



Anatomic Studies on *Verbascum pestalozzae* Boiss. and *Verbascum pycnostachyum* Boiss. & Heldr.

Verbascum pestalozzae Boiss. ve *Verbascum pycnostachyum* Boiss. & Heldr. Üzerinde Anatomik Araştırmalar

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ABSTRACT

Objectives: The genus *Verbascum* L. (Scrophulariaceae) known as “sığır kuyruğu” in Anatolia is represented by 248 species, 193 of which are endemic. The flowers contain essential oil, mucilage, and glycosides. Some species of *Verbascum* have some folkloric uses as expectorants, sedatives, and treatment for dysmenorrhea and rheumatism. Its use for healing wounds in animal care has also been reported. In this study, the anatomic structures of root, stem, and leaves of *Verbascum pestalozzae* Boiss. and *Verbascum pycnostachyum* Boiss. & Heldr. are given for the first time. According to the results, some of the differences between stem and leaf anatomic features of these species were described and the data were supported by detailed photographs.

Materials and Methods: Specimens were collected from C3 Antalya: Voucher specimens of *V. pestalozzae* and *V. pycnostachyum* were deposited in the Herbarium of the Biology Department, Akdeniz University in Antalya, Turkey and Herbarium of the Faculty of Pharmacy, Anadolu University in Eskişehir, Turkey. The materials were identified as *V. pestalozzae* and *V. pycnostachyum* using flora of Turkey and the East Aegean islands. For anatomic studies, samples were collected from the natural habitats and kept in 70% alcohol. In the research, root, stem and leaves of mature and flowered plants were used. Investigations were performed on the cross-sections of the root, the flowering stem, and the leaf. The anatomic structures of the species were drawn using a Leitz SM-LUX binocular microscope. All sections were embedded in glycerin gelatin and stained using Sartur reactive, and photographs were taken through a light microscope (Olympus BX51T).

Results: The cross sections taken from root, stem, and leaves of *V. pestalozzae* and *V. pycnostachyum* were examined and the anatomic features belonging to these plants were determined and compared.

Conclusion: The anatomic properties belonging to the two species were generally compatible with findings of Metcalfe and Chalk and others signified in the genus. We believe that our results provide additional evidence for taxonomists to help separate the species. The lack of a taxonomic, morphologic, and anatomic study on the species made it important for the systematic introduction of the research.

Key words: Scrophulariaceae, *Verbascum*, anatomy, endemic

ÖZ

Amaç: Anadolu’da “Sığır kuyruğu” olarak adlandırılan *Verbascum* L. (Scrophulariaceae) cinsi, ülkemizde 193’ü endemik olmak üzere 248 tür ile temsil edilmektedir. Çiçekleri müsilaj, uçucu yağ ve glikozitler taşır. Bazı *Verbascum* türleri halk arasında göğüs yumuşatıcı ve balgam söktürücü olarak, adet sancısını gidermede, yatıştırıcı, romatizma ağrılarını giderici ve ayrıca hayvan yaralarını tedavi etmekte kullanılmaktadır. *Verbascum pestalozzae* Boiss. ve *Verbascum pycnostachyum* Boiss. & Heldr. türlerinin kök, gövde ve yaprak anatomik yapıları ilk kez bu çalışmada verilmiştir. Araştırmalar sonucunda türlerin gövde ve yaprak anatomik özellikleri belirlenmiş ve veriler detaylı fotoğraflarla desteklenmiştir. Gen merkezi Anadolu olmasına rağmen *Verbascum* ile ilgili yeterli çalışmaların bulunmaması ve taşıdığı sekonder metabolitler ile potansiyel bir tıbbi bitki olması çalışmanın önemini artıracaktır.

Gereç ve Yöntemler: *V. pestalozzae* ve *V. pycnostachyum*’a ait örnekler C3 Antalya’dan toplanmış, Akdeniz Üniversitesi Biyoloji Bölümü ve Anadolu Üniversitesi Eczacılık Fakültesi Herbaryumu’na konulmuştur. Türlerin teşhisi Türkiye florası ve Doğu Ege adaları kullanılarak belirlenmiştir. Anatomik çalışmalar için, örnekler doğal habitatlardan toplanmış ve %70 alkolde tutulmuştur. Araştırmada, olgun ve çiçekli bitkilerin kök, gövde ve yaprakları kullanılmıştır. Kök, çiçekli gövde ve yaprak kesitleri üzerinde incelemeler yapılmış. Türlerin anatomik yapılarının çizimleri Leitz SM-LUX binoküler mikroskop kullanılarak yapılmıştır. Kesitler gliserin jelatin ile daimileştirilip, Sartur reaktifile boyanmış ve fotoğraflar ışık mikroskopu (Olympus BX51T) ile çekilmiştir.

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Bulgular: *V. pestalozzae* ve *V. pycnostachyum*'ün kök, gövde ve yapraklarından alınan kesitler incelenmiş ve bu bitkilere ait anatomik özellikler karşılaştırılmıştır.

Sonuç: İki türe ait anatomik özellikler genellikle Metcalfe ve Chalk ve diğer çalışmalarda belirtilen bulgularla uyumludur. Sonuçlarımızın, taksonomistlere ek kanıt sağladığına ve türlerin ayrılmasına yardımcı olabileceğine kanısındayız. Türler üzerinde taksonomik, morfolojik ve anatomik bir çalışma olmaması, araştırmamızın sistematik olarak tanıtımı için önemli hale getirmiştir.

Anahtar kelimeler: Scrophulariaceae, *Verbascum*, anatomi, endemik

INTRODUCTION

The genus *Verbascum* Linnaeus (1753: 177) (excl. *Celsia* Linnaeus 1753: 621) (Scrophulariaceae) has about 360 species from all over the world (Heywood 1993, Mabberley 2008). Represented with 248 species in Turkey, the genus has been divided into 13 partly artificial groups with 130 additional hybrids. Among them, 193 species are endemic to Turkey, with an endemism percentage of about 80%. The genus *Verbascum* L. (Scrophulariaceae) known as "Siğir Kuyruğu" in Anatolia.¹⁻⁵

Many plant species among the flora of Turkey play important roles in traditional medicine. There are approximately 9300 plant species in Turkish flora, some of which are widely used in folkloric medicine due to their antimicrobial and anticarcinogenic properties.^{6,7} One of the well-known *Verbascum* species is *V. thapsus* L., which has been used for the treatment of several diseases including asthma, spasmodic cough, migraine, and earache. Moreover, *V. thapsus*, *V. fruticosum* Post, *V. undulatum* Lam. and *V. georgicum* Benthams have anti-malarial and anti-viral effects, which have been investigated in both *in vitro* and *in vivo* studies.⁶

It is reported that leaves and flowers of *Verbascum* species have expectorant, mucolytic, and demulcent properties, and they are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis, and asthma in Anatolia.^{8,9} *Verbascum* species are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. Furthermore, these species demonstrate several inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. Macerated oil prepared from the flowers is used for reducing earache, and applied externally for eczema and other types of inflammatory skin disorders.¹⁰

Verbascum species have some folkloric uses such as a sedative, and treatment for dysmenorrhea and rheumatgia. Its use for healing wounds has also been reported in animal care. Iridoid and neolignan type glycosides, oleanan type terpenes, flavonoids, polysaccharides, saponins, steroids and alkaloids were major compounds isolated from *Verbascum* species.¹¹ In several bioactivity studies on *Verbascum* sp., anti-proliferative,¹² anti-inflammatory,¹³ antioxidant,^{14,15} anti-histaminic, anti-fungal, anti-bacterial,¹⁶ wound healing,¹⁷ anti-microbial¹⁸ and anti-cancer effects¹⁹ have been shown in crude extracts of roots, leaves, flowers, and aerial parts.

Verbascum genus is one of the largest genus with regard to the number of species in Turkey and is also known for several problems in diagnosis and taxonomy.

In addition, the anatomic structures of root, stem and leaves of and *Verbascum pestalozzae* Boiss. (endemic), "Boz Siğir

Kuyruğu" and *Verbascum pycnostachyum* Boiss. & Heldr. "Eğirdir Siğir Kuyruğu"^{2,4} are given in this study for the first time.

EXPERIMENTAL

Plant material

The flowering aerial parts of *Verbascum pestalozzae* were collected from C3 Antalya: Konyaaltı, Doıran, between Saklıkent and Karçukuru (36° 49' 01" N, 30° 21' 54" E), on lime stone rocks, 2100 m, 17.07.2008, ESSE 15069! Göktürk 7338; *Verbascum pycnostachyum* were collected from C3 Antalya, between Korkutelı and Fethiye (37° 02' 53" N, 30° 06' 26" E), steppe, 1370 m above the sea level, at the end of July 2007, Eskişehir (ESSE) 14730!, Göktürk 6093; (Figures 1-3). Voucher specimens of *V. pestalozzae* and *V. pycnostachyum* were deposited in the Herbarium of the Biology Department, Akdeniz University in Antalya, Turkey and Herbarium of the Faculty of Pharmacy, Anadolu University in ESSE, Turkey. The materials were identified as *V. pestalozzae* and *V. pycnostachyum* using flora of Turkey and the East Aegean islands.²

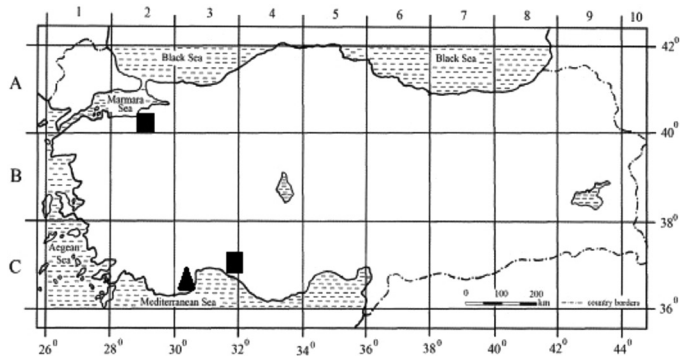


Figure 1. Distribution map of *V. pestalozzae* (▲) and *V. pycnostachyum* (■) in Turkey



Figure 2. *V. pestalozzae*

Anatomical

For anatomic studies, samples were collected from the natural habitats and kept in 70% alcohol. In the research, root, stem, and leaves of mature and flowered plants were used. Investigations were performed on the cross-sections of the root, the flowering stem, and the leaf. The anatomic structures of glandular and covering hairs were drawn using a Leitz SM-LUX binocular microscope. All sections were embedded in glycerin gelatin and stained using Sartur reactive, and photographs were taken through a light microscope (Olympus BX51T).

RESULTS AND DISCUSSION

The cross sections taken from root, stem, and leaves of *V. pestalozzae* and *V. pycnostachyum* were examined and the anatomic features belonging to these plants were determined and compared (Figures 4-10, Table 1).



Figure 3. *V. pycnostachyum* habit (picture: R.S. Göktürk)

Root

The root in both species is composed of periderm on the outside and felloderm where 4-5 radial rows are broken down and felloderm with 2-3 rows of tissue. Outer felloderm cells are tissue debris of the primary cortex that has been shattered or crushed in place. Secondary phloem formed of elliptical-shapeless, round-shaped, irregular-arranged and 4-6 row cells under the periderm is taken part. Cambium is uncertain. The secondary xylem part covers a large area and consists of tracheal elements with large and small sizes in a

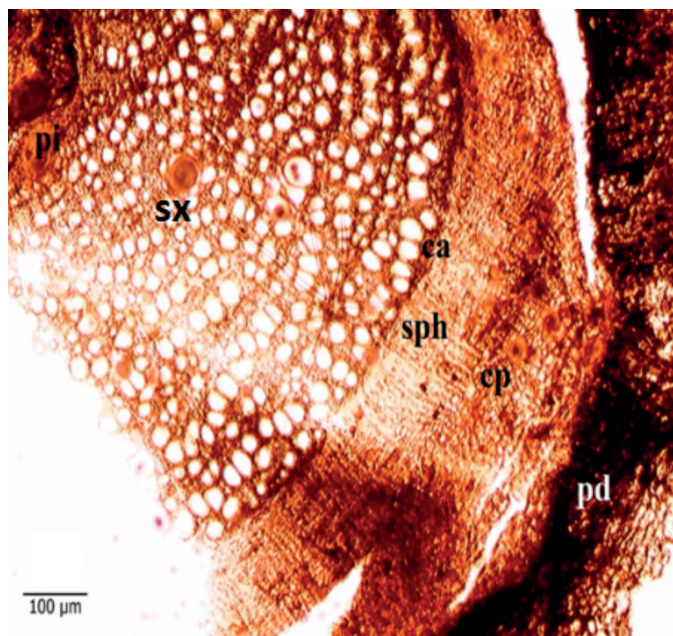


Figure 4. *V. pestalozzae*; cross-section of root

cp: Cortex parenchyma, pd: Periderm, pi: Pith, sph: Secondary phloem, sx: Secondary xylem

Table 1. Anatomic differences of the species

		<i>V. pestalozzae</i>	<i>V. pycnostachyum</i>
Root	Pith	Parenchymatic	Parenchymatic
Stem	Sclerenchyma	3-6 celled	1-5 celled
	Phloem	Thick, 3-8 celled	Thick, 8-15 celled
Leaf	Vascular bundles	Abaxial surface is projected under the vascular bundle	Protrusion
	Upper parenchymatic cells	Crescent-shaped	Horne shaped
	Lower epidermis parenchymatic cells	5-6 celled	25-30-celled
Eglandular hairs in the stem and leaf		5-10 celled	10-15 celled
Glandular hairs in the stem and leaf		Candelabriform, stellate, multicellular	Candelabriform, stellate, multicellular
		Head 1 stalk 2 celled; Head 2 stalk 1 celled; Head 3 stalk 2 celled; Head 1 stalk 3 celled; Head 2 stalk 3 celled; pellucid glands	Head 1 stalk 1 celled Head 2 stalk 1 celled Head 3 stalk 2 densely

sclerenchymatic tissue. Medullary rays are 2-3 rows of cells. The pith region, covering a narrow area, is parenchymatic in *V. pestalozzae* and *V. pycnostachyum* (Figures 4, 7).

Stem

When cross-sections were taken on the stems of two *Verbascum* species, secondary growth was observed. The epidermis is formed by a single-row, thick membrane elliptic or round cells. The upper and lower walls are thick but the lateral sides are thin. Its upper surface is covered with cuticle (Figures 5, 8). Covering hair and glandular trichomes were observed. Covering hairs of *V. pestalozzae* are candelabriform, stellate, and multicellular. Glandular trichomes were of 6 types; head 1 stalk 2 celled; head 2 stalk 1 celled; head 3 stalk 2 celled; head 1 stalk 3 celled; head 2 stalk 3 celled; pellucid glands (Figures 11b). Covering hairs are candelabriform, stellate, and multicellular in *V. pycnostachyum*. Its glandular trichomes are of three types; head 1 stalk 1 celled, head 2 stalk 1 celled, head 3 stalk 2 celled (dense, Figures 10). Parenchymatic cortex in 5 or 20 rows is found in both species under the epidermis. Collenchyma cells under the epidermis in the primary cortex were seen, and parenchyma cells including oval-shaped chloroplast inside the epidermis were found. Druse crystals were observed in

parenchymatic cells. The endodermis, consisting of flattened cells, can hardly be distinguished from the cortex parenchyma. There were large, small, and discontinuous sclerenchyma bunches, 3 or 6 rows in secondary phloem of *V. pestalozzae*, but 1-5 rows in *V. pycnostachyum*. Phloem is much narrower in *V. pestalozzae* with 3-8 rows, but it is much wider in *V.*

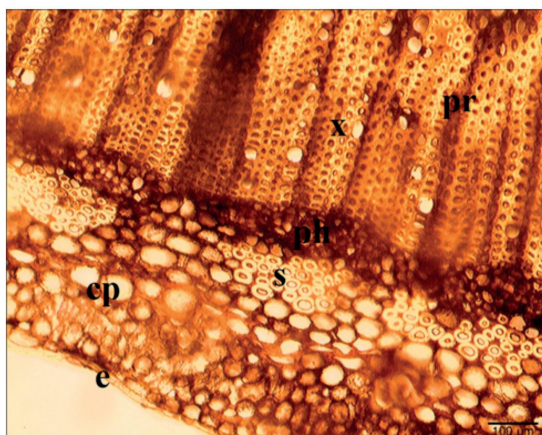
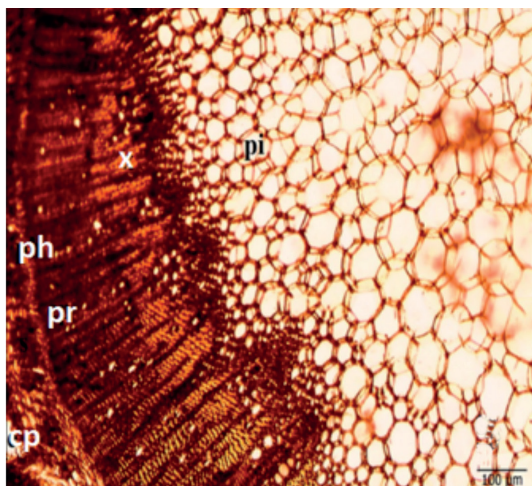


Figure 5. *V. pestalozzae*; cross-section of stem
cp: Cortex parenchym, e: Epidermis, ph: Phloem, pi: Pith, pr: Pith ray, s: Sclerenchyma, x: Xylem

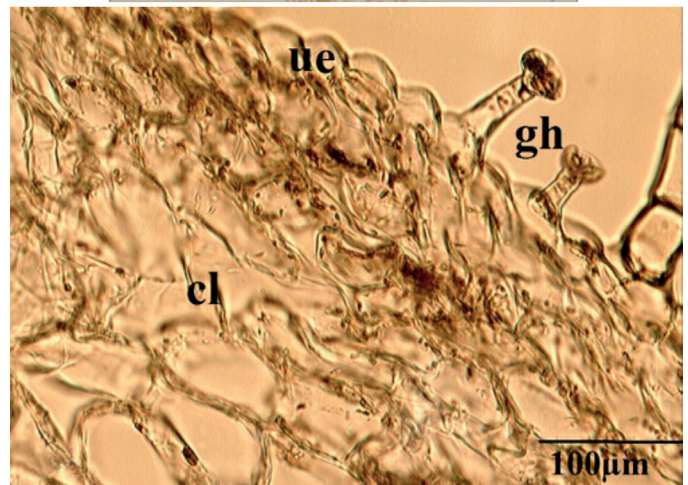
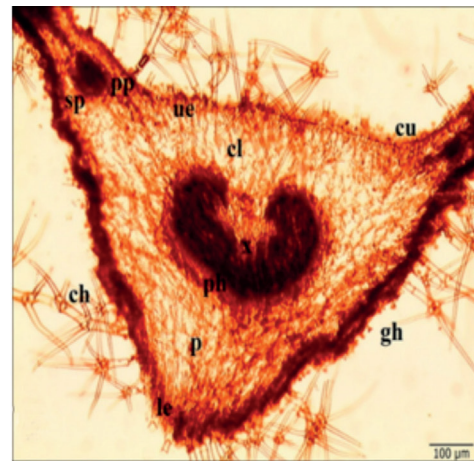


Figure 6. *V. pestalozzae*; cross-section of leaf, respectively
ch: Covering hair, cl: Collenchyma, cu: Cuticle, gh: Glandular hair, p: Parenchyma, ph: Phloem, ue: Upper epidermis, x: Xylem

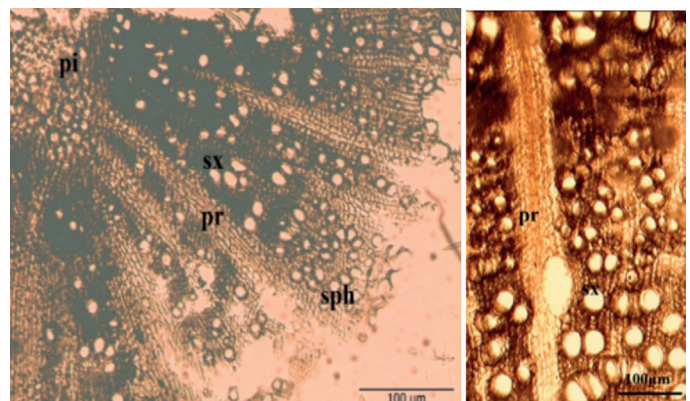


Figure 7. *V. pycnostachyum*; cross-section of root
pi: Pith, pr: Pith ray, sph: Secondary phloem, sx: Secondary xylem

pycnostachyum with 8-15 rows, but has a circle shape consisting of flattened, shapeless or oval cells. Cambium is uncertain. Bunches in both types of secondary xylem narrowed towards the primary xylem. Sclerenchymatic cells in this part, formed from trache and tracheids in both types, created regular rows in a radial direction. Pith bunches were in the form of large polygon or round- shaped and parenchymatic cells where their walls were lignified. Druse crystals were clearly found in these cells (Figures 5, 8).

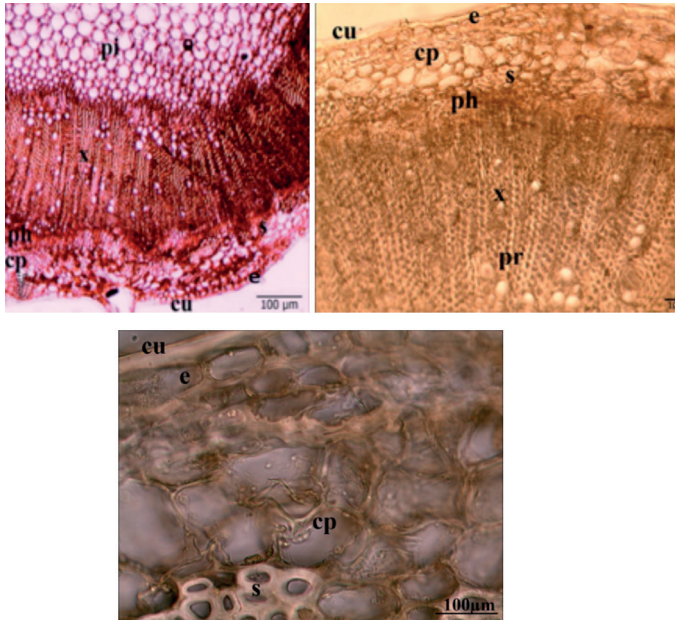


Figure 8. *V. pycnostachyum*; cross-section of stem

cp: Cortex, parenchym, cu: Cuticle, e: Epidermis, ph: Phloem, pi: Pith, pr: Pith ray, s: Sclerenchyma, x: Xylem

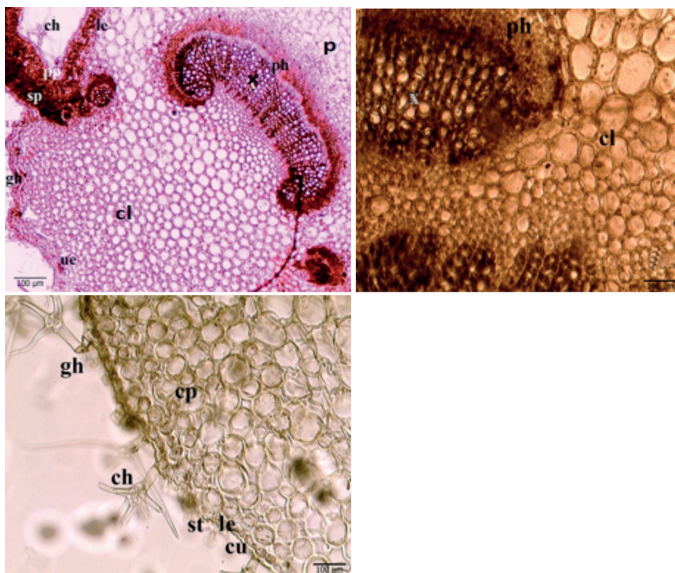


Figure 9. *V. pycnostachyum*; cross-section of leaf, respectively

ch: Covering hair, cl: Collenchyma, cp: Cortex, parenchym, cu: Cuticle, gh: Glandular hair, le: Lower epidermis, p: Parenchyma, ph: Phloem, pp: Palisade parenchyma, st: Sp-spongy parenchyma, ue: Upper epidermis, x: Xylem

Leaf

In the cross-section of the main and inter vascular tissues, the outer and inner layers of *V. pestalozzae* and *V. pycnostachyum* were clearly protrusive. The epidermis includes a single flattened row, rectangular, round or oval-shaped cells. Outer epidermal cells are larger than the inner epidermal cells, covered with a thin cuticle layer, which is curled over. Outer membranes are thicker than inner and longitudinal membranes, but the inner membranes of epidermal cells in the middle vein area became thicker. Covering hairs and glandular hairs were the same as the stem and seen in both epidermis. Covering hairs are candelabriform, stellate, and multicellular in *V. pestalozzae*. Glandular hairs were of 6 types. The covering hairs of *V. pycnostachyum* were candelabriform, stellate, and multicellular. Glandular hairs were of 3 types. Stoma (amphistomatic) found on both surfaces of the leaf were much denser on the lower surface. The transverse cross-section is higher than the epidermal cells (hygromorphic stoma). In all types of mesophyll was arranged as two or three-rows under the outer epidermis. It was formed with plentiful chloroplasted palisade parenchyma and three or five rows of sponge parenchyma, which was underneath (bifacial leaf). Vascular bundles were collateral. These were well developed in a continuous crescent in *V. pestalozzae* but interrupted and horn-shaped in *V. pycnostachyum*. Xylem was located in the upper epidermis, while the floor was located in the lower epidermis. In xylem, tracheal elements are arranged radially and thin-walled parenchymal cells are present. Phloem is placed under the xylem. Clear parenchymatic cells were arranged in 2 or 3 rows under the outer epidermis, five or six rows in *V. pestalozzae* after collenchyma, and 5 or 6 rows in *V. pycnostachyum*. Parenchymatic tissue was found in *V. pycnostachyum* with 25-30 rows under the phloem till the inner epidermis, but much narrower in *V. pestalozzae* with 5-10 rows. Thick lateral veins in both sides of the middle vein were arranged till the edge of palm and the middle vein made a deep outgrowth. The lateral veins had the same anatomical structure as the middle veins, but vascularity was much more reduced (Figures 6, 9-12).

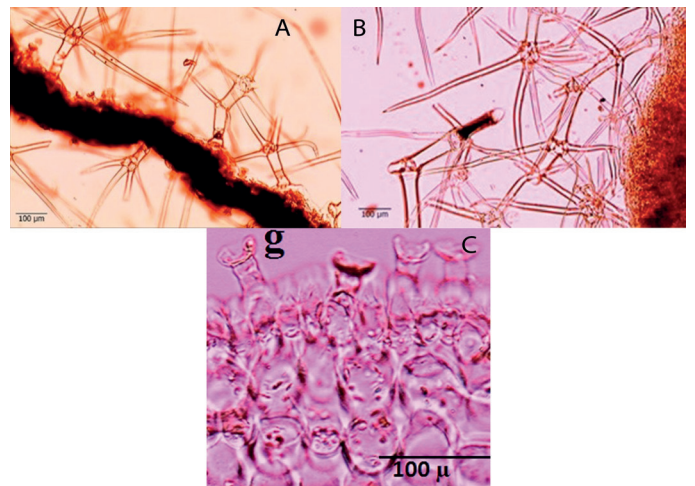


Figure 10. Hairs of stem and leaf. (A) *V. pestalozzae* eglanular hairs of leaf; (B) *V. pycnostachyum* eglanular hairs of leaf, glandular hairs of stem (C)

g: Glandular hairs

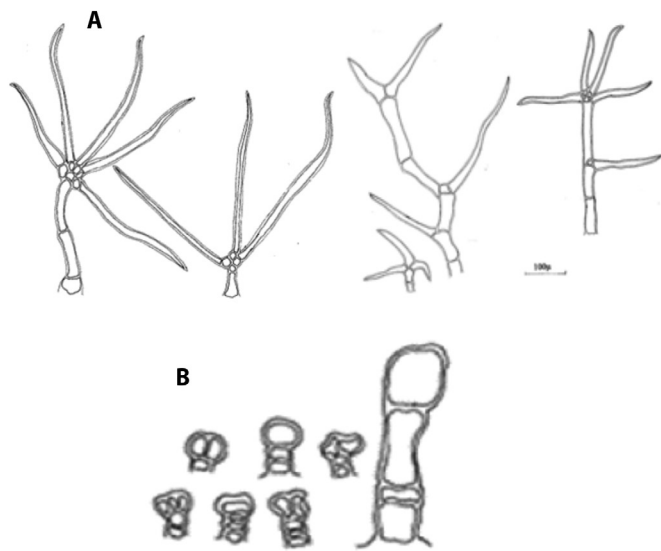


Figure 11. Hairs of stem and leaf of *V. pestalozzae* and *V. pycnostachyum* (A) eglandular hairs (B) glandular hairs

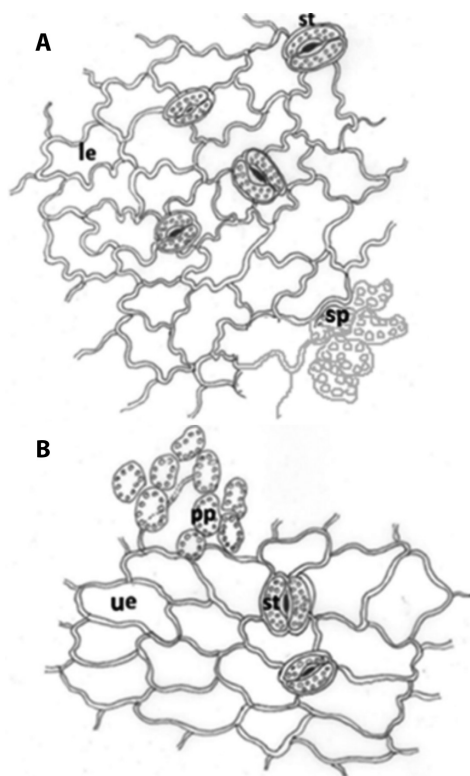


Figure 12. *V. pestalozzae* and *V. pycnostachyum*; (A) surface view of lower epidermis, (B) surface view of upper epidermis

ue: Upper epidermis, le: Lower epidermis, st: Stomata, pp: Palisade parenchyma, sp: Spongy parenchyma

According to the results, the root, stem, and leaf anatomic structures of each *Verbascum* species, *V. pestalozzae* differs anatomically from each other. These differences can be summarized as follows: in the stem, there are narrower sclerenchymatic cells and phloem than those in the leaf of *V.*

pestalozzae; having a protrusion below, continuous crescent vascular bundles, less parenchymatic cells in outer and inner epidermis, stellate type hairs and differences in the number of cells in secretion hairs.

CONCLUSIONS

The anatomic properties belonging to the two species are generally compatible with the findings of Metcalfe and Chalk²⁰ and others signified in the genus.²¹⁻²⁴ We believe that our results provide additional evidence for taxonomists and can help to separate the species. The lack of a taxonomic, morphologic, and anatomic studies on the species made it important for the systematic introduction of the research.

Conflict of Interest: No conflict of interest was declared by the authors.

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