*, *Benincasa hispida*, *Cucurbita maxima*, *Luffa acutangula* and *Cucumis sativus* confirm their affinity as they show marked similarities and overlaps in the number of layers of cell and tissues and also in their distribution and differentiation. However, the distinct numbers of petiole-vascular bundles varied in the five species with *Lagenaria vulgaris* having 20, 10 in *Benincasa hispida*, 12 in *Cucurbita maxima*, *Cucumis sativus* and *Luffa acutangula* having 9 in numbers. This provides an additional information for maintaining their distinction into five different taxa. This also agrees with the taxonomic values of petiolar vascular bundle as earlier highlighted by Metcalfe and Chalk (1979) [20].

Epidermal trichomes are abundant on the leaf and petiole of the species studied. The trichomes are multicellular, uniseriate and unbranched. The trichomes are nonglandular covering type. The trichomes are broad at the base and gradually tapering into pointed tip. The trichome arises from large dilated epidermal basal cells, the trichome bearing epidermal cell are encircled by three or more subsidiary cells. Glandular trichomes are seen in *Lagenaria vulgaris* and *Luffa acutangula*. They are club shaped and short. They have a stalk cell, a neck cell and Multicellular spherical glandular head. Calcium carbonate crystal bodies are seen in some of the trichomes. Stomata are common. They are widely elliptic or circular in outline, the stomatal pore narrow and straight.

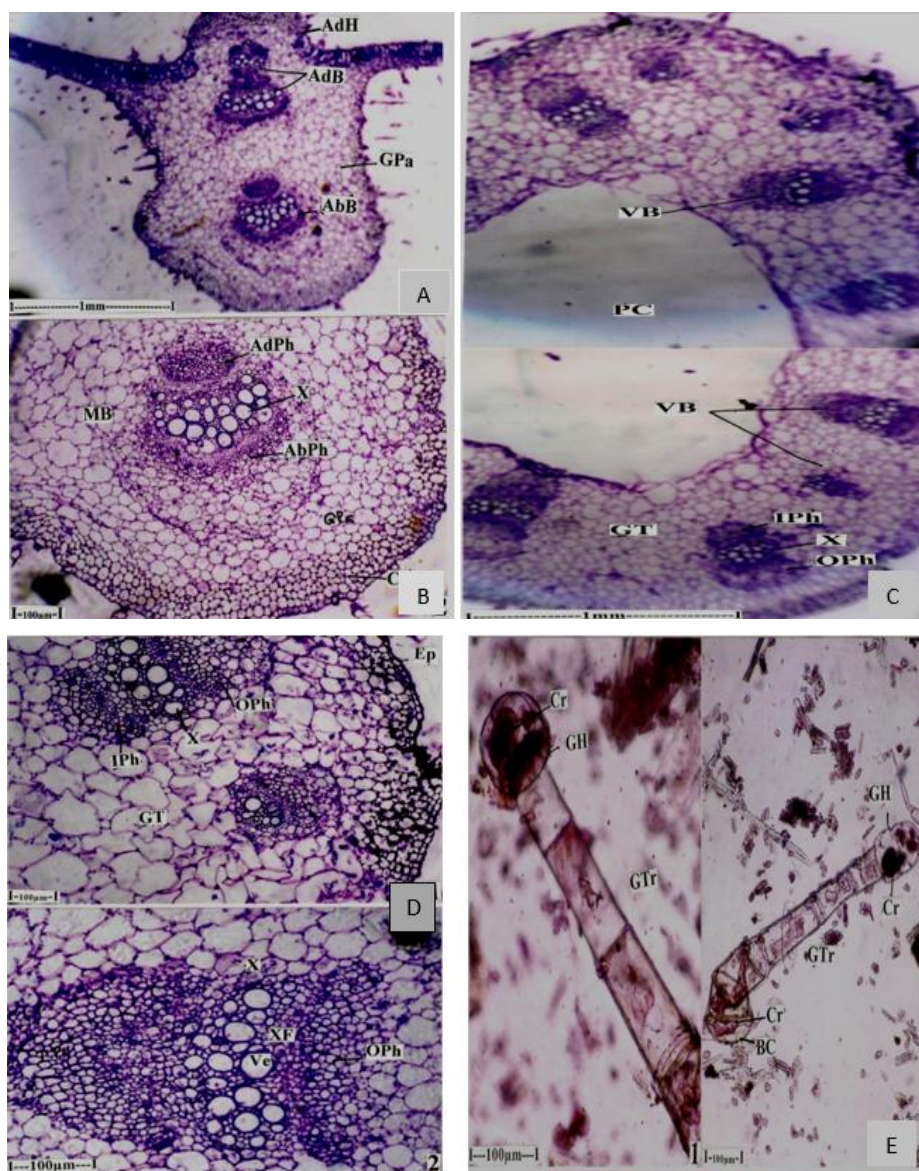


Fig 1 A-E: Anatomical features of *Lagenaria vulgaris*. A: T.S of midrib- entire view, B: Abaxial vascular bundle of the midrib- enlarged, C: T.S of Petiole-, D: vascular bundle of the petiole- enlarged, E: Two glandular trichomes with terminal spherical head, (AbB: Abaxial Bundle; AbPh: Abaxial Phloem; AdB: Adaxial Bundle; AdH: Adaxial Hump; AdPh: Adaxial Phloem; Gpa: Ground parenchyma; MB: Median Bundle, GT: Ground Tissue, IPh: Inner Phloem, OPh: Outer Phloem, Ve: Vessels, XF: xylem Fibre, X: Xylem, BC: Basal Cell, Cr: Crystals, GTr: Glandular Trichome, GH: Glandular Head, Cr: Crystals).

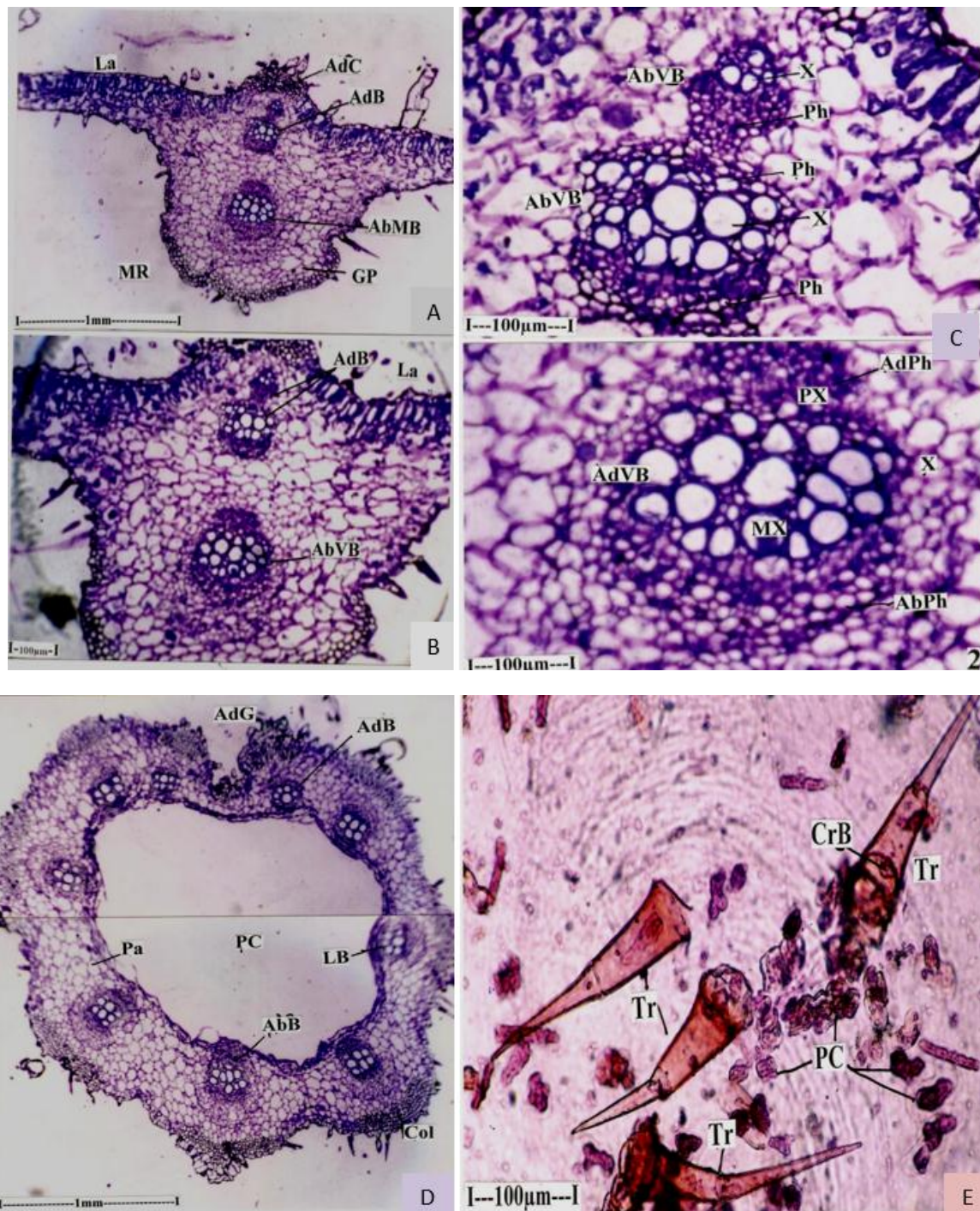


Fig 2 A-E: Anatomical features of *Benincasa hispida*. A: T.S of leaf through midrib, B: T.S of mid rib- enlarged, C: Abaxial and Adaxial vascular bundle of the midrib, D: T.S of Petiole- upper and lower portions (entire view), E: Isolated nonglandular epidermal trichomes. (AdC: Adaxial Cone, AdB: Adaxial Bundle, AbMB: Abaxial median bundle, GP: Ground Parenchyma, La: Lamina, MR: Midrib, AbUB: Abaxial upper vascular bundle, AbLB: Abaxial lower vascular bundle, AbPh: Abaxial phloem, AdVB: Adaxial vascular bundle, AbVB: Abaxial vascular bundle, AdPh: Adaxial phloem, MX: Metaxylem, Ph: Phloem, X: xylem, AdB: Adaxial Bundle, AbB: Abaxial Bundle, AdG: Adaxial Groove, Col: Collenchyma, LB: Lateral Bundle, Pa: Parenchyma, PC: Pith Canal). (BC: Basal Cell, CrB: Crystal Body, PC: Palisade Cells, Tr: Trichomes)

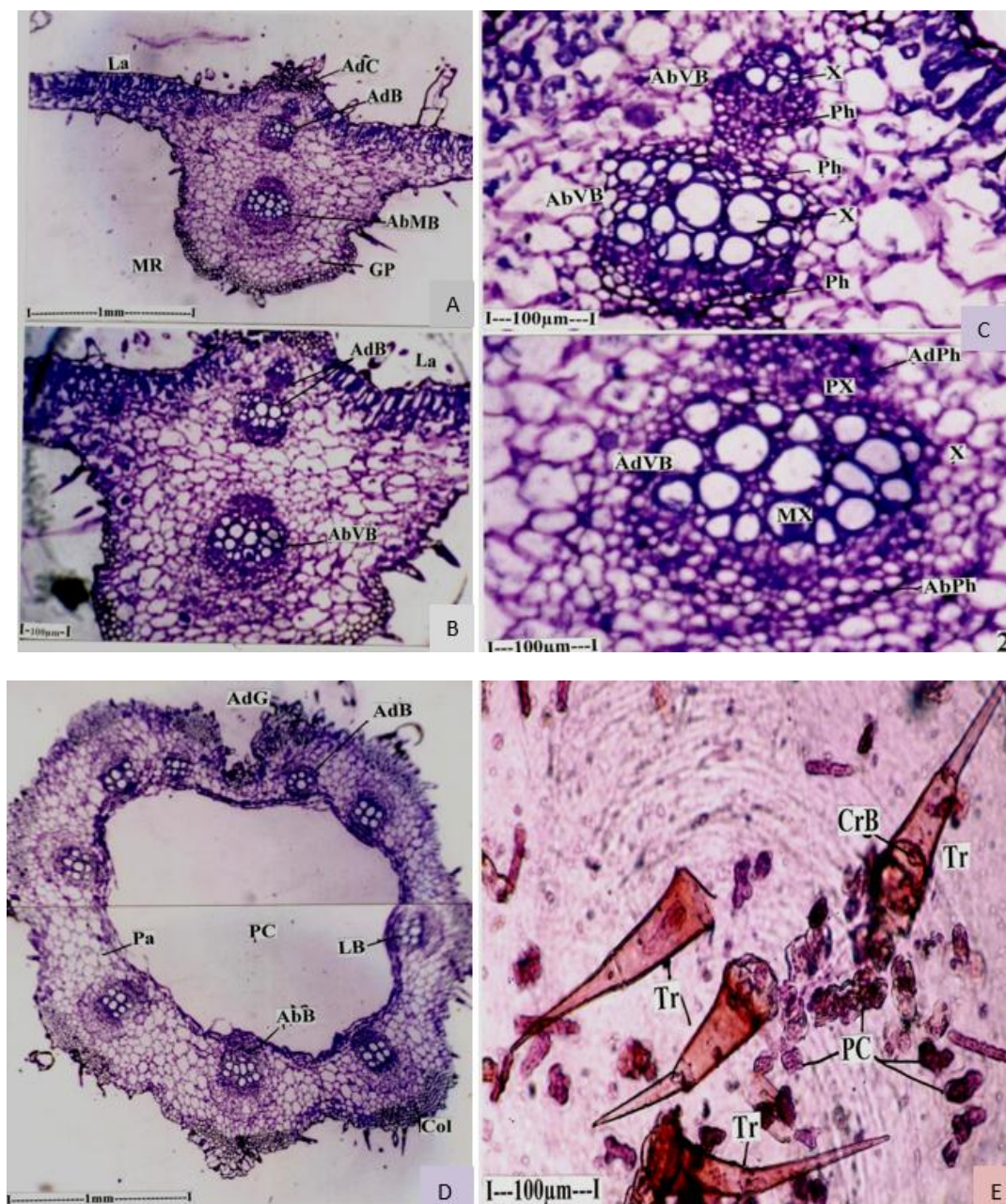


Fig 3 A-G: Anatomical features of *Cucurbita maxima*, A: TS of leaf through midrib and enlarged, B: T.S of lamina, C: T.S of lamina bearing epidermal trichome, D: TS of Petiole – entire view, E: Vascular bundle of the Petiole –enlarged, F: Epidermal fragment showing Trichome bearing epidermal cells, G: Marginal trichomes(AbMB: Abaxial Median Bundle, AdPh: Adaxial Phloem, AdB: Adaxial Bundle, AdLB: Adaxial Lateral Bundle, AbB: Abaxial Bundle, AbPh: Abaxial Phloem, AdS: Adaxial Side, Col: Collenchyma, La: Lamina, LB: lateral Bundle, IPh: Inner Phloem, GP: Ground Parenchyma, MR: Midrib, OP: Outer Phloem, Tr: Trichome, X: Xylem, AdEp: Adaxial Epidermal, BC: Basal Cell, MT: Mesophyll cells, PM: Palisade Mesophyll, Tr: Trichome, SM: Spongy Mesophyll, CCA: Central canal, Ad G: Adaxial Groove, IEp: Inner Epidermis; I Ph: Inner Phloem; GP: Grand Parenchyma; MX: Metaxylem; OEp: Outer Epidermis; OPh: Outer Phloem; Px: Parenchyma; VB: Vascular Bundle, BC: Basal cells; Ep: Epidermis; Tr: Trichomes)

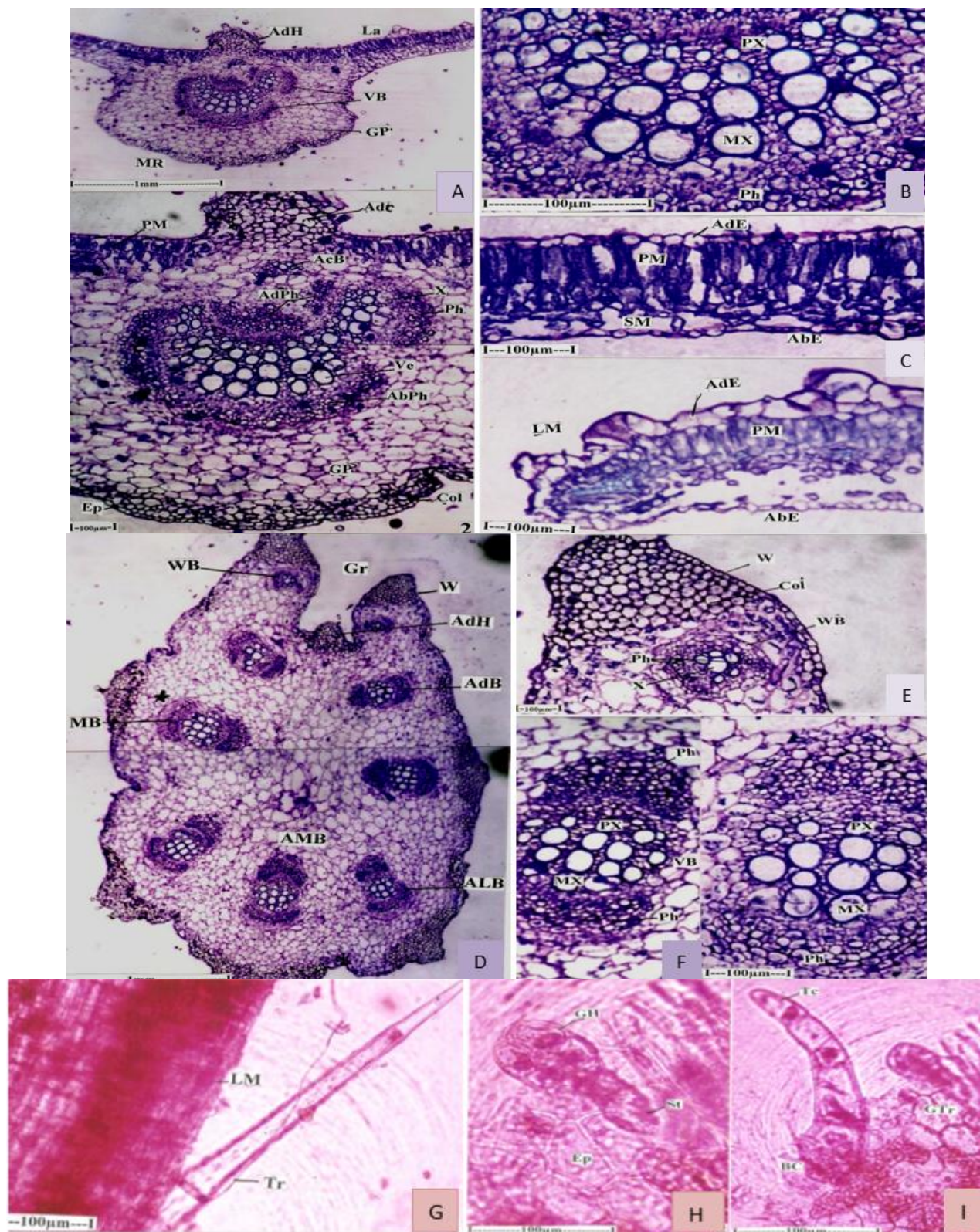


Fig 4 A-I: Anatomical features of *Luffa acutangula*. A: TS of leaf through midrib- enlarged, B: Vascular bundle of the midrib, C:T.S of lamina and T.S of leaf margin, D: T.S of petiole – entire view, E: Wing portion of petiole with vascular bundle, F: vascular bundle of petiole- enlarged, G: Epidermal nonglandular trichome, H: glandular trichome, I : non glandular and glandular trichome, (AbPh: Abaxial Phloem, AdH: Adaxial Hump, AcB: Accessor Bundle, AdT: Adaxial collenchyma of the hump, col: collenchyma, GP: Ground parenchyma, La: lamina, MR: Midrib, PM: palisade Mesophyll, Ph: Phloem, VB: Vascular Bundle, Ve: vessel, X: Xylem, AbE: abaxial epidermis, AdE: adaxial epidermis, MX: metaxylem, LM: leaf margin, PX: Protoxylem, SM: spongy Mesophyll, AdB: Adaxial Bundle, AMB: abaxial median bundle, ALB: Adaxial lateral bundle, GR: Groove, MB: Median Bundle, W: wing, WB: wing bundle, BC: basal cell, Ep: epidermal, GH: glandular Head, Gtr: Glandular trichome, St: Stalk cell, Tc: terminal cell.)

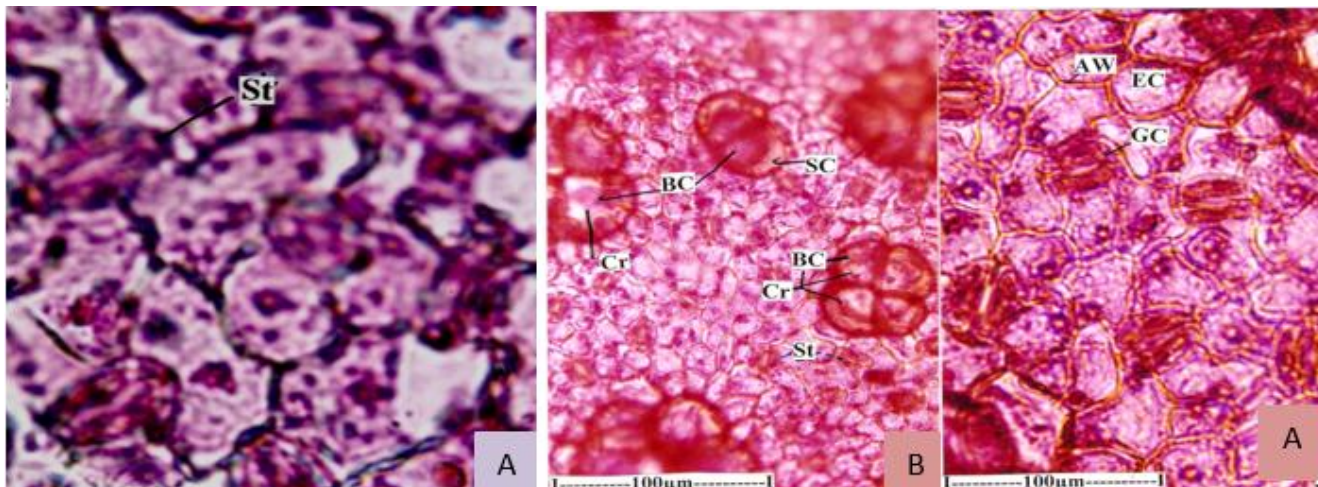
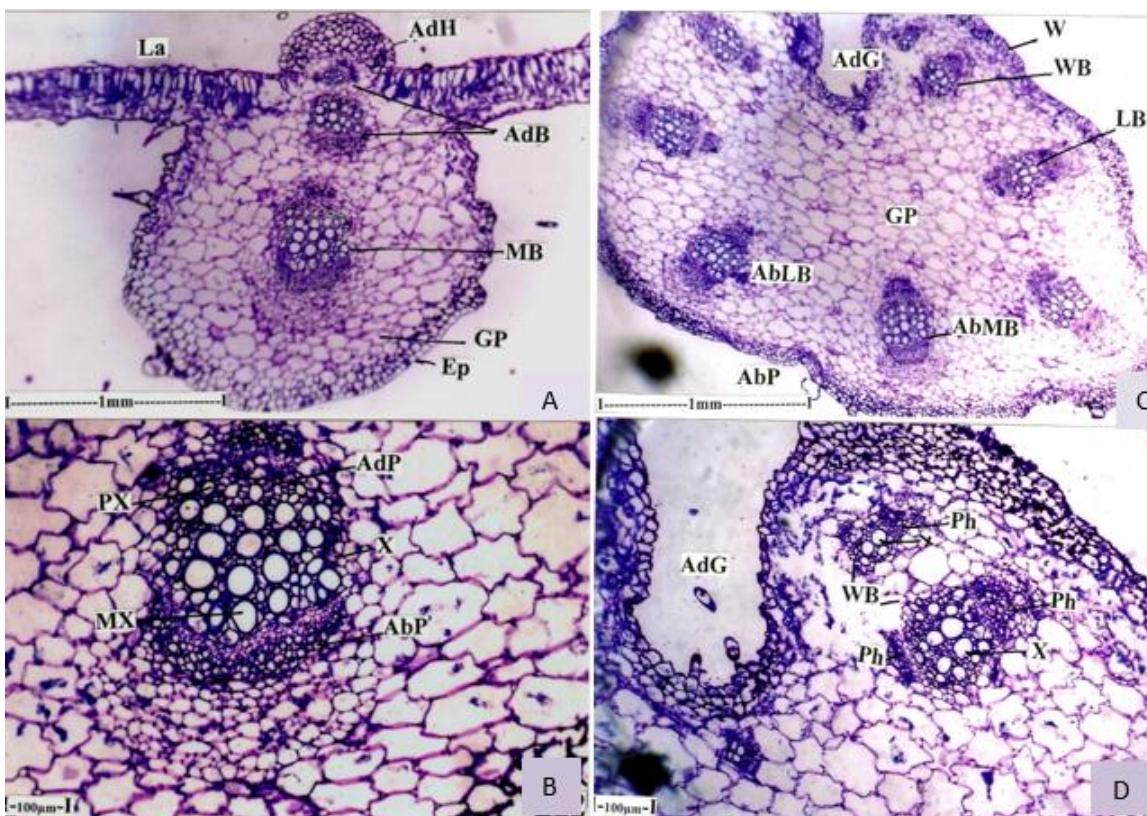


Fig 5 (A): Actinocytic stomata of the lamina, (B) Epidermal trichome bearing cells with calcium carbonate crystals, (BC: Basal cell, SC: Subsidiary cell, St: Stomata, AW: Anticlinal walls, EC: Epidermal cells; Cr: Crystal; GC: Guard cells)



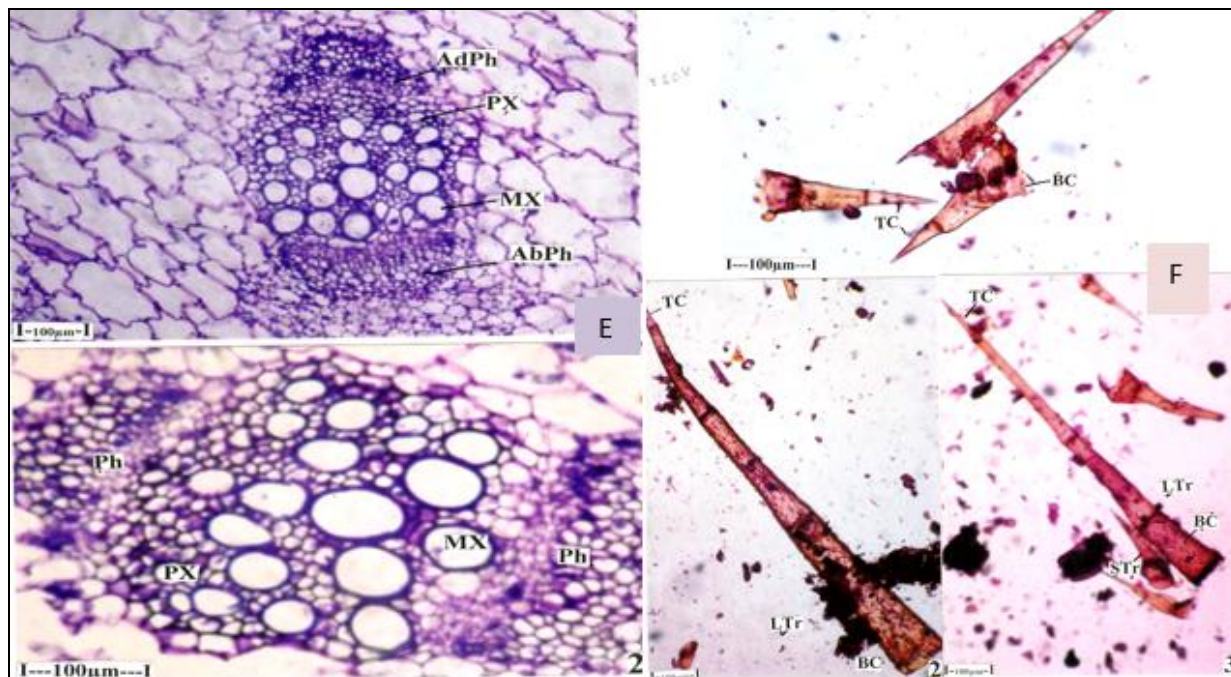


Fig 6 A-F: Anatomical features of *Cucumis sativus*, A: T.S of leaf through midrib, B: Median vascular bundle of the midrib-enlarged, C: T.S of Petiole – entire view, D: Petiole – wing bundle-enlarged, E: Abaxial median bundle and Adaxial lateral bundle of the petiole, F: long and short nonglandular Trichomes. (AbP: Abaxial Phloem, AdP: Adaxial Phloem, AdB: Adaxial Bundle, AdH: Adaxial Hump, Ep: Epidermis, GP: Ground Parenchyma, La: lamina, MX: Metaxylem, X; Xylem, AbLB: Abaxial Lateral Bundle, AbMB: Abaxial Median Bundle, AdG: Adaxial Groove, LB: Lateral Bundle, Ph: Phloem, W: Wing, WB: Wing Bundle, PX: Protoxylem, BC: Basal Cell, LTr: Long Trichome, STr: Short Trichome, TC: Terminal cell)

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