

# Comparative petiole and stem anatomy of species of the genera *Bryophyllum* and *Kalanchoe* in South-western Nigeria

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**Abstract:** The petiole and the stem anatomy of *Kalanchoe* and *Bryophyllum* species were studied to document characters that are of taxonomic importance among the species. The species are *Kalanchoe crenata*, *Kalanchoe lanceolata*, *Bryophyllum pinnatum*, *Bryophyllum delagoense* and *Bryophyllum houghtonii*. Transverse sections of the median region of the petioles and the stem of each of the species were made using a Reichert sliding microtome at 8-15 $\mu$ m thickness. Unifying and diagnostic features were also identified. Unifying features such as starch grains present on the petiole, parenchymatous pith in the stem were recorded. Diagnostic features such as biseriate to multi seriate epidermis in the stem of *Bryophyllum pinnatum* were also recorded. The unifying characters suggest that the species of the two genera should be combined into one genus *Kalanchoe* with sections under it.

**Keywords:** Parenchymatous, uniseriate, biseriate, triseriate, Amphicribal.

## INTRODUCTION

Members of the family Crassulaceae are perennial herbs, sub-shrubs, shrubs (seldom tree-like or aquatic) usually with fleshy leaves, at times with succulent stems, rhizomes, underground caudices or succulent roots (t' Hart, 1997; Moran, 2009). Crassulaceae is the third largest succulent plant family, after Aizoaceae and Cactaceae with 1,500 species from 35 genera (Heywood *et al.*, 2007). Some members of the Crassulaceae family are edible and many of them are used as medicine around the world (Arnold *et al.*, 2002; Gonzalez *et al.*, 2018). Genera *Kalanchoe* Adans. and *Bryophyllum* Salisb. belong to the the subfamily Kalanchoideae (Descoings, 2003; Thiede & Eggli, 2007). The genus *Kalanchoe* consist about 150 species found majorly in the dry regions of East and South-West Africa and some of its adjacent Islands, and in South-East Asia (Descoings, 2006).

*Bryophyllum* Salisb. on the other hand contains about 230 species (Descoings, 2003; Thiede & Eggli, 2007). Species of the genus *Bryophyllum* are garden escapes in many parts of the world while few are aggressive invaders, as a result of their ease of reproduction and propagation (Gledhill, 2008; Walters, 2011).

The anatomy of plants is useful in the taxonomic characterization of plants because they are not usually affected by the changes in the environment and therefore, highly conservative in taxa variations (Stace, 1980). Chernetsky (2012) used morphological and anatomical structure of leaves of 35 species of the genus *Kalanchoe* Adans. in solving taxonomic problems in subfamily Kalanchoideae. Chernetsky & Weryszko-Chmielewska (2008) described the structure of the leaves of *Kalanchoe pumila* Bak. Sharma & Naresh (2014) carried out pharmacognostical studies on *Bryophyllum pinnatum*.

There has been questions raised on the taxonomy of the subfamily Kalanchoideae. Some say that the genera *Kalanchoe* and *Bryophyllum* should be distinguished as separate within the subfamily (Zepkova, 1976, 1977, 1980; Takhtajan, 1966, 1987; Vinogradov *et al.*, 1976, 1978) while some say that the two genera should be merged into one genus *Kalanchoe* with sections under it (Jacobsen, 1981; Descoings, 2003; Descoings, 2006; Chernetsky, 2011). There is also little information about the petiole and stem anatomy of the species of genera *Bryophyllum* and *Kalanchoe* especially in Nigeria.

## MATERIALS AND METHOD

Five species, being two from genus *Kalanchoe* and three from genus *Bryophyllum* were used for this work. They are *Kalanchoe crenata* (Andr.) Haw, *Kalanchoe lanceolata* (Forsk.) Pers, *Bryophyllum pinnatum* (Lam.) Oken, *Bryophyllum delagoense* (Eckl. and Zeyh.) Schinz, *Bryophyllum houghtonii* (D.B Ward) P.I.Forst.

Specimens from these species were collected from different locations on Obafemi Awolowo University, Campus, Ile ife, Osun state, Nigeria with GPS location, N 07° 31.252' E 04° 31.307'.

### Petiole anatomical study

A transverse section of the median region of the petioles of each of the species was made using a Reichert sliding microtome at 8-15 µm thickness. These were stained with Safranin O for 5 minutes, rinsed and counterstained with Alcian blue for 3-5 minutes. The sections were then rinsed and passed through various dilutions of Ethanol- 50%, 70%, 80%, 90% and Absolute, for dehydration and differentiation. They were afterwards mounted in dilute glycerine solution and examined under the microscope.

### Stem anatomical study

Transverse Section (TS) of the stem of each of the species were made with a Reichert sliding microtome at 8-15µm thickness. These were stained with Safranin O for 5 minutes, rinsed and counterstained with Alcian blue for 3-5 minutes. After this, the sections were thoroughly rinsed and passed through various dilutions of Ethanol- 50%, 70%, 80%, 90% and Absolute, for dehydration and differentiation. They were then mounted in dilute glycerine solution and examined under the microscope.

## RESULTS

### Transverse section of petiole

#### *Kalanchoe crenata* (Andr.) Haw

The outline of the petiole was slightly concave on the adaxial surface and convex on the abaxial surface. The epidermis was uniseriate on the adaxial surface with thin undulating, striated cuticle on them. Abaxial surface was uniseriate with striated and undulating cuticle. The cortex consisted of 0-2 layers of angular collenchyma cells and 8-10 layers of thin walled parenchyma cells. The vascular bundle shape was Oval. Vascular bundle was Amphicribal (Table 1; Fig. 1 A-C).Cortical bundles present. Starch grains present.

#### *Kalanchoe lanceolata* (Forsk.) Pers,

The outline of the petiole was convex on the adaxial and abaxial surface. The epidermis was uniseriate with thick cuticle. The cortex consisted of 1-3 layers of angular collenchyma cells and 19-20 layers of thin walled parenchyma cells. Vascular bundles was arc-shaped. Vascular bundle was amphicribal. Cortical bundles present. Tannins more abundant, starch grains also abundant (Table 1; Fig. 1 D-G).

#### *Bryophyllum pinnatum* (Lam.) Oken

The outline of the petiole was convex on the adaxial surface, and concave on the abaxial surface. The epidermis was uniseriate with thin cuticle. The cortex consisted of 0-2 layers of angular collenchyma cells and 9-11 layers of thin walled parenchyma cells. Vascular bundle shape was semi-circular. Vascular bundle was amphicribal. Cortical bundles present. Starch grains scattered all over the surface of the cells (Table 1; Fig. 2 A-B).

#### *Bryophyllum delagoense* (Eckl. and Zeyh.) Schinz.

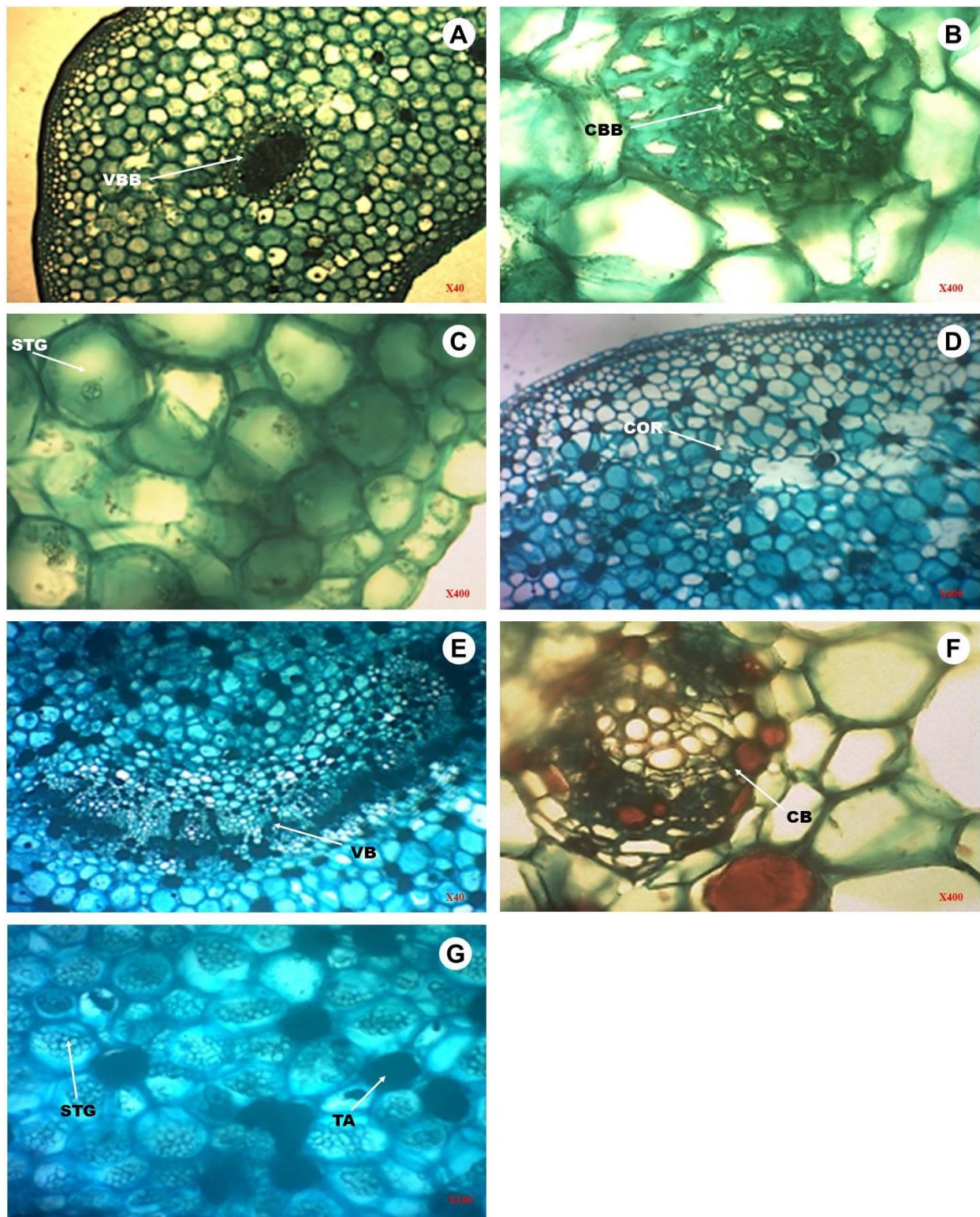
Apetiolate

#### *Bryophyllum houghtonii* (D.B Ward) P.I.Forst

The outline of the petiole was round on both the adaxial and abaxial surface. The epidermis was uniseriate. The cortex consisted of 0-2 layers of angular collenchyma and 8-11 layers of thin walled parenchyma cells. Vascular bundle was arc-shaped and amphicribal. Cortical bundles present. Starch grains was found present in the cortex (Table 1; Fig. 2 C-F).

**Table 1.** Summary of the petiole characters of the *Kalanchoe* and *Bryophyllum* species studied.

Characters	<i>Kalanchoe crenata</i>	<i>Kalanchoe lanceolata</i>	<i>Bryophyllum pinnatum</i>	<i>Bryophyllum delagoense</i>	<i>Bryophyllum houghtonii</i>
<b>Outline of the petiole</b>	Slightly concave on the adaxial surface. Convex on the abaxial surface.	Convex on the adaxial surface. Convex on the abaxial surface	Convex on the adaxial surface Concave on the abaxial surface	Apetiolate	Round on both adaxial and abaxial surface.
<b>Layers of Epidermis</b>	Uniseriate	Uniseriate	Uniseriate	Apetiolate	Uniseriate
<b>Cortex</b>	0-2 layers of angular collenchyma 8-10 layers of parenchyma cells	1-3 layers of angular collenchyma 19-20 layers of parenchyma cells	0-2 layers of angular collenchyma. 9-11 layers of parenchyma cells	Apetiolate	0-2 layers of angular collenchyma 8-11 layers of parenchyma cells
<b>Vascular bundle shape</b>	Oval	Arc-shaped	Semi-circular	Apetiolate	Arc-shaped
<b>Vascular bundle type</b>	Amphicribal	Amphicribal	Amphicribal	Apetiolate	Amphicribal
<b>Cortical bundles</b>	Present	Present	Present	Apetiolate	Present
<b>Starch grains</b>	Present	Present and abundant	Present	Apetiolate	Present

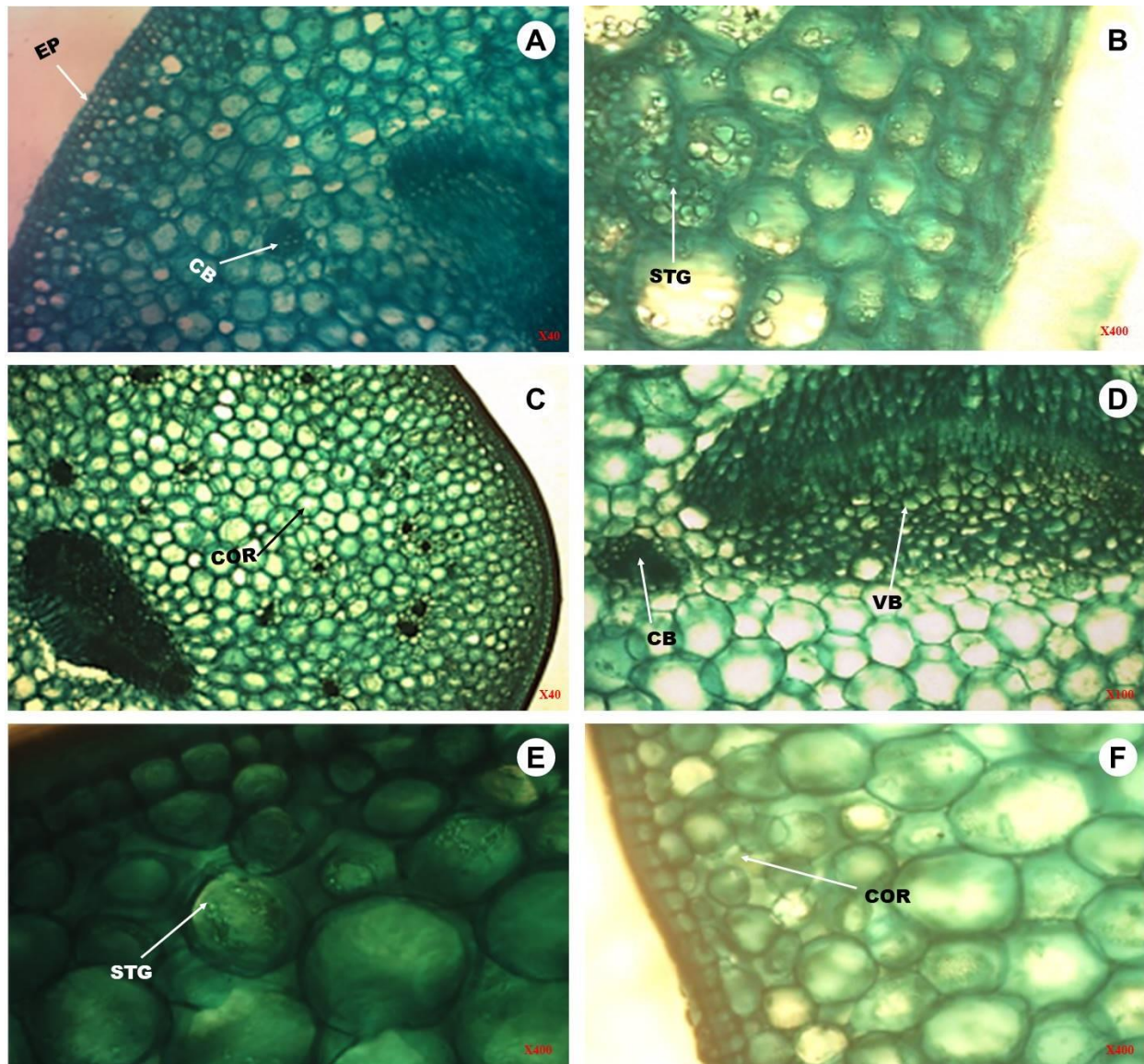


**Figure 1.** Transverse section of petiole of *Kalanchoe* species: **A**, Outline of *K. crenata*; **B**, Cortical bundle of *K. crenata*; **C**, Starch grains of *K. crenata*; **D**, Outline of *K. lanceolata* showing the cortex; **E**, Vascular bundle of *K. lanceolata*; **F**, Cortical bundle of *K. lanceolata*; **G**, Starch grains and Tannins of *K. lanceolata*. [Legend: STG- Starch grains, VB- Vascular bundle, CB- Cortical bundle, TA- Tannins]

#### *Transverse section of stem*

##### *Kalanchoe crenata* (Andr.) Haw

The epidermis was uniseriate and consisted of rectangular shaped cells covered by a thick and striated cuticle. The cortex was made up of 0-2 layers of angular collenchyma cells and 14-16 layers of parenchyma cells. Starch grains present within the cortex. Vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the xylem. The xylem was not lignified and had solitary or small groups of tracheary (vessels and tracheids) elements. Pith was filled with parenchyma cells. Tannins present (Table 2; Fig. 3 A-C).



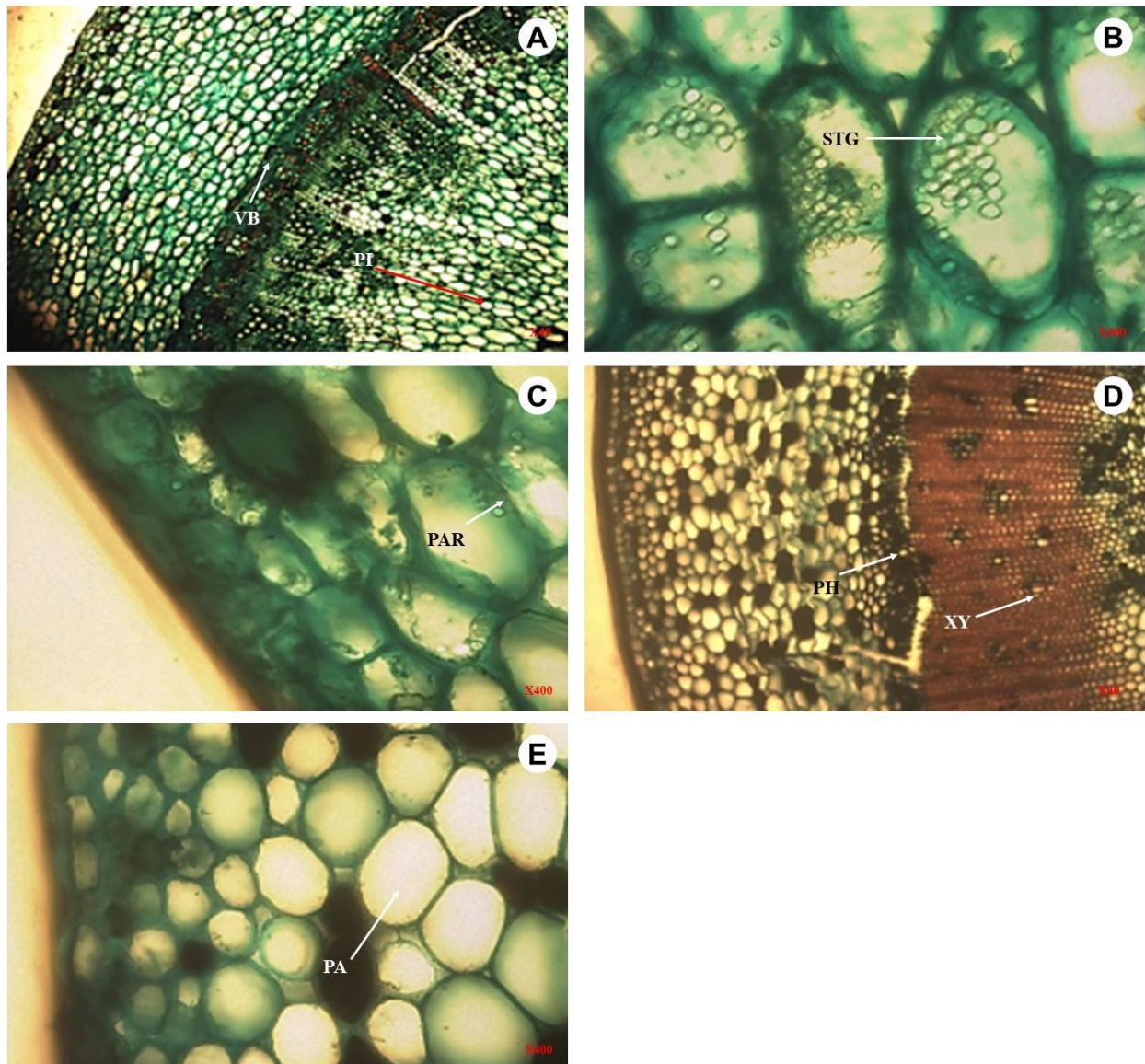
**Figure 2.** Transverse section of petiole of *Bryophyllum* species studied: **A**, Adaxial Outline of petiole of *B. pinnatum*; **B**, Starch grains of *B. pinnatum*; **C**, Outline of petiole of *B. houghtonii*; **D**, Cortical bundle of *B. houghtonii*; **E**, Starch grains of *B. houghtonii*; **F**, Cortex of *B. houghtonii*. [Legend: EP- Epidermis, CB- Cortical bundle, STG- Starch grains, VB- Vascular bundle, COR- Cortex]

**Table 2.** Summary of the stem characters of the *Kalanchoe* and *Bryophyllum* species studied.

Character	<i>Kalanchoe crenata</i>	<i>Kalanchoe lanceolata</i>	<i>Bryophyllum pinnatum</i>	<i>Bryophyllum delagoense</i>	<i>Bryophyllum houghtonii</i>
<b>Epidermis</b>	Uniseriate	Uniseriate	Biseriate to multi seriate	Uniseriate	Uniseriate to multi seriate
<b>Cortex</b>	0-2 layers of angular collenchyma cells. 14-16 layers of parenchyma cells	1-3 layers of angular collenchyma cells. 8-12 layers of parenchyma cells.	3-4 layers of lamellar collenchyma cells. 19-21 layers of parenchyma cells.	1-2 layers of lamellar collenchyma cells. 8-9 layers of parenchyma cells.	0-1 layers of lamellar collenchyma cells. 9-16 layers of parenchyma cells
<b>Vascular bundle</b>	Arranged in form of a ring, represented by a cylinder of phloem external to the xylem. Xylem is not lignified with solitary or small groups of tracheary elements.	Arranged in form of a ring, represented by a cylinder of phloem external to the xylem. Xylem is lignified with solitary or small groups of tracheary elements.	Arranged in form of a ring, represented by a cylinder of phloem external to the xylem. Xylem is lignified with solitary or small groups of tracheary elements.	Arranged in form of a ring, represented by a cylinder of phloem external to the xylem. Xylem is lignified with solitary or small groups of tracheary elements.	Arranged in form of a ring, represented by a cylinder of phloem external to the xylem. Xylem is lignified with solitary or small groups of tracheary elements.
<b>Pith</b>	Filled with parenchyma cells.	Filled with parenchyma cells	Filled with parenchyma cells	Filled with parenchyma cells	Filled with parenchyma cells
<b>Cortical bundle</b>	Absent	Absent	Absent	Absent	Present

*Kalanchoe lanceolata* (Forsk.) Pers

The epidermis was uniseriate and was composed of rectangular shaped cells compactly arranged covered by a thin non striated cuticle. The cortex consisted of 1-3 layers of angular collenchyma cells and 8-12 layers of parenchyma cells. Abundant tannins present in the cortex. Vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the xylem. The xylem was lignified and had solitary or small groups of tracheary (vessels and tracheids) elements. The pith was filled with parenchyma cells (Table 2; Fig. 3 D-E).



**Figure 3.** Transverse section of stem of *Kalanchoe* species studied: **A**, Adaxial Outline of stem of *K. crenata*; **B**, Starch grains in the cortex of *K. crenata*; **C**, Parenchyma cells in the cortex of *K. crenata*; **D**, Outline of stem of *K. lanceolata* showing the xylem and phloem; **E**, Parenchyma cells in the cortex of *K. lanceolata*. [Legend: VB- Vascular bundle, PI- Pith, STG- Starch grains, PA- Parenchyma cells, XY- Xylem vessels, PH- Phloem vessels]

*Bryophyllum pinnatum* (Lam.) Oken

Epidermis was biseriata to multiseriata, and consisted of circular shaped cells covered by non-striated thick cuticle. Cortex consisted of 3-4 layers of lamellar collenchyma cells and 19-21 layers of parenchyma cells. Vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the xylem. The xylem was lignified and had solitary or small groups of tracheary elements (vessels and tracheids). Pith cavity filled with parenchyma cells (Table 2; Fig. 4 A-B).

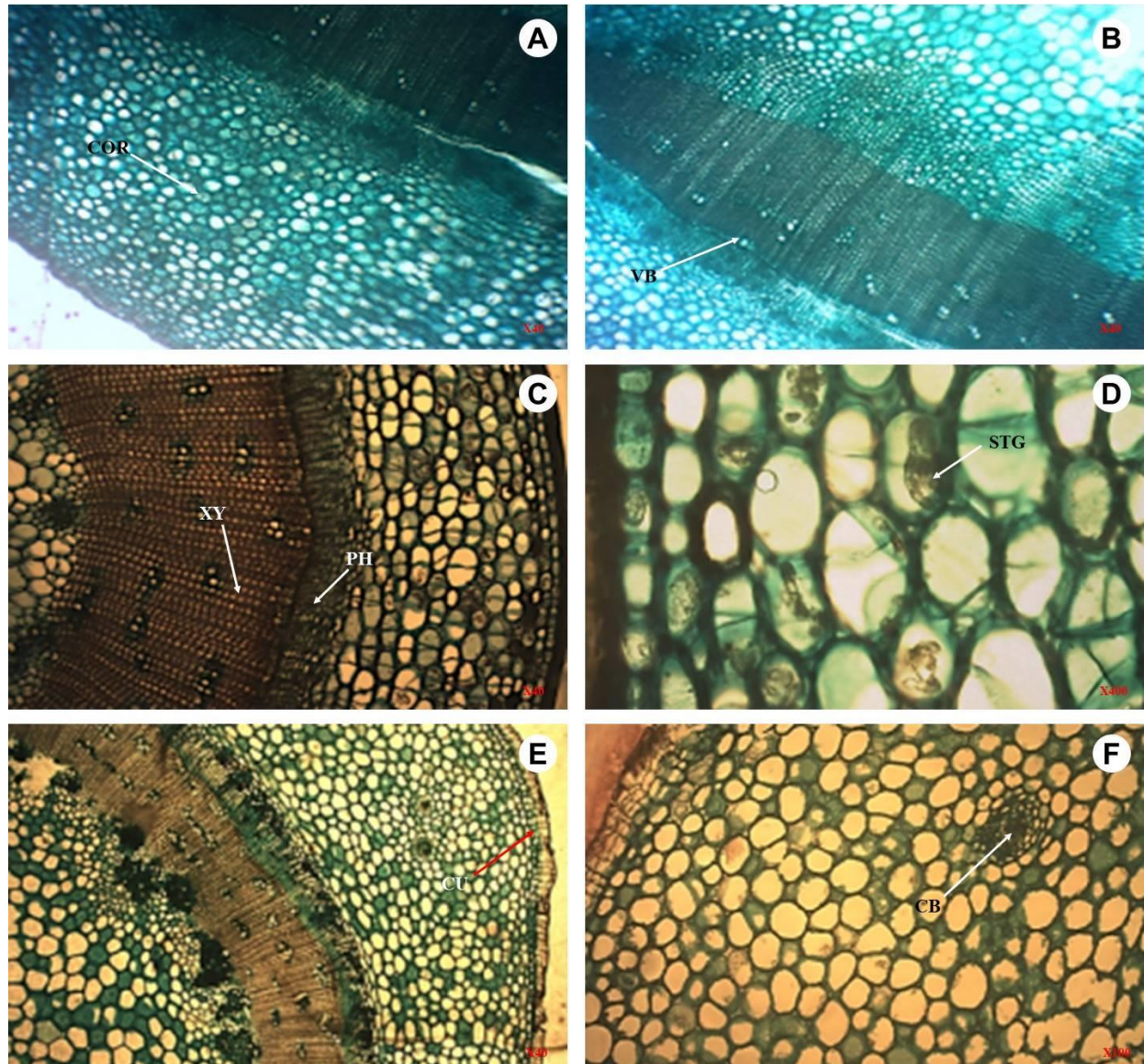
*Bryophyllum delagoense* (Eckl. and Zeyh.) Schinz

The epidermis was uniseriate and consisted of short cylindrical, oval, and rectangular cells covered by thick, gently undulating striated cuticle. The cortex consisted of 1-2 layers of lamellar collenchyma cells and 8-9 layers of parenchyma cells. Vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the

xylem. The xylem was lignified and had solitary or small groups of tracheary elements (vessels and tracheids). The pith was filled with parenchyma cells. Starch grains also present in the pith (Table 2; Fig. 4 C-D).

*Bryophyllum houghtonii* (D.B Ward) P.I.Forst

The epidermis was uniseriate to multiseriate composed of rectangular shaped cells covered by thick and striated cuticle. The cortex consisted of 0-1 layer of lamellar collenchyma cells and 9-16 layers of parenchyma cells. Cortical bundles present. Starch grains also present in the cortex. Vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the xylem. The xylem was lignified and had solitary or small groups of tracheary (vessels and tracheids) elements. The pith was filled with parenchyma cells (Table 2; Fig. 4 E-F).



**Figure 4.** Transverse section of stem of *Bryophyllum* species studied: **A**, Adaxial Outline of stem of *B. pinnatum*; **B**, Vascular bundle of *B. pinnatum*; **C**, Outline of *B. delagoense*; **D**, Starch grains of *B. delagoense*; **E**, Outline of stem of *B. houghtonii*; **F**, Cortical bundle in *B. houghtonii*. [Legend: COR- Cortex, VB- Vascular bundle, CU- Cuticle, STG- Starch grains, CB- Cortical bundle, XY- Xylem, PH- Phloem]

## DISCUSSION

The anatomy of the petiole was discovered to be taxon specific and useful in the identification of distinct plant taxa by Ingole & Patil (2003). Baruah (2007) used the anatomy of the petiole of some *Cinnamomum* species to aid their taxonomic discrimination. Uniseriate epidermis was observed in all the species studied. Sharma & Naresh (2014) who carried out a pharmacognostic study on the root, stem and leaf of *B. pinnatum* in Kalyan, India also observed uniseriate epidermis in the petiole of *B. pinnatum*. Vascular bundle was generally Amphicribal, that is, when the xylem is surrounded by the phloem tissues. This is in agreement with findings of Sandoval-Zapotitla *et al.* (20019) who also observed amphicribal vascular bundles in the genus *Echeveria* (Crassulaceae). Starch grains were present in all the

species studied. Sharma & Naresh (2014) also observed starch grains in the cortex of *B. pinnatum* in a research carried out in Kalyan, India.

The epidermis of the stem of all the species studied was uniseriate except in *B. pinnatum* which was biseriate to multiseriate and *B. houghtonii* which was uniseriate to multiseriate epidermis. Shahrestani *et al.* (2020) also reported uniseriate epidermis for species of *Sedum* and *Phedimus* (Crassulaceae) studied. In all the species studied, vascular bundles were arranged in form of a ring, represented by a cylinder of phloem external to the xylem. The xylem was lignified and had solitary or small groups of tracheary (vessels and tracheids) elements except in *K. crenata* where it was not lignified. This can be used to distinguish *K. crenata*. Shahrestani *et al.* (2020) also observed that the xylem vessels in the some *Sedum* and *Phedimus* (Crassulaceae) species form a continuous ring.

Cortical bundles was recorded for only *B. houghtonii*, this delimits the species from the other members of genus *Bryophyllum* and genus *Kalanchoe* in this study. Cortical bundles are recorded for some thick-stemmed taxa, however these are simply leaf traces running vertically for a distance, as Thiede & Eggli (2007) pointed out. Rudall (2007) also observed that some stems possess cortical or medullary bundles and are associated with leaf vasculature. The pith is parenchymatous in all the five species of the genera *Bryophyllum* and *Kalanchoe* studied. Thiede & Eggli (2007) also observed that the pith of members of the family are parenchymatous.

## CONCLUSION

The five species studied have unifying features such as amphicribal vascular bundle in the petiole, starch grains present on the petiole, vascular bundle arranged in form of a ring and represented by a cylinder of phloem external to the xylem, parenchymatous pith in the stem.

Diagnostic features such as biseriate to multi-seriate epidermis in *B. pinnatum*, uni to multiseriate epidermis in *B. houghtonii*, presence of cortical bundle only in *B. houghtonii* can be used to delimit the species.

## ACKNOWLEDGEMENTS

The author will like acknowledge Mr. Isaac Ifeoluwa OGUNLOWO for his help in the collection of plant samples. We will like to acknowledge Mr. Biodun OMOLE for his help in the sectioning of the plant sample.

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