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ANATOMICAL EXAMINATION OF IRANIAN ENDEMIC *STACHYS ACEROSA* BOISS. (LAMIACEAE): IMPLICATIONS FOR THE SEPARATION OF MORPHOLOGICALLY SIMILAR SPECIES

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Abstract

An anatomical study was conducted in *Stachys acerosa* Boiss. is endemic to Zagros Chain Mountains in Iran. In the current study the samples were collected from Oshtorankuh Mountain and examined. Vegetative and reproductive organs anatomically using light microscope. The distinguishing feature of *S. acerosa* is the presence of sclerenchyma at the angles of the stem. Five types of trichomes - simple nonglandular uniseriate uni- and multicellular, short and long capitate glandular, and glandular peltate trichomes were found on the surface of vegetative and reproductive organs. The leaves are amphistomatic and isobilateral; and the mesophyll is composed of four-layers palisade parenchyma. Two of them present at the upper side and another two layers at lower side and in between palisade layers two-layered spongy parenchyma were existed. The present study shows that the certain anatomical features of *Stachys* taxa are valuable for species delimitation.

Introduction

The genus *Stachys* L., consisting of about 300 species, is one of the largest genera of the family Lamiaceae. It is a sub-cosmopolitan genus (Mabberley 1997, Harley *et al.* 2004). Iran has one of the richest diversities of *Stachys*, with 34 species belonging to 12 sections and 2 subgenera. Seventeen taxa (50%) are endemics in Iran (Rechinger 1982).

Stachys acerosa Boiss. belongs to subgenus *Stachys*, section *Aucheriana*. It is cushion-forming perennial with a four-sided stem which sparsely covered with appressed short simple hairs up to 1 mm and short subsessile glandular hairs of 0.2 - 0.5 mm length. The leaves of *S. acerosa* are subsessile to sessile, hairy as the stem. The calyx is also sparsely covered with appressed long simple hairs and subsessile glandular hairs at margin. Corolla and bracts are 18-20 and 8 - 10 mm long, respectively. Detail information on anatomical properties of *S. acerosa* cannot be found in the existing literature. Up to now, Salmaki *et al.* (2011) and Dehshiri and Azadbakht (2019) studied only leaf and stem anatomy of *S. acerosa*, respectively. An attempt, therefore, has been taken to study the anatomy of *S. acerosa* for the first time to elucidate its taxonomic implications.

Materials and Methods

Thirty plant individuals of the *S. acerosa* were collected in Oshtorankuh Mountain, during the flowering stage. The voucher specimens were prepared according to standard herbarium techniques and stored in the Herbarium of the Boroujerd Branch, Islamic Azad University, Iran (Iran: Lorestan province, Aligoudarz, Oshtorankuh Mts, 3000 m, July 1st, 2013, Dehshiri 5342).

Fresh material was fixed in formalin–acetic acid–alcohol and glycerol-acetone-formalin and stored in 70% alcohol for anatomical studies. We sampled 10 leaves per individual. About micro-characters dimensions, such as trichomes, cuticles, etc., we measure 10 point per materials. Hand-

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cut sections were taken from leaves and stems in the middle parts of plants. All sections were stained with methylene blue-carmine dye. They were embedded in glycerin. Measurements and photographs of anatomic sections were then observed under an Olympus CX-21 binocular light microscope.

Results and Discussion

Stem: The upper part of stem is orbicular while the middle part is quadrangular on cross section. The cuticle is 3 to 5 µm thick. The epidermis consists of cubical or rectangular cells. The dimensions of the epidermis cell are 4 to 6 µm (Table 1). Three to four layers of sclerenchyma are observed below the epidermis at the angles. The rest of the cortex consists of the 3-7-layered elliptic to orbicular parenchymatic cells. Vascular tissues form four bundles, making almost a cylinder, which is pronounced in the angles. There was sclerenchyma outside the phloem. Pith region occupies a considerable part of the stem and is filled with large hexagonal or orbicular parenchyma cells in the center of stem (Fig. 1). The diameter of the pith cells are 19 to 28 µm (Table 1).

Table 1. Anatomical measurements of various cells and tissues of *Stachys acerosa.*

Material	Min-Max (µm)	Mean ± S.D. (µm)
Stem		
Length of non-glandular trichome	50 - 170	110 ± 61
Length of glandular trichome	20 - 70	45 ± 25
Cuticle thickness	3 - 5	4 ± 0.9
Epidermal thickness	4 - 6	5.2 ± 0.7
Diameter of pith cell	19 - 28	22.8 ± 3.3
Leaf		
Length of non-glandular trichome	30 - 115	70 ± 31
Length of glandular trichome	15 - 60	36.25 ± 16.3
Adaxial cuticle thickness	2 - 4	3 ± 0.9
Abaxial cuticle thickness	3 - 5	4 ± 0.6
Adaxial epidermis thickness	2 - 4	3.2 ± 0.7
Abaxial epidermis thickness	2 - 4	3.6 ± 0.9
Petiole		
Length of non-glandular trichome	65 - 175	120 ± 54
Length of glandular trichome	30 - 80	55 ± 25
Adaxial cuticle thickness	8 - 9	8.6 ± 0.5
Abaxial cuticle thickness	6 - 9	7.8 ± 1.2
Adaxial epidermis thickness	3 - 5	4 ± 0.6
Abaxial epidermis thickness	3 - 4	3.4 ± 0.5
Diameter of cortex cell	25 - 31	28.2 ± 2.1

Min = Minimum, Max = Maximum, S.D. = Standard deviation

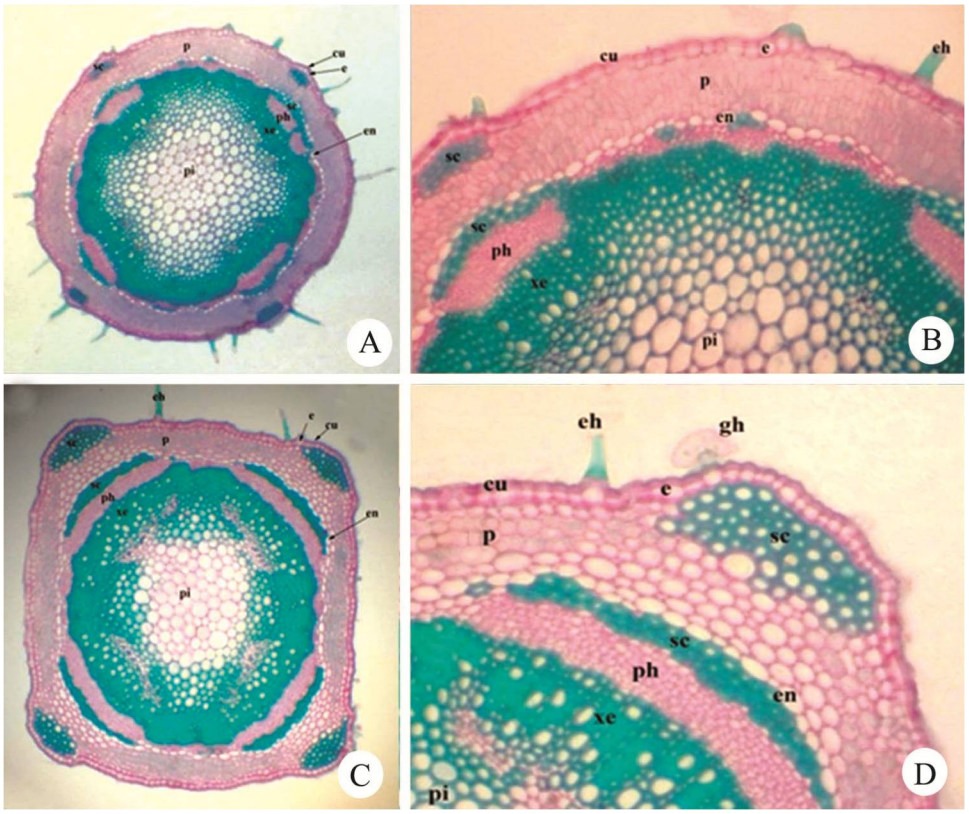

Fig. 1. Stem cross section of *Stachys acerosa*. A, B - upper part (× 10, 40); C, D - middle part (× 10, 40) (cu – cuticle, e - epidermis, eh - eglandular hair, gh - glandular hair, sc - sclerenchyma, p - parenchyma, en – endodermis, ph – phloem, xe – xylem, pi – pith).

Leaf: The thick of the adaxial cuticle is 2 to 4 µm, while that of the abaxial cuticle is 3 to 5 µm. The adaxial and abaxial epidermal cells are 2 to 4 µm thick (Table 1). The upper epidermal cells are larger than lower ones. The leaf showed isobilateral anatomical structure. The palisade parenchyma consists of two layers, on both sides of spongy parenchyma. The spongy parenchyma cells consist of two layers. Vascular bundles are collateral, surrounded by a parenchymatic sheath. There is a sub-epidermal two-layered collenchyma which is formed under nerves in the cross section of leaf. Sclerenchymatic cells are not visible in the phloem tissue. The leaves are amphistomatic. The anomocytic stomata are located on the same level with the epidermal cells (Fig. 2).

Petiole: The transverse section of petiole shows one-layered epidermis with cuboidal or rectangular cells, numerous densely packed hexagonal or orbicular parenchymatous cells, one large central vascular bundle and four to six lateral vascular bundles. The thick of the adaxial cuticle is 8 to 9 µm, while that of the abaxial cuticle is 6 to 9 µm. The thick of the adaxial epidermal cells are 3 to 5 µm, whereas those of the abaxial epidermal cells are 3 to 4 µm (Table 1). Collenchyma is situated under epidermis. It is one-layered on abaxial and adaxial surfaces and three-layered at the ends of the petiolar wings. The diameter of the cortex cells are 25 to 31 µm (Table 1). The vascular bundle is collateral (Fig. 3).

Fig. 2. Leaf anatomy of *Stachys acerosa*. A, B - cross section (× 10, 40), C, D - leaf surface (× 40) (ad - adaxial, ab - abaxial, cu - cuticle, ue - upper epidermis, le - lower epidermis, pp – palisade parenchyma, sp -spongy parenchyma, co - collenchyma, vs - vascular sheath, pc - parenchyma cells, p - phloem, x - xylem, st - stomata).

Calyx: There were anomocytic stomata only in abaxial epidermis. The adaxial epidermis cells were smaller than abaxial ones. Palisade parenchyma consists of two layers of densely compacted and elongated cells, on both sides of two layers of spongy parenchyma. They were close together around vascular bundles. In vascular bundles, there was sclerenchyma outside the phloem (Fig. 4).

Corolla: The epidermal cells were hexagonal. Beneath the epidermis, there were the 3-4 layers of parenchyma cells. The vascular bundles were arranged in one row (Fig. 5).

Bract: Adaxial and abaxial epidermis is single-layered and epidermal cells of the bracts were nearly cuboidal or rectangular. The palisade parenchyma consists of three layers on both sides of

one-layered spongy parenchyma. The vascular bundles are collateral with palisade parenchyma toward the upper and lower sides of bract. The bundle is wrapped with a vascular parenchyma sheath (Fig. 5).

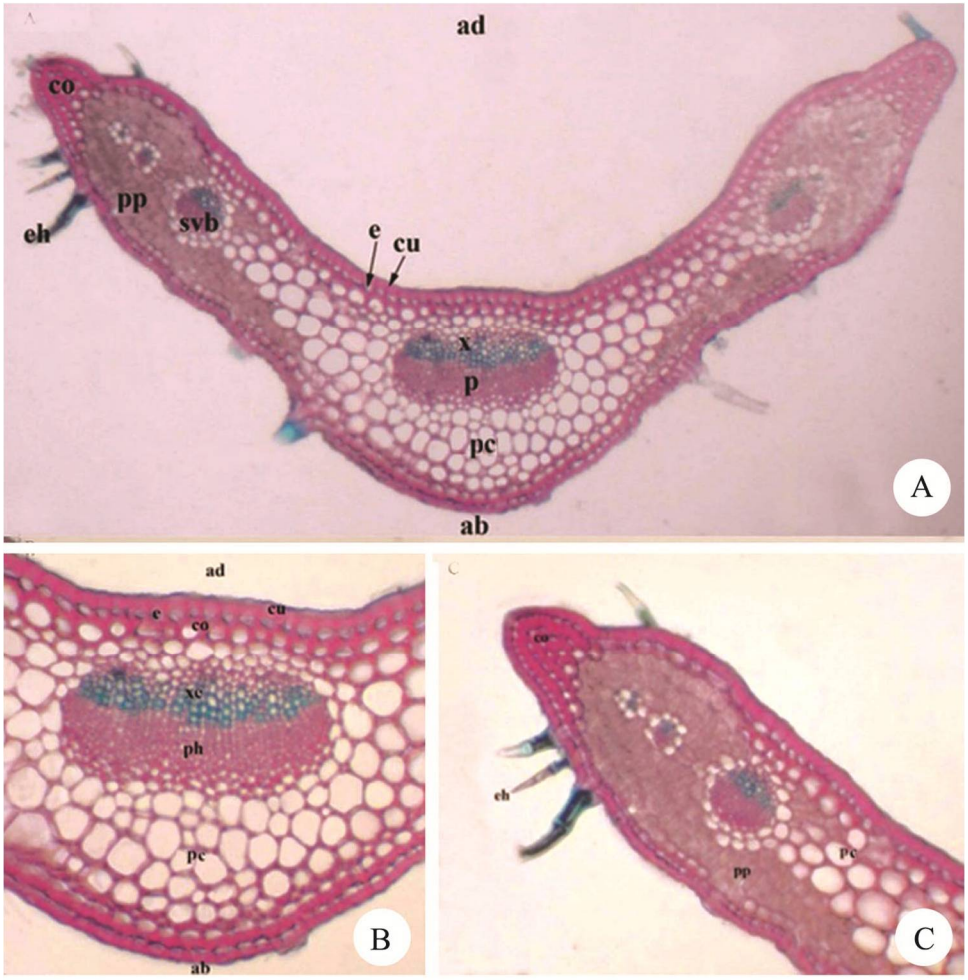

Fig. 3. Petiole anatomy of *Stachys acerosa* (x 10, 40). (ad - adaxial, ab - abaxial, cu - cuticle, e - epidermis, eh - eglandular hair, pp - palisade parenchyma, svb - small vascular bundle, co - collenchyma, pc - parenchyma cells, p, ph - phloem, x, xe - xylem).

Trichome and cuticle: On the vegetative and reproductive organs surface, five types of trichomes were found: simple nonglandular uniseriate uni- and multicellular, short and long capitate glandular, and glandular peltate trichomes (Fig 6, Tables 1, 2). Stem, calyx, corolla, and bract are covered by a thin-layered cuticle, but petiole shows thick cuticle. The cuticle on the leaf upper epidermis is thinner than the lower epidermis (Table 1).

Metcalfe and Chalk (1950) reported that the stems of the family Lamiaceae species are rectangular and that the collenchymatous tissue covers broad area at the corners, and

scleranchymatous tissue surrounding the vascular tissue. The same observations were made during the anatomical studies on other Lamiaceae members in Iran (Hatamneia *et al.* 2008, Dehshiri and Azadbakht 2012a, 2012b, Yousefi *et al.* 2014, Bahadori *et al.* 2016). The distinct feature of *S. acerosa* is the presence of sclerenchyma at the angles of the stem. This character has not been found in any *Stachys* species investigated yet.

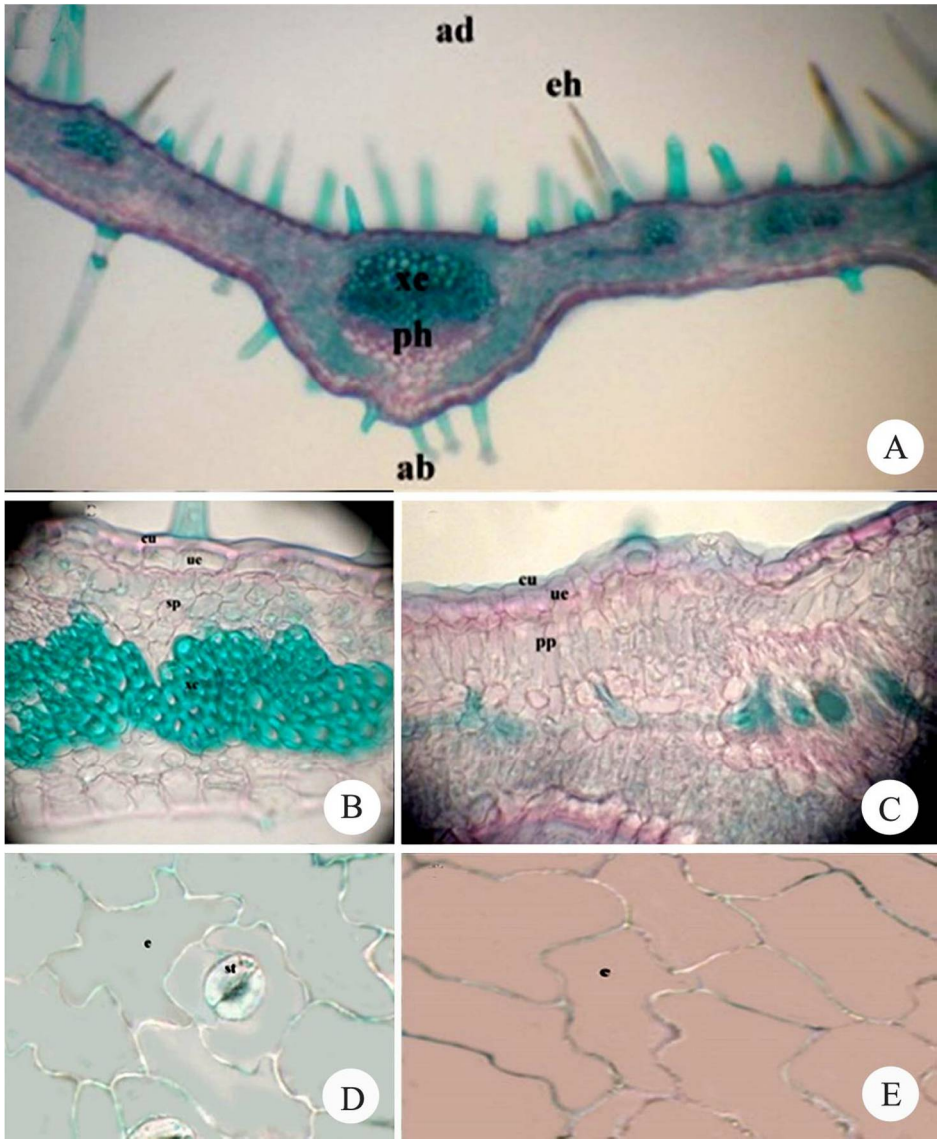

Fig. 4. Calyx anatomy of *Stachys acerosa*. A, B, C - cross section (× 10, 40), D - Abaxial surface of calyx (× 40), E - Adaxial surface of calyx (ad - adaxial, ab - abaxial; cu - cuticle, ue - upper epidermis, eh - eglandular hair, pp - palisade parenchyma, sp - spongy parenchyma, ph - phloem, xe - xylem, e - epidermis, st - stomata).

Leaves of Lamiaceae are generally dorsiventral or isobilateral. While the former is the predominant type in *Stachys*, the latter is restricted to section *Aucheriana*. The numbers of palisade layers have taxonomic value in the family Lamiaceae (Venditti *et al.* 2013, Kaya *et al.* 2015). It has been suggested that intense illumination and scarce water can enhance development of palisade tissue, and consequently increase photosynthetic activity (Fahn 1982). The anatomy of leaves and bracts, calyx is similar and isobilateral. The anomocytic stomatal structure of leaf is similar with calyx within *S. acerosa*.

Table 2. Distribution of trichome types in *Stachys acerosa.*

Material	Glandular trichome			Nonglandular trichome	
	Peltate	Glandular capitates		Unicellular	Multicellular
		Short stalk	Long stalk		
Stem	+	+	+	+	+
Leaf	+	+	+	+	+
Petiole	-	+	-	+	+
calyx	-	+	+	+	+
Corolla	-	+	-	+	+
Bract	-	+	+	+	+

- Absent, + Present.

The petiole anatomy showed variability in different Lamiaceae species (Gupta and Bhambie 2008, Akçin *et al.* 2011). Salmaki *et al.* (2011) reported that *S. acerosa* leaves are subsessile to sessile and had not studied petiole anatomy. Our results show that the petiole is crescent-shaped and vascular bundles surrounded by parenchyma. The adaxial phloem that was recorded exclusively in subgenus *Betonica* was represented as a valuable distinguishing character, while it did not exist in the vascular bundles of the petiole of species from the subgenus *Stachys* such as *S. acerosa*.

In Lamiaceae, nonglandular and glandular trichomes are distinguished, with peltate and capitate trichomes as the basic types of glandular trichomes. Capitate trichomes are divided to short stalk and long stalk. Non-glandular trichomes can be divided into two main groups: unicellular and multicellular (Cantino 1990, Fahn 2000, 2002). The glandular trichomes' structure can vary widely among species, as does the composition of the essential oils they produce for protection of the plant aerial parts against herbivores and pathogens (Werker 1993). The function of nonglandular trichomes depends on their morphology, the organ on which they are situated and their direction and orientation. When nonglandular trichomes are sparsely spread all over the plants, they may serve as a mechanical barrier (Werker 2000). Falciani *et al.* (1995) have studied the micromorphology and distribution of trichomes of *Stachys* species growing in Italy and described different glandular and nonglandular trichomes. Salmaki *et al.* (2012) reported that stems, leaves and calyx have only short glandular hairs but our results show that long glandular hairs are also present. Giuliani and Maleci Bini (2012) found that *Stachys* subgenus *Betonica* possessed only peltate trichomes, while *Stachys* subgenus *Stachys* has different types of large capitate hairs (Grujić Jovanović *et al.* 2014). Our results show that peltate trichomes were restricted to stem and leaves and not recorded by Salmaki *et al.* (2009, 2012). They reported leaves sparsely covered by short simple trichomes up to 0.1 and 1 mm, respectively. Our results show that the first demonstrate is correct.

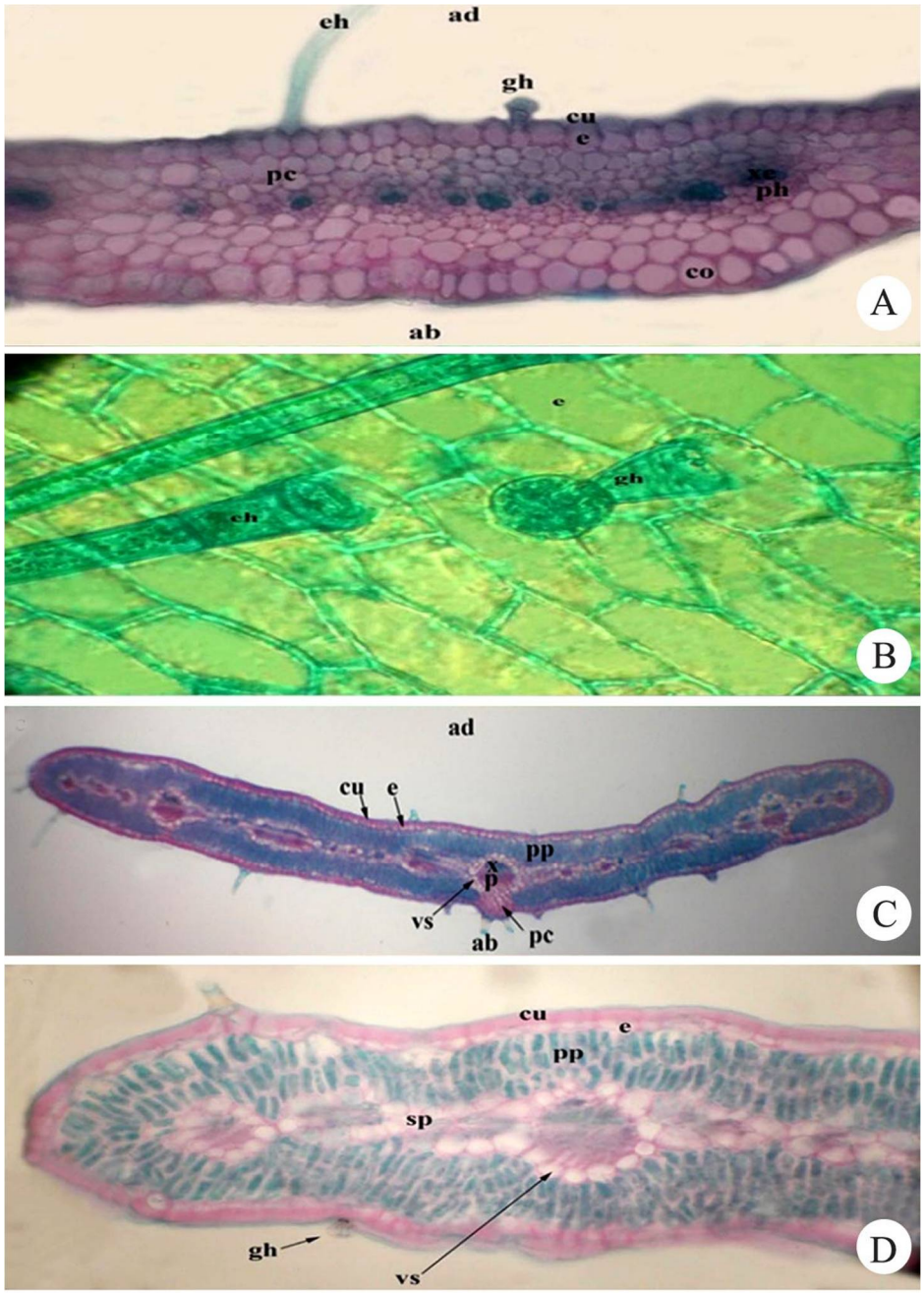

Fig. 5. Corolla and bract anatomy of *Stachys acerosa*. A - cross section of corolla (×10), B - Adaxial surface of corolla (× 40), C, D - cross section of bract (× 10, 40) (ad - adaxial, ab - abaxial; cu - cuticle; e -epidermis; eh - eglandular hair, gh - glandular hair, pc -parenchyma cells, co - collenchyma; ph - phloem, xe – xylem, pp - palisade parenchyma, sp - spongy parenchyma, vs - vascular sheath).

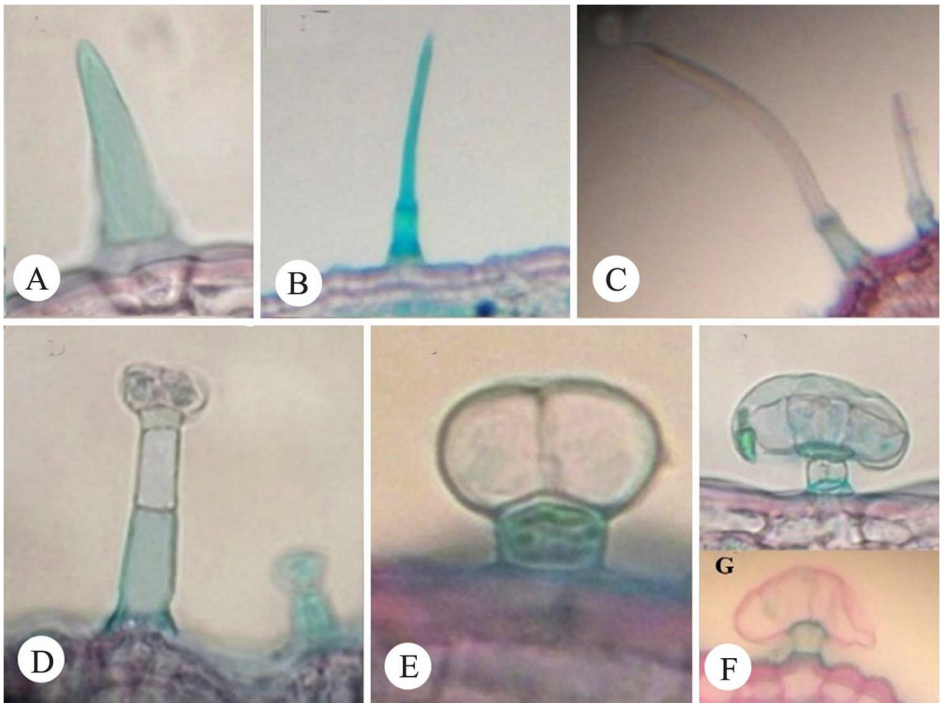

Fig. 6. Light micrograph of trichomes of *Stachys acerosa* (× 40). A - simple nonglandular uniseriate unicellular trichomes, B, C - simple nonglandular uniseriate multicellular trichome, D - long capitate glandular trichomes, E - short capitate glandular trichome, F, G - glandular peltate trichomes).

The results of the present study offer useful data for evaluating the taxonomy of *Stachys* at infrageneric levels. A remarkable result from this study was anatomical identification of endemic species *S. acerosa* with distinguishing characters. Furthermore, the present results support that the different types of trichomes play an important role as diagnostic characters among *Stachys* species. These findings combined with anatomy of the vegetative and reproductive organs can contribute to species identification.

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