

RESEARCH PAPER

Some Diagnostic and Anatomical Characteristics of Five Taxa from Boraginaceae Juss. Family Erbil, Sulemania and Duhok -Iraq.

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ABSTRACT:

Some anatomical characteristics of five taxa belong to Boraginaceae family (*Asperugo procumbens* L._Boiss, *Buglossoides arvensis* L._Boiss, *B. tenuiflorum* L. (fil)_Boiss, *B. incrassatum* Guss_Boiss and *Symphytum kurdicum* Boiss.et Haussk). The study was performed in the mountain region at the north of Iraq were examined the field trips have done at the growth period during the year 2019. Some parts of specimens were anatomically examined by paraffin method, the diagnostic characters of stems, leaves and leaf petioles were identified. Glandular and non-glandular hairs of each species have been determined, the different trichomes in adaxial and abaxial of both leaf surfaces and stems were present. The shapes of stem in cross sections were different between the species, lysigenous canals were present in the stem of *B. tenuiflorum* and *S. kurdicum* and the drusses were present only in stem of *B. incrassatum*. The main vascular bundle found in the midrib, the leaf margin shapes were different between species.

KEY WORDS: Anatomy of Boraginaceae, Leaf anatomy of *Symphytum*, Glandular hairs of leaf *Asperugo*, Stem anatomy of *Buglossoides*.

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1. INTRODUCTION:

There is a remarkable revolution in the investigation of vascular plant anatomy and its use in classification in the last 60 years, the anatomical characters are as valuable as morphological ones and must not be neglected. In taxonomy, every anatomical aspect of plant has been studied without insisting on some and ignoring others, therefore, the quality of information combined are enormous (Stace, 1980). Radford *et al.* (1974) stated that anatomical characters have been employed for systematic purposes well over a hundred years and its results included that anatomical data tend to be most useful at the genus level

and at higher taxonomic categories and the anatomical characters are no more or less reliable than characters from other investigation parts of the plants, also the similarities in structural specialization do not necessarily imply close relationships but may be the result of parallel and convergent evolution and also when anatomical information are coupled with evidences from other investigation parts of the plant will a natural classification be attained.

Metcalf and Chalk (1950; 1965) mentioned that the practical applications alone provide sufficient cause to justify the use of anatomical methods in taxonomic investigations. It may be pointed out that a complete anatomical survey is necessary as a preliminary to the interpretation of Palaeobotanical remains. Furthermore, mentioned adscription for vegetative anatomical characters for many of dicotyledon families including Boraginaceae family with

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identification of some important anatomical characteristics which are considered the merit feature of the family. Cronquist, (1981) hinted to some of the anatomical characteristics of the Boraginaceae through the family description as well.

There is no anatomical study in Iraq pertaining with the understudied taxa of the family Boraginaceae, so this study was the first study according to available literature. The terms in this study depended on the terminologies that come from (Metcalf and Chalk, 1950), (Esau, 1965) and (Radford *et al.*, 1974).

2. MATERIALS AND METHODS

The plant parts as stem, leaf and petiole were obtained from collected samples of Boraginaceae taxa (*Asperugo procumbens*, *Buglossoides arvensis*, *B. tenuiflorum*, *B. incrassatum* and *Symphytum kurdicum*) that existed in different districts from mountain region Kurdistan region of Iraq. The following steps are conducted for preparation the permanent slides of cross sections of the leaves parts and stems; a small parts of the samples were taken in size 0.5-1 cm, then placed in a small vial contain F.A.A. solution for 24 hour at room temperature (Saeed, 2003).

The samples placed in series of ascending concentration of Ethanol 95% for one hour and 100% for three to four hours twice in each concentration. Then the samples were placed in solution of absolute xylene for 3-4 hours twice, then placed in mixture of melted paraffin and xylene 1:1 for half (0.5) hours in an oven at 60 °C. Finally the sample placed in melted pure paraffin only for overnight in an oven at 60 °C. For paraffin wax templates preparation poured with melted paraffin previously covered by thin film of glycerol to prevent adhesion of the paraffin to the metal, as adopted by (Najmaddin and Mahmood, 2016). Sectioning was done by using Rotary Microtome and the samples were cut with 8µm thickness, The slides were dried by putting on hot plate at 45°C and left at room temperature for overnight for completing affixing process (Johansen, 1941). The sections were stained by using Safranin and light green. The sectioning done according to the procedure that modified by (Al-Khazrajy and Aziz, 1990). The sections were examined and the photographs were taken by light microscope Olympus with digital camera system.

3. RESULTS:

1. Cross section of lamina:

The epidermis of the leaf was covered by continuous of waxy layer (cuticle), the cuticle thickness is vary among the taxa, it is thick in most taxa, but appears thin in another such as *A. procumbens* and *B. tenuiflorum* (fig. 3 and 4)

In cross section, the epidermal cells as in species of Boraginaceae were arranged in single irregularly layer, the lumen of cells were appear in spherical, semi-spherical, polygonal, oblong, narrowly oblong, semi quadrate and ovate, which have different size in the same layer of single blade. The external cell walls were convex and thick, the other sides of the cell walls were concave, convex or straight and thin (fig. 3 and 4).

All studied species of the family were dorsiventral and possess heterogeneous mesophyll. The vascular bundles were collateral closed system. The palisade cells were oblong in shape with obvious intercellular spaces, were arranged in (1) layer as in *A. procumbens* and *S. kurdicum*; (2-3) layers as in *B. tenuiflorum*, *B. arvensis* and *B. incrassatum*. The spongy cells have different irregular shapes with ovoid to ovoid oblong, quadrate and rectangular with obvious intercellular spaces, were arranged in (4-7) layers depended on the location and lamina thicknesses, most mesophyll cells were containing tannin also. (fig. 3 and 4)

The trichomes play a role in plant taxonomy, especially for delimitation among morphologically similar taxa in a single genus. In all studied taxa the trichomes occur in peculiar forms and size in different positions and parts of the plants and there were non- glandular trichomes were unicellular in all studied species but they were various shapes and size (fig. 1), conical bristle, they found in leaves of *A. procumbens*. The dimensions of hairs were (24.4-79)×(4-67)µm. Conical falcate, they were presence in the leaves of *B. tenuiflorum*. The dimensions of hairs were (12.2-118)×(6.9-88.2)µm. While cylindrical filiform of pilose, this types present on the leaves of the most studied taxa, as in *S. kurdicum* and *B. incrassatum*. The dimensions of hairs were (370.1-11.3)×(4-59.7)µm and (120.3-16.4)×(6.536.3)µm respectively. The Glandular hairs, they were presence in all studied species, there were unicellular and multicellular hairs; which were various shapes and size (fig. 2), they were sessile glandular hairs consist of multicellular globoid head (trichomes consisting

of small bulbous bases bearing long, hypha-like terminal portions), such as found in the leaves of *A. procumbens*, and *B. incrassatum*; the dimensions of head were $(3.7-79.7) \times (2.2-67.6) \mu\text{m}$. the sessile capitate unicellular hairs, they present in the leaves of *B. arvensis*, *B. tenuiflorum*, and *S. kurdicum*; the dimensions of head were $(6.2-105) \times (4.6-71.8) \mu\text{m}$.

2. Leaves tip and margin:

The leaves margin outline were varying in Boraginaceae member. The margin was straight rounded tip as in *S. kurdicum*, while rounded downward tip as in *B. arvensis* and *B. tenuiflorum*, when rounded slightly downward tip in *A. procumbens* and *B. incrassatum*. The unicellular glandular trichomes found in *B. incrassatum* and *B. tenuiflorum* and the unicellular non-glandular trichomes presence in *S. kurdicum*, while *A. procumbens* *B. arvensis* do not have trichomes (fig. 5).

3. Petiol:

The current anatomical study reveals, that the leaves petiole of high signification taxonomic character, it is owing to; among all studied taxa only two species were petiolate such as *A. procumbens* and *S. kurdicum*, the other studied species was sessile, each of these two species have their particular shape and vascular strand. The petiole has only one large vascular bundle in center; the abaxial was V shape and the adaxial was concave with two lateral projection, each lateral projection contains small vascular bundle of lateral veins (fig. 6).

4. Midrib:

The midribs outline of the studied species were various in shapes, the midrib outline was V-shaped in the abaxial and concave in adaxial as in *A. procumbens*, while the abaxial was rounded and concave in adaxial such as *B. arvensis*, *B. incrassatum* and *B. tenuiflorum*, whereas the midrib is rounded in abaxial surface and adaxial surface in *S. kurdicum*.

The midrib vascular strand was collateral closed arrangement type, relatively larger than other lamina vascular strands, approximately similar in shape in all species, they appear in gibbous or crescent shape, consist of (8-13) xylem straps arrange perpendicularly above phloem tissue. In all species the vascular strand surrounded by (2-3) parenchymatous layers (bundle sheath). Also the species of this family have lacunar collenchyma in adaxial and abaxial

layer. The unicellular trichomes found in *Buglossoides arvensis*; *B. incrassatum* and *B. tenuiflorum*, while *A. procumbens* and *S. kurdicum* do not have trichomes (fig. 7).

5. General Anatomical Description of Stem:

The cross sections outline of stems were varying in shapes in studied taxa of the family, the outline was irregular, with three small projection in *A. procumbens*, circular to semi-circular with four projection (two small and two larger) in *B. arvensis*, circular to semi-circular with one small projection in *B. incrassatum*, circular to semi-circular with one small projection in *B. tenuiflorum*, and irregular with one small projection in *S. kurdicum* (fig. 8).

Non-glandular trichomes were unicellular, Cylindrical bristle like obtuse tip, there were found only in the stem of *B. arvensis*, the dimensions of hairs were $(16.1-) \times (50.8) \mu\text{m}$. Conical falcate presence in the stem of *B. tenuiflorum*, $(12.2-118) \times (6.9-88.2) \mu\text{m}$. Cylindrical filiform of pilose, in the stem of *S. kurdicum* and *B. incrassatum*, $(370.1-11.3) \times (4-59.7) \mu\text{m}$ and $(120.3-16.4) \times (6.536.3) \mu\text{m}$ respectively. Sessile glandular hairs consist of multicellular globoid head, they were found in the stem of *B. incrassatum*, $(3.7-79.7) \times (2.2-67.6) \mu\text{m}$ and Sessile capitate unicellular hairs, they present in the stems of *B. arvensis*, *B. tenuiflorum*, and *S. kurdicum*. The dimensions of head were $(6.2-105) \times (4.6-71.8) \mu\text{m}$. Hypoderms in studied taxa contain lysigenous ducts in *B. tenuiflorum* and *S. kurdicum*, while *B. incrassatum* contains druses (fig. 9). The vascular strands in all studied taxa were continuous circular ring except in *S. kurdicum* which the vascular strands were arranged in interrupted circular ring-like (fig. 10-13).

4. DISCUSSION:

The anatomical study of different parts of Boraginaceae taxa, there were many significant anatomical characteristic are obtained to be taken account of separating the under the genera and species levels, as Al-dabbagh and Saeed, (2019) believed that

the dissimilarity in epidermal tissue, type of trichomes, stem outline, leaf margin, and midrib outline have great roles in separating species especially among closed species within a single genus.

In this study, the microscopic investigation of micromorphology showed various types of trichomes, about five types of both glandular and

non-glandular hairs have been observed in studied taxa.

The epidermal of all Boraginaceae species were contained different types of trichomes in different part of the plant body. This differences are evidence of high taxonomic value of segregation genera and species as mentioned by Najmaddin (2016). (Stace, 1980) believed that the trichomes have significance value at all levels in plant classification, from family to even varieties.

The epidermal cells of the stem, there are single layer according to number of epidermal layers.

The mesophyll layer has limited role in identifying the species; generally it is dorsiventral for example composed of the palisade and spongy parenchyma layers as mentioned by Najmaddin (2016).

The midrib and the margin outline, with their contents have the taxonomic value of the genera and species levels, especially the midrib outlines have particular shape of each genus and species as mentioned by (Al-dabbagh and Saeed, 2020).

The anatomical characters of petioles were best feature of difference between *A. procumbens* and *S. kurdicum*, while another species are sessile.

The outline of the stem clearly showed important variation in the shape. In all species the stem epidermis was single layers. Hypoderms in studied taxa have different pattern in tissue contain, *A. procumbens* composed large parenchymatous cells with very thin walls, while in *B. arvensis*, *B. incrassatum*, *B. tenuiflorum* and *S. kurdicum* composed 3-8 layer of lacunar collenchymatous tissue, with 2-3 layers of parenchymatous tissue toward the pith, the cortex in *B. tenuiflorum* and *S. kurdicum* contain lysigenous, whereas *B. incrassatum* contains druses, these results are matches with what cited by (Metcalfe and Chalk, 1950; 1965). The Boraginaceae taxa contain tannin.

5. CONCLUSION:

The current study demonstrated that the anatomical characteristics have a great role in taxonomic value among studied genera and species of Boraginaceae family, and it has shown approximately some anatomical differences between the studied species especially in morphologically closed taxa within single genus.

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Conflict of Interest (1)

No conflict of Interest

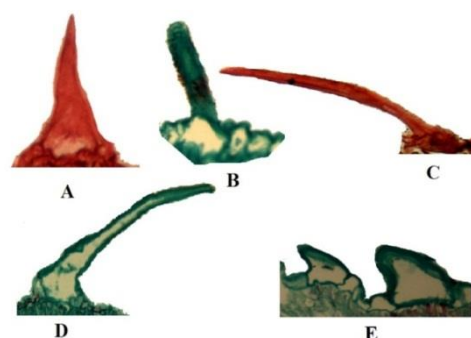


Figure 1: Non-glandular hair types in studied taxa of Boraginaceae:

A- conical bristle; B- Cylindrical bristle-like obtuse tip; C and D- cylindrical filiform of pilose; E- Conical falcate, 40X

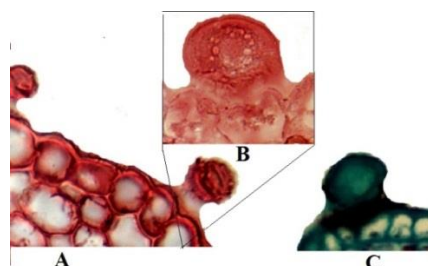


Figure 2: Glandular hair types in studied taxa of Boraginaceae:

A and B- Sessile glandular hairs consist of multicellular globoid head 40X; B- magnified portion 100X; C- Sessile capitate unicellular hairs 40X.

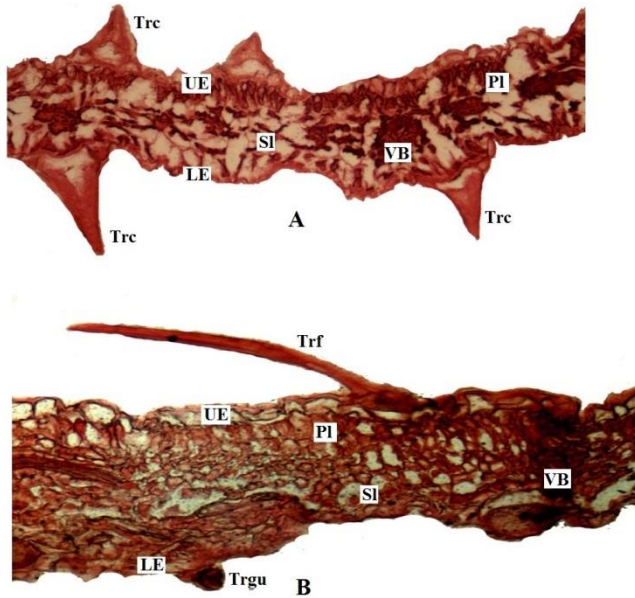


Figure 3: Cross sections of leaf of the studied species of Boraginaceae:
 A- *A. procumbens*; B- *B. incrassatum*; C- *B. arvensis*.
 UE- upper epidermis; LE- lower epidermis; VB- vascular bundle; PI- palisade layer; SI- spongy layer; Trc- Conical bristle; Trf- cylindrical filiform of pilose; Trgu- Unicellular glandular hair and Trgm- Multicellular glandular hair.(40x).

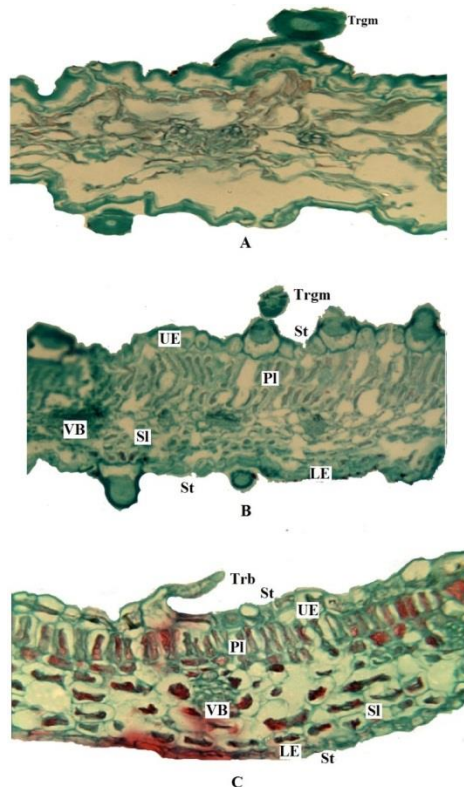


Figure 4: Cross sections of leaf of the studied species of Boraginaceae:
 A- *B. arvensis*; B- *B. tenuiflorum*; C- *S. kurdicum*.
 UE- upper epidermis; LE- lower epidermis; VB- vascular bundle; PI- palisade layer; SI- spongy layer; Trc- Conical bristle; Trf- cylindrical filiform of pilose; Trgm- Multicellular glandular hair and St- stomata.(40x).

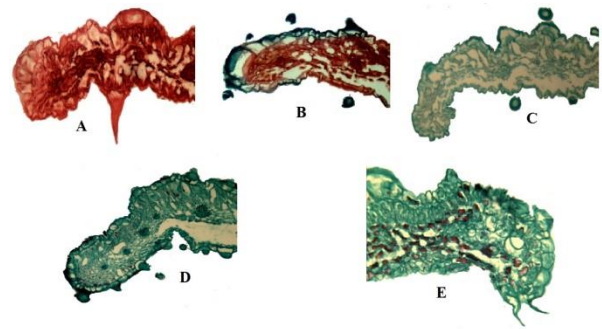


Figure 5: Leaf blade tip and margin outlines in studied taxa of Boraginaceae:
 A- *A. procumbens*; B- *B. incrassatum*; C- *B. arvensis*; D- *B. tenuiflorum*; E- *S. kurdicum*.

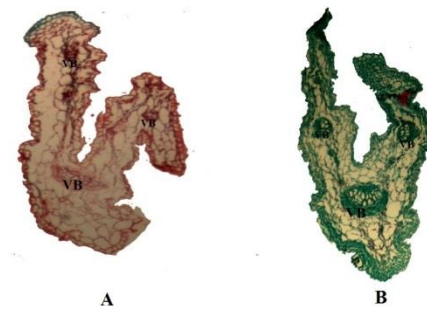


Figure 6: Cross sections of petioles of studied Boraginaceae species. A- *A. procumbens*; B- *S. kurdicum*. (10x).

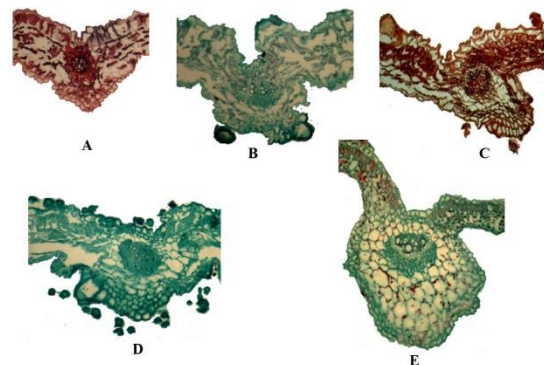


Figure 7: Leaves midrib outline in studied taxa of Boraginaceae:
 A- *A. procumbens*; B- *B. arvensis*; C- *B. incrassatum*; D- *B. tenuiflorum*; E- *S. kurdicum*. (40x).

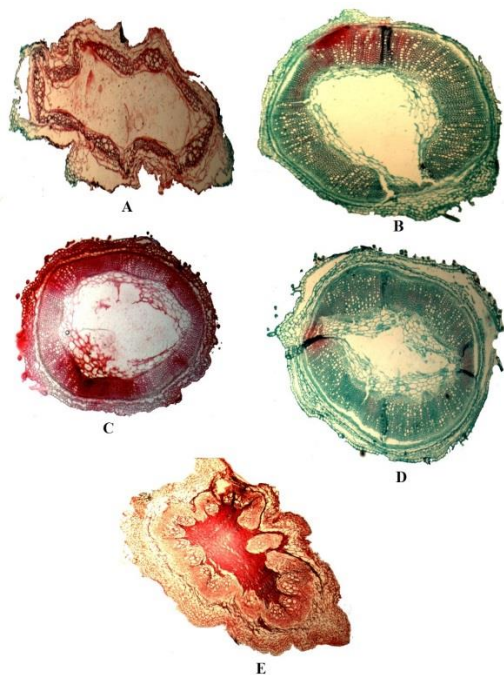


Figure 8: Cross sections of stem outlines of the studied taxa of Boraginaceae: A- *A. procumbens*; B- *B. arvensis* ; C- *B. incrassatum*; D- *B. tenuiflorum* and E- *S. kurdicum*. (10x).

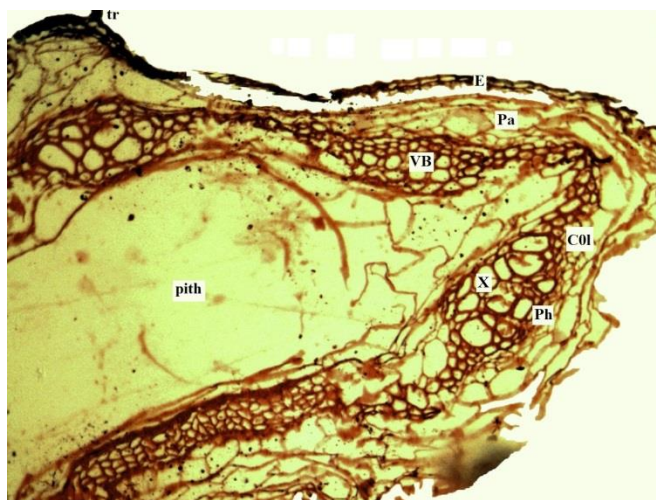


Figure 9: Portions of cross section of the studied *A. procumbens* species stem.

E= Epidermis; tr= trichome Pa= Parenchyma; VB= Vascular bundle; Col=Collenchyma; X= Xylem and Ph= Phloem, (Magnified 40x).

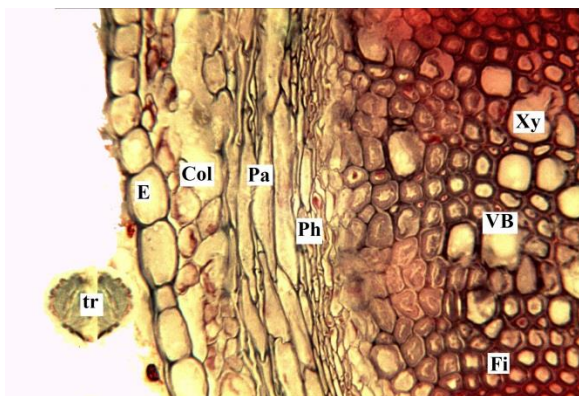


Figure 10: Portions of cross section of the studied *B. arvensis* species stem.

E= Epidermis; tr= trichome Pa= Parenchyma; VB= Vascular bundle; Col=Collenchyma; Xy= Xylem; Fi= Fiber and Ph= Phloem, (Magnified 40x).

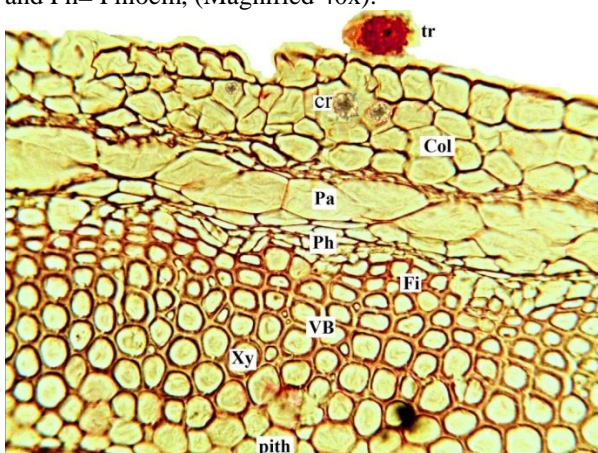


Figure 11: Portions of cross section of the studied *B. incrassatum* species stem.

cr= crystal; tr= trichome Pa= Parenchyma; VB= Vascular bundle; Col=Collenchyma; Xy= Xylem; Fi= Fiber and Ph= Phloem, (Magnified 40x).

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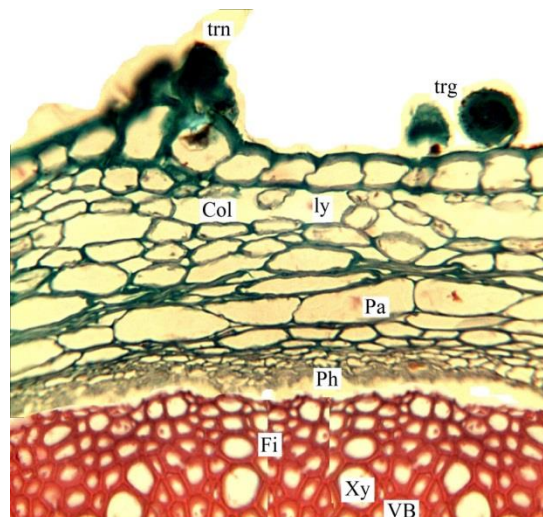


Figure 12: Portions of cross section of the studied *B. tenuiflora* species stem.

trn= non-glandular hair; trg= glandular hair; ly= lysigenous; Pa= Parenchyma; VB= Vascular bundle; Col=Collenchyma; Xy= Xylem; Fi= Fiber and Ph= Phloem, (Magnified 40x).

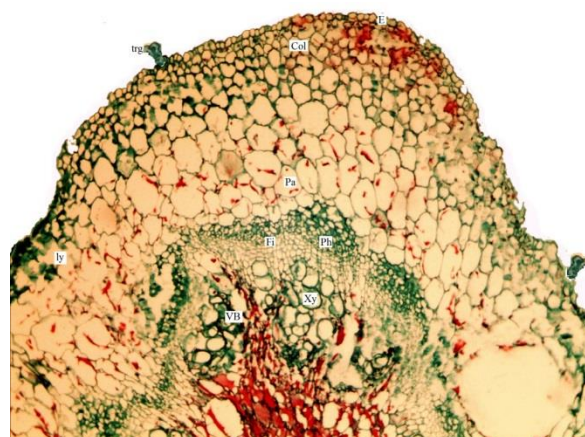


Figure 13: Portions of cross section of the studied *S. kurdicum*.species stem.

trg= glandular hair; ly= lysigenous; Pa= Parenchyma; VB= Vascular bundle; Col=Collenchyma; Xy= Xylem; Fi= Fiber and Ph= Phloem, (Magnified 40x).

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