ANATOMICAL INVESTIGATION OF FOUR TAXA OF SCUTELLARIA ALBIDA L. (LAMIACEAE)

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Abstract

The root, trunk and leaf anatomy of four taxa of Scutellaria albida namely, S. albida subsp. albida, S. albida subsp. velenovskyi, S. albida subsp. colchica and S. albida subsp. condensata were examined. The aim is to determine whether these characters can be used for systematic purposes. The roots displays a typical stele structure in all studied taxa. But their number of pith rays are different in each taxa. The stems of S. albida taxa have square like transection, collateral vascular bundles, parenchymatous pith and show 1-2 layered epidermis coated with thick cuticle. S. albida subsp. condensata has papillate epicuticular wax on its stem cuticle layer. In all taxa angular collenchyma, and cambium forming phloem outward and xylem inward are observed. The leaves are hypostomatic, have single rowed epidermis coated with thick cuticle and show dorsiventral mesophyll in all studied taxa. S. albida subsp. velenovskyi has echinate epicuticular wax on its leaf cuticle layer, but the others have smooth cuticle layer. The midrib shows one collateral bundles in all taxa but the shape of median veins of the leaf blade show variation in each taxa. Some of the anatomical characters viz. the number of pith rays of roots, the number of palisade parenchyma layer, plant cuticles covered by wax, the shape of median veins of the leaf blade and stomata index, provide information of taxonomical significance for these taxa.

Introduction

Scutellaria L. (skullcap), a member of the Lamiaceae family, has approximately 300 species and this genus prefers living in temperate climates (Paton, 1990a). Scutellaria is viable plant for every continent except for Antarctica with its main centre of diversity in Irano-Turanianregion of Asia. Eastern Mediterranean and the Andes are secondary center of the diversity (Paton, 1990b, Bruno *et al.*, 2002). Turkey is accepted as the gene center of Lamiaceae family to which the Scutellaria genus belongs (Başer, 1993). Scutellaria is represented by twenty-four species, thirteen subspecies and one hybrid in the flora of Turkey. Thirteen (%41) of them are endemic to Turkey (Çiçek, 2008)

Scutellaria has been used in spices, fragrances, traditional and folk medicines in different parts of the world for centuries. They are well known among people as powerful medicinal herbs which are mild relaxants that affect the neural and muscular-skeletal systems (Werker *et al.*, 1985; Duke, 1989; Zargari, 1990; Stojakowska and Kisiel, 1999; Graham *et al.*, 2000; Kim *et al.*, 2001; Hui *et al.*, 2002; Weber, 2009). A lot of *Scutellaria* species were studied in hygiology. There are lots of scientists who studied their therapeutic activities, for example, inflammatory, antioxidant, anti-HIV, spasmolytic, anti-anticonvulsant, antifungal, antidiarrhea, anticancer, antipyretic, antibacterial, and antiviral activities (Chou *et al.*, 2003; Shang *et al.*, 2010).

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Scutellaria albida subsp. *velenovskyi* (Rech. f.) Greuter & Burdet was considered as a different species in Turkish literature and has named as *Scutellaria velenovskyi* (Greuter *et al.*, 1984). Çiçek (2008) considered as a polymorphism within the species because of geographic isolation of different populations. Few studies of detailed examination and the conflictions mentioned above lead us to study these four taxa anatomy; since anatomical characters play an important role in the taxonomical studies of medical plants (Agbagwa and Ndukwu, 2004; Kharazian, 2007).

In this study, anatomical structures of roots, stems and leaves of four taxa of *Scutellaria albida* namely, *S. albida* subsp. *albida* L., *S. albida* subsp. *velenovskyi*, *S. albida* subsp. *colchica* (Rech.f.) J.R. Edm. and *S. albida* subsp. *condensata* (Rech.f.) J.R. Edm. were examined and compared. The vegetative anatomy was proved to be an important source of additional characters for resolving taxonomic difficulties in *Scutellaria albida*. So, this study aims to clarify the questions on this polymorphic species and add new databases.

Material and Methods

Plant samples were collected between 2013 and 2014 from their natural environments during their flowering period (Table 1).

Taxa	Specimen location and habitat
S. albida subsp. albida	Kastamonu: From Cide to Azdavay, between 53 km, Asarkaya tunnel entry and exit, 547 m, rock hill, N 41°49.503' E 033°24.035'; 30.07.2013
S. albida subsp. velenovskyi	Aydın: Kuşadası, Davutlar, Dilek Peninsula National Park, Kalamaki stream, 202 m, dry river bed edges, N $37^\circ40'46.0"$ E $027^\circ09'48.8"$; 07.07.2013
S. albida subsp. colchica	Artvin: From Yusufeli to Artvin, between 30 km, 444 m, rock slopes, N 40°55.979' E 041°46.192'; 21.07.2014
S. albida subsp. condensata	Bitlis: From Van to Tatvan, between 23 km, 1679 m, <i>Quercus</i> sp. slopes, N 38°28' 32" E 042°31' 02"; 19.07.2014

Table 1. The location and collection data of the material studiedfrom Turkey.

Plant samples were stored in the Manisa Celal Bayar University Herbarium. The taxonomic descriptions of the species were made according to Paton (1990a). The plant specimens were kept in 70% alcohol. The paraffin wax method (Algan, 1981) was used in order to fix the materials. Then the prepared paraffin blocks were sectioned with a Leica RM2125RT rotary microtome. In order to make structural characterization, the transverse sections (5-10 μ m thick) were stained with safranin-fast green (Johansen, 1940). Using entellan, the slides were fixed. Finally slides were looked over with Olympus BX50 research microscope and photographs were taken on Leica DW 3000 with a Leica DFC 295 camera. Ten peripheral slides were prepared for each taxon and the stomata index was calculated (Meidner and Mansfield 1968).

Results and Discussion

Anatomy of roots

The outermost surface of root's transverse sections are made up of the periderm's cells which are squashed or breaking up in all investigated taxa. There is a multi-layered parenchymatic cortex which has compactly arranged, rectangular, oval or orbicular parenchymatic cells adjacent to the

peridermis. Endodermis layer is seen clearly only in S. albida subsp. condensata (Fig. 1D). Pericycle located adjacent to the endodermis is sporadically divided with supporting tissue members in S. albida subsp. condensata. Above the phloem of all taxa, there is a sclerenchymatical sheath. The root of S. albida subsp. velenovskyi has irregular layered sclerenchymatical cells scattered throughout the cortex (Fig. 1B). There is small phloem region in the roots except for S. albida subsp. condensata which has large phloem region and the members of the phloem are seen clearly. 1–3 layered cambium rings are hardly visible and indistinguishable for all taxa of S. albida. A very large area of the transverse section occurs with the component of xylem in all investigated taxa. Trachea members are circular or hexagonal and they are regularly settled in increasingly accurate diameter from the centre to the cortex with the exception of S. albida subsp. condensata (Fig. 1) because its vessels are irregularly placed. The central vessels are larger than peripheral vessels. Pith rays of S. albida subsp. albida and S. albida subsp. velenovskyi are 2–9 layered, S. albida subsp. colchica are 2–4 layered and S. albida subsp. condensata are 1–2 layered (Fig. 1). They are heterogeneous. The pith of all taxa investigated is occupied by xylem elements. Representative anatomical characters of four taxa investigated are summarised in Table 2.



Fig 1. Cross section of the roots. A: *S. albida* subsp. *albida*, B: *S. albida* subsp. *velenovskyi*, C: *S. albida* subsp. *colchica*, D: *S. albida* subsp. *condensata* cp::cortex_parenchyma, x::xylem, en: endodermis,,pr::pith_rays, pe: periderm, ph: phloem, t::trachea, Bar: 50 µm

Root anatomical characters	S. albida subsp. albida	S. albida subsp. velenovsky	S. albida subsp. colchica	S. albida subsp. condensata
Peridermis cell				
width (µm)	41.2 ± 11.49	34.05 ± 10.66	43.33 ± 10.67	31.35 ± 8.90
length (µm)	15.8 ± 4.35	18.99 ± 5.57	19.91 ± 3.69	24.00 ± 5.41
Parenchyma cell				
width (µm)	42.53 ± 14.23	47.33 ± 16.64	37.87 ± 7.70	38.53 ± 10.38
length (µm)	16.95 ± 3.72	26.00 ± 7.02	21.25 ± 4.25	25.43 ± 6.75
Vessel (µm)	40.22 ± 13.95	50.19 ± 14.74	38.89 ± 15.12	27.10 ± 8.53
Pith rays	2–9	2–9	2–4	1–2

Table 2. Root features of four Scutellaria albida taxa.

Anatomy of stems

Transverse section of the stem is square like in four taxa of this species. Epidermis of all investigated taxa of this paper are coated with a thick cuticle layer. At the corner of S. albida subsp. *condensata* the cuticle layer is very thick. Furthermore, on the cuticle layer of it, papillate epicuticular wax is evident (Fig. 2). But papillate epicuticular wax has not been observed on others cuticle layers. The epidermal structure consist of 1-2 layered rectangular, oval or squarish cells. These cells are tabular. Limited number of stomata set into the epidermis. On the surface of the protective tissue of four taxa of S. albida, there are plenty of hairs most of which are glandular. Adjacent to the epidermal cells, there are multi-layered lamellar and lacunar collenchyma cells at the corners of the stems of all taxa. The cortex region is very narrow in all taxa and composed of irregular ovoidal, circular like or squashed parenchymatic cells with intercellular spaces. Measurements of these cells and the number of layers are given in Table 3. The sclerenchyma cells (1-3 layers) cover vascular bundles both at the corners and also between the corners of the stem. But, the sclerenchyma cells are indistinguishable between the corners of *S. albida* subsp. condensata. All vascular bundles located at the corners of stems of S. albida taxa are next to each other; yet, bundles between the corners in the stems are separated from each other by parenchymatic cells. At the corners, the vascular bundles are larger than the bundles between corners for all the taxa. Their cambiums are hardly visible. The stem of all taxa investigated in this paper have large pith which are composed of orbicular or hexagonal parenchymatic cells and there are intercellular spaces. The diameter of the cells in the centre of the pith is quite larger than of the cells located under the vascular bundle on four taxa of this species (Fig 3).



Fig. 2A: Cross-section of the leaf of S. albida subsp. velenovskyi, B: Cross-section of the stem of S. albida subsp. condensata. cu: cuticle with epicuticular wax. Bars: 10 µm

Stem anatomical	S. albida subsp.	S. albida subsp.	S. albida subsp.	S. albida subsp.
characters	albida	velenovsky	colchica	condensata
Epicuticular wax	absent	Absent	absent	papillate
Epidermis cell				
width (µm)	23.11 ± 5.68	12.40 ± 3.28	20.08 ± 3.1	20.87 ± 6.80
length (µm)	15.02 ± 2.60	17.50 ± 2.52	15.46 ± 3.42	12.57 ± 1.98
Cortex thickness	218.12 ± 13.02	257.63 ± 7.22	182.00 ± 7.96	263.81 ± 15.89
in the corner (µm)				
Cortex thickness in the	59.31 ± 20.02	52.35 ± 9.87	118.33 ± 12.79	94.14 ± 18.25
marginal (µm)				
Number of cortex layer	3–6	3–8	5-8	6–10
Corner collenchyma	66.09 ± 3.24	105.11 ± 34.24	51.06 ± 12.12	103.10 ± 8.61
thickness (µm)				
Vascular bundle				
Phloem length (µm)	18.01 ± 2.47	24.20 ± 8.95	27.41 ± 5.98	46.15 ± 12.76
Xylem length (µm)	206.88 ± 44.61	223.74 ± 33.62	100.9 ± 29.21	198.13 ± 35.62
Trachea size (µm)	23.24 ± 5.28	40.96 ± 11.50	22.08 ± 4.54	26.39 ± 6.42
Pith cell size (µm)	95.69 ± 25.09	92.02 ± 36.99	71.76 ± 31.17	101.89 ± 37.85

Table 3. Stem features of four Scutellaria albida taxa.



Fig. 3. Cross-section of the stems. A: *S. albida* subsp. *albida*, B: *S. albida* subsp. *velenovskyi*, C: *S. albida* subsp. *colchica*, D: *S. albida* subsp. *condensata*. co: collenchyma, cp: cortex parenchyma, e: epidermis, p: pith, t: trachea. Bar: 50 μm

Anatomy of leaves

Outer surface of the transverse section of the leaf blade is covered with a thick cuticle layer in the studied taxa. In their upper epidermis cuticle layers are thicker than those of the lower epidermis. Only *S. albida* subsp. *velenovskyi* has echinate epicuticular wax on the cuticle layer. The others have smooth cuticle layer. The glandular and eglandular hairs are located on both epidermises of all the studied taxa. Mesomorph stomata can only be seen in lower epidermis in the studied taxa. Their epidermis cells are rectangular, oval or squarish. Upper epidermal cells are large and tabular, but lower epidermal cells are small in four taxa of this species (Fig. 4). Their leaves are bifacial. The mesophyll is made up of elongated rectangular palisade parenchyma and isodiametric spongy parenchyma cells. Measurements and number of layers of these cells are given in Table 4. While the palisade paranchyma cells are arranged tightly and there are large intercellular spaces between the sponge parenchyma cells. There is one large arc-shaped vascular bundle in the center and vascular bundles are surrounded by a parenchymatic bundle sheaths in all studied taxa.



Fig. 4. Cross-section of the leaves. A,B: *S. albida* subsp. *albida*, C,D: *S. albida* subsp. *velenovskyi*, E,F: *S. albida* subsp. *colchica*, G,H:*S. albida* subsp. *condensata*. le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, t: trachea, ue: upper epidermis, vb: vascular bundle. Bars: 50 µm

Leaf anatomical	S. albida subsp.	S. albida subsp.	S. albida subsp.	S. albida subsp.
characters	albida	velenovsky	colchica	condensata
Upper cuticle (µm)	2.25 ± 0.69	3.41 ± 0.59	3.65 ± 1.51	2.40 ± 0.47
Lower cuticle (µm)	1.32 ± 0.09	3.71 ± 1.76	2.10 ± 1.00	2.89 ± 0.67
Epicuticular wax	absent	Echinate	absent	absent
Upper epidermis				
width (µm)	32.30 ± 7.72	30.13 ± 15.09	29.86 ± 9.94	30.73 ± 8.73
length(µm)	24.39 ± 3.41	24.39 ± 4.40	28.62 ± 5.05	22.39 ± 5.91
Lower epidermis				
width (µm)	9.29 ± 4.36	16.00 ± 6.39	11.08 ± 4.59	13.12 ± 4.82
length(µm)	8.35 ± 2.29	14.23 ± 3.11	11.58 ± 2.63	11.00 ± 3.25
Number of the palisade cell line	1–3	1–2	1–5	1–3
Palisade cell				
width (µm)	11.72 ± 2.18	13.05 ± 2.39	18.11 ± 3.03	11.38 ± 2.46
length(µm)	24.92 ± 6.34	29.07 ± 6.03	36.35 ± 11.19	30.33 ± 9.31
Spongy cell				
width (µm)	15.28 ± 3.55	17.90 ± 4.28	17.98 ± 3.66	14.49 ± 3.95
length(µm)	12.91 ± 2.81	13.51 ± 2.56	16.22 ± 3.39	09.78 ± 2.64
Midrip trachea	08.28 ± 2.15	02.23 ± 0.44	13.09 ± 2.23	15.07 ± 4.09
Stomata index	24.19 ± 1.02	25.97 ± 0.93	18.75 ± 0.78	18.51 ± 0.85

Table 4. Leaf features of four Scutellaria albida taxa.

Members of genus *Scutellaria* can live in various habitats. Many species become evident characteristics of moist environments (Metcalfe and Chalk, 1950). The smallest group of the genera, including *Scutellaria albida*, shows characteristic of arid environments such as xeromorphic characters. This study describes the characteristics of four subspecies obtained from anatomical investigations. The anatomy of four taxa of *S. albida* is broadly similar, but they also have some important distinctions among the taxa.

Metcalfe and Chalk (1950) studied the root anatomy in some of Lamiaceae taxa. They found that the roots of Lamiaceae family have 2–12 or more-rowed pith rays. The present findings are congruent with the study made by Metcalfe and Chalk's (1950); Dinç *et al.* (2008); Baran and Özdemir (2009); Kahraman *et al.* (2010a,b); Celep *et al.* (2011). On the other hand, *Lamium lycium* Boiss. has 1-4 rowed rays (Baran and Özdemir, 2009). The pith rays of *Salvia chrysophylla* Stapfare composed of 1-24 rows (Kahraman *et al.*, 2010a) and the pith rays of *Salvia ballsiana* (Rech.f.) Hedge are composed of 1–3 (-4) rows (Kahraman *et al.*, 2010b). *Lamium truncatum* Boiss. has 1-4 rowed rays (Celep *et al.*, 2011). The present study indicates that *S. albida* subsp. *albida* and *S. albida* subsp. *velenovskyi* have 2–9, *S. albida* subsp. *colchica* has 2–4 and *S. albida* subsp. *condensata* has 1–2 rows of ray cells (Fig. 1). Therefore, the pith rays number is useful discriminative characters for the subspecies of *S. albida*.

The mechanical supportable tissue is seen in the root of all taxa. These are sclerenchymatical cells located sporadically over the phloem. Moreover, roots of studied *S. albida* subsp. *velenovskyi* showed irregular layered sclerenchymatical cells scattered throughout the cortex. Gönüz and Özörgücü (1999) have obtained the similar results for the root of *Origanumonites* L.L.. Çobanoğlu (1988), Özdemir and Şenel (2001) have pointed the same sclerenchymatic ring and sheath on root of *Salvia palaestina* Bentham and *S. forskahlei* L. In the root of *Scutellaria orientalis* subsp.

bicolor (Özdemir and Altan, 2005) sclerenchymatic ring and sheath is observed over phloem. The stems of all taxa of *S. albida* have sclerenchymatical sheath on phloem. Similar results were obtained for the stem of *S. orientalis* subsp. *santolinoides* (Hausskn. ex Bornm.) J.R.Edm. and *S. orientalis* subsp. *bicolor* (Özdemir and Altan, 2005). Meanwhile, the sclerenchyma is very important supportable tissue as recorded for species of *Hypenia* (Mart. ex Benth) Harley, one of the genera that grow in dry environments (Faria, 2008). *S. albida* taxa are arid environment plants. So, they have some sclerenchymatic cells in roots and in some parts of the stems.

Having square stem and features of endoderm are diagnostic characteristics of Lamiaceae members, as explained in this work for *S. albida* taxa and also for other species of this genus, such as *Scutellaria agretis* A. St.-Hil. ex Benth. (Oliveria *et al.*, 2013), *Scutellaria orientalis* subsp. *pinnatifida* (Candan and Cali, 2012), *S. orientalis* subsp. *bicolor* and *S. orientalis* subsp. *santolinoides* (Ozdemir and Altan, 2005; Metcalfe and Chalk, 1950). Evident collenchyma in the four angles is frequently described for Lamiaceae (Cronquist, 1981). In the studied taxa, there are collenchymatic cells at each corner of the stems.

Stem epidermises of all investigated taxa are covered with a thick cuticle layer. At the corner of *S. albida* subsp. *condensata* the cuticle layer is thicker than the other taxa and papillate epicuticular wax is evident on this cuticle layer. Furthermore, outermost surface of the leaf of *S. albida* subsp. *velenovskyi* is covered with echinate epicuticular wax. The presence of epicuticular wax is noticed for the first time for the genus. Duarte and Lopes (2007) observed striate cuticle on *Plectranthus neochilus* Schltr (Lamiaceae) leaf blade. Plant cuticles are covered with waxes, many of which are of great systematic significance and it can be taxonomically useful characteristic for *Scutellaria*. Waxes are an essential structural element of the surface and of fundamental functional and ecological importance for the interaction between plants and their environments (Barthlott *et al.*, 1998). Classification and terminology of epicuticular waxes are proposed based on high resolution SEM analysis of 13000 species of seed plants by Barthlott *et al.* (1998). But there is no record of epicuticular wax for the representatives of *Scutellaria*. The function of the epicuticular wax is known to be anti-adhesive, hardly wettable and preventing insect attachment (Barthlott *et al.*, 1998).

Four taxa of S. albida have dorsiventral and hypostomatic leaves. However number of palisade layer shows some differences among the taxa. The mesophyll of S. albida subsp. colchica formed by 1-5 layers of palisade parenchyma. On the other hand, others formed fewer palisade layers (Table 4). Thick palisade tissue of mesophyll is considered as xerophytic characters. This feature was reported in Teucrium sandrasicum O. Schwarz (Dinc et al., 2008), T. montanum L. and T. polium L. (Dinc et al., 2011). Stomata may have found on one or both sides of the leaf blade in Lamiaceae family (Metcalfe and Chalk, 1950). S. albida has a hypostomatic leaf. Such leaf, it was also described in S. altissima L. (Thaler et al., 1992), S. orientalis subsp. bicolor and S. orientalis subsp. santolinoides (Ozdemir and Altan, 2005). Having hypostomatic leaf is also interpreted as xeromorphic type. S. albida exhibits diacyticstomata as predominantly other Lamiaceae family member (Metcalfe and Chalk, 1950). The stomata index showed variation in S. albida taxa. Stomata index of S. albida subsp. albida and S. albida subsp. velenovskyi are nearly same, and that of S. albida subsp. colchica and S. albida subsp. condensata are nearly same (Table 4). All studied taxa have projective median veins. However, when it is observed at the crosssections of taxa, the shape of median veins of the leaf blade show some differences (Fig. 4). S. albida subsp. albida has concave to flat median vein at abaxial side. S. albida subsp. velenovskyi has flat to concavemedian veinabaxial side. S. albida subsp. colchica and S. albida subsp. condensata have concave median vein at abaxial side. Therefore, these characters can also be used for distinguishing the subspecies in Scutellaria albida.

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