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Investigations Anatomical on Turkish Vulnerable *Scrophularia* species: Scrophularia lepidota Boiss. (Scrophulariaceae)

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Research Article	ABSTRACT							
	In this study, the root, stem and leaf anatomy of <i>Scrophularia lepidota</i> , a narrowly distributed endemic species							
History	specific to our country, whose morphological features are known, was investigated. Plant materials were							
Received: 21/12/2024	collected from Ziyarettepe, Ulaş district of Sivas, Türkiye and anatomically analyzed. In the root cross-section,							
Accepted: 17/03/2025	root is in the secondary structure, there are 5-8 cell layered periderm in outside and under periderm tissue,							
	there are 6-10 cell layered pericycle. The stem is in primary structure, covered by uniseriate epidermis and							
	stellate epidermal trichomes. Under the epidermis there is cortex tissue which consists of 6-8 cell layers. The							
	leaves are equifasiyal according to the mesophyll layer, and amphistatic according to the presence of stoma. The							
	midrib is oval-circular in shape, and is surrounded by a single cell layered bundle sheath. There are idioblastic							
	cells in leaf cross section. The determined anatomical features of S. lepidota were compared with previous							
	anatomical studies on other species of the genus Scrophularia and the results were discussed. In this study, the							
	anatomical features of S. lepidota, which is endemic to our country, were revealed for the first time. In addition,							
	by determining the anatomical features of S. lepidota, a contribution was made to the general anatomical							
This article is licensed under a Creative features and systematics of the genus Scrophularia.								
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Introduction

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Scrophulariaceae Juss. family, which has 200-250 genera and approximately 3000-4000 herbaceous and a few shrub species worldwide, is known as the seventh largest flowering plant family in the world [1,2]. Although the genera belonging to the Scrophulariaceae family are cosmopolitan, they also spread widely in the temperate regions of the Northern Hemisphere to tropical regions, but varies especially in Africa [2-4]. The species of this family are generally autotrophic, semi or rarely fully parasitic plants [5].

Scrophularia L. is the genus that gives its name to the Scrophulariaceae family, and this genus is represented in the world with approximately 300 taxa that generally spread naturally in the Mediterranean Region [6]. The primary diversification center of the genus Scrophularia is Southeast Asia [7,8]. According to Turkish Flora there are 59 species in which 22 of them are endemic with the endemism ratio 37.2 % belonging to the genus Scrophularia spread naturally in Turkey [9]. According to Turkey Plant List (Vascular Plants) with the addition of new species recently discovered in Turkey, Scrophularia is represented by 65 species and 85 taxa in Turkey [10].

Anatomical studies on the members of the genus Scrophularia in the world and in our country are not sufficient. Anatomical studies on members of the genus Scrophularia mostly focused on leaf characteristics. The first brief description of idioblasts found in the leaves of Scrophularia species was made by Volkens (1887) in Scrophularia deserti Delile, and later on other species by Metcalfe and Chalk (1950) [11,12].

Holm (1929) stated that there was not much difference between root and stem anatomies as a result of his examination on five Scrophularia species that were close to each other [13]. Pennel (1929, 1935) studied the root, stem and leaf anatomy of 25 species and showed that there are anatomical and morphological differences between these species [14,15]. In another study by Lersten and Curtis (1997), idioblasts and their intracellular secretory structures were examined in detail. As a result of this study conducted on 172 genera and 237 species in the Scrophulariaceae family, secretion structures with different sizes and shapes were discovered [6].

In a recent study published by Lersten and Curtis (1997, 2001), the distribution of idioblast and leaf endocrine system in different Scrophulariaceae taxa was examined, and many important differences were found among the taxa examined [6]. Another explanation of idioblasts in species of the genus Scrophularia is Makbul et al. (2006). Makbul et al. (2006) investigated the anatomical features of some Scrophularia taxa and the distribution of idioblasts and found many important differences between the taxa examined [16].

The morphological and anatomical features of 14 Scrophulariaceae taxa naturally distributed in Turkey were studied by Kaplan and İnceoğlu (2003) but there are no members of the genus *Scrophularia* among these taxa [17]. Although the anatomical characters are very important in the genus *Scrophularia*, there are not enough studies and publications on the species of the genus in Turkey [16]. The subject of this study, *Scrophularia lepidota* Boiss, is a perennial herbaceous plant. It is an endemic plant that spreads naturally only in Sivas and Erzincan provinces in Turkey [18]. The endangered status of the *S. lepidota* species Ekim et al. (2000) in the "Red Book of Plants of Turkey" prepared by taking IUCN criteria into account (VU-May be Harmful) [19].

In this study, the root, stem and leaf anatomy of *S. lepidota*, a narrowly distributed endemic species specific to our country, whose morphological features are known, was investigated.

Material and Methods

In order to obtain plant material, a field study was carried out in Sivas where the species is distributed and the species was reached. Some of the plants collected as a result of the field work were transformed into herbarium material and stored in Cumhuriyet University Faculty of Science Herbarium (CUFH) under the code number M. Tekin 1426. Locality of the collected specimen: B6 Sivas: Ulaş district, Ziyarettepe, 39° 33' 08,9" N, 37° 01' 12,1" E and the date of collection was 11.05.2013.

Anatomical Studies

For anatomical study, fresh plant samples taken from at least 3 different individuals during the field study were preserved in 70% ethyl alcohol. The root, stem and leaf sections of these specimens were manually cut using a razor blade and stained with Safranin-Alcian blue mixture (double staining) to separate the pectin and lignin containing parts. The stained sections were covered with glycerin-gelatin and made into permanent preparations [20]. Photographs of the anatomical sections of all organs were taken with an Olympus DP70 digital camera connected to an Olympus brand BX51 model light microscope. Previous anatomical analysis studies on the Scrophulariaceae family and *Scrophularia* genus were utilized in anatomical studies [8,16].

Dyes and Solutions Used in Anatomical Studies Preparation of Safranin-Alcian Blue

This staining method is used to see the difference between lignified and non-woody parts in the anatomical structures of plants. Safranin and Alcian blue are prepared separately.

Safranin solution

1 g safranin + 100 ml distilled water; Alcian blue solution: 1 g alcian blue + 3% acetic acid + 100 ml distilled water + 1 thymol crystal (acetic acid is added until pH 2.5).

The procedure followed for staining; sections are soaked in 6 volumes of Safranin: 4 volumes of Alcian blue for 1-2 minutes [21]. The sections removed from the stain are washed in 70% ethyl alcohol and made into permanent preparations using glycerin-gelatin.

Preparation of Glycerin-Gelatin

To prepare glycerin-gelatin, 50 ml glycerin, 8 g gelatin, 0.1 g phenol and 52 ml distilled water are used. The gelatin pieces are left in warm water for 30 min. to swell, glycerin and a small amount of phenol are added and the mixture is heated in a water bath for 15 minutes below 75 °C with stirring until homogeneous. If the heating is done above 75 °C, the gelatin turns into metagelatin and does not harden at room temperature. The mixture is cooled and stored in the refrigerator. When used, it is melted in an oven at 60 °C. In the anatomical study, layers, tissues and cells were identified and indicated on the photographs taken from the sections of different organs of the plants.

Results

Morphological Features

Dense lepidote, many-stemmed, perennial, 10-35 cm; scales yellowish to whitish-gray, margins stellate-pilose. Leaves thick, $3-7 \times 0.3-0.7$ cm; margins ± inverted, lower leaves linear-spathulate, narrowing to long petiole; upper leaves (and bracts) linear-oblong, ± sessile; tip of all leaves obtuse or slightly or deeply 3(-5)-dentate or wedge-shaped, otherwise nearly flat.







Figure 1. Gypsiferous hills where *Scrophularia lepidota* naturally occurs (A); *Scrophularia lepidota* in its natural habitat (B) *Scrophularia lepidota*' s basal leaves (C) and flower state (D) Symous 1-5-flowered; peduncle 4-10 mm; bracteoles oblong-linear, obtuse to spatulate, 2-3 mm with pedicels exceeding 1-2 mm. Calyx lobes with lepidote and glandular hairs, ovate to obovate, $3.5-4 \times 3.5-4.5$ mm; scaly margins yellow to buff, 1-2.5 mm wide, lacerate, \pm undulate. Corolla greenish to maroon, 7 mm, all lobes with yellow-green margins. Stamens exserted; staminodes ovate to roundish, lobed. Capsule globose, $4-5 \times 4-4.5$ mm. Inflorescence 5-6. On dry gypsiferous land, 1200-1500 m [23].

Root Anatomy

The root is circular and secondary in cross-section. Outermost is the periderma consisting of 5-8 cell rows. The cells of the peridermal layer are rectangular, sometimes irregularly shaped.



Figure 2. Root anatomy of *Scrophularia lepidota* (A, B, C, D)(ca: cambium; pd: periderm; ph: phloem; pl: pericycle; tr: trachea; xy: xylem)

The cells are usually brown and do not contain intercellular spaces. In the section, it is observed that the outer layers of the periderma are fragmented in places. The fellem layers of the periderma are prominent, whereas the phellogen and phelloderma are not. Immediately below the periderm is the pericycle layer consisting of 6-10 cell rows. The cells of the pericycle layer are rectangular, squarish or generally irregular in shape (Figure 2A, B). There is no space between the cells. Below the pericycle layer is the phloem layer, which usually consists of 9-12 cell rows. Phloem cells are usually irregularly shaped and sometimes rectangular (Figure 2C.D). Phloem cells close to the pericycle layer are larger. while cells located close to the cambium are smaller. The cambium layer separates phloem and xylem. The cambium is composed of 3-4 cell rows and highly flattened irregularly shaped cells (Figure 2B, C, D). The center of the root cross-section is filled by xylem elements. In the xylem, the tracheae close to the cambium are larger in diameter, while those in the center are narrower wider in diameter, while those in the center are narrower (Figure 2C,D).

Stem Anatomy

The epidermis is single-row oval, oblong-oval and rarely square. There are stellate hairs originating from the epidermis (Figure 3A, B). The body is in the primary stage of development. Below the epidermis is the cortex layer with 6-8 cell rows and circular or oval cells. There are intercellular spaces between the cortex cells. Below the cortex is the phloem layer with 3-5 cell rows. Below the phloem is the xylem (Figure 3A, B, and C). Between the phloem and xylem is a layer of sclerenchymatic fibers with 7-13 cell rows, which surrounds the stem as an inner ring. The cambium layer is not prominent. In the radial direction there are pith rays composed of uniseriate parenchymatic cells embedded in the phloem and xylem (Figure 3A, B, and C). The pith region is composed of usually circular, sometimes oval cells with large intercellular spaces, occupying a large area in the stem cross-section (Figure 3A, B, and C).



Figure 3. Stem anatomy of *Scrophularia lepidota* (A, B, C, D) (co: cortex; eh: cover hair; ep: epidermis; ph: phloem; pt: pith; sc: sclerenchyma; st: stomata; xy: xylem)

Leaf Anatomy

In cross-section, the leaf is surrounded by a single row of epidermis above and below.



Figure 4. Leaf cross-section photomicrographs of *S. lepidota* (A, B, C, D).Mesophytic stomata in leaf lower epidermis (E); idioblastic cell in leaf mesophyll (F) Detail of stellate cover hair on leaf upper epidermis (G); stellate cover hair on leaf lower epidermis (H) (cl: collenchyma; eh: cover hair; id: idioblast; le: lower epidermis; pc: parenchymatic cells; ph: phloem; pp: palisate parenchyma; sp: sponge parenchyma; st: stomata; ue: upper epidermis; vb: conduction bundle; xy: xylem; as: substomatal space; eh(H): stellate cover hair; cu: cuticle)

It is covered with stellate hairs, more densely on the lower surface of the leaf (Figure 4G, H). The mesophyll layer is quite thick. The leaf is equifacial in terms of mesophyll. There are 3-4 cell rows of palisate parenchyma on the upper and lower epidermis. Palisate cells are usually oblong, oblong-oval and sometimes cylindrical in shape. Sponge parenchyma with 2-4 cell rows is located between the two palisate layers. Sponge parenchyma cells are usually circular, oval or sometimes irregularly shaped (Figure 4A, B, and C). The midrib (leaf main conduction bundle) is oval-circular in shape and surrounded by bundle sheath cells consisting of a single layer of cells.

The midrib is located closer to the upper epidermis in the leaf cross-section and occupies a small area in the cross-section. In the area where the midrib is located, there is a single row of collenchyma just above the lower epidermis. Between the midrib and this single-row collenchyma layer are parenchymatic cells, which are usually circular in shape (Figure 4A, B, and D). The leaf is amphistomatic in terms of stomata and the stomatal cells are located at the same level (mesophytic) as the epidermis cells (Figure 4A, B, C, D, and E).

In the leaf transverse section, idioblastic cells, which were very dense but considerably larger in size than the neighboring palisade parenchyma cells, were also found just below the upper epidermis (Figure 4F).

Discussion

In this study, the anatomical features of *S. lepidota* species were revealed. In terms of many anatomical features, *S. lepidota* has the general anatomical features of Scrophulariaceae family and *Scrophularia* genus given by Metcalfe and Chalk (1950) [12].

For example, Metcalfe and Chalk (1950) reported that the leaves of Scrophulariaceae are usually amphistomatic in terms of stomata and sometimes only the lower epidermis may have stomata (hypostomatic). As a result of this study, the leaves of S. lepidota are also amphistomatic in terms of stomata.Furthermore, Metcalfe and Chalk (1950) reported that the endodermis layer is usually seen in stem anatomy sections of species of the genus Scrophularia, but this is not a distinctive character; in addition, sclerenchymatic fibers corresponding to the pericycle layer are found as a band in the stem cross-section. In this study, unlike Metcalfe and Chalk (1950), no endodermis layer was observed in S. lepidota stem transverse sections. In addition, although Metcalfe and Chalk (1950) stated that sclerenchymatic fibers can be seen as bands in members of the genus Scrophularia, in the stem of S. lepidota, the sclerenchymatic fiber layer forms a thick layer that surrounds the stem cross-section as an inner ring [12].

Table 1. Comparison of the results of the stem and leaf anatomy of <i>Scrophularia lepidota</i> species in this study with the
results of the stem and leaf anatomy of six Scrophularia species studied by Makbul et al. (2006)

	This study			Makbul et al. (2006)				
	Features/Species	S. lepidota	S. ilwensis	S. capillaris	S. nodosa	S. libanotica var. pontica	S. lucida	S. cinerancens
St	em Epidermis cell shape	Oval, oblong-oval, rarely square	Rectangular or circular	Rectangular or circular	Not specified	Rectangular or circular	Rectangular or circular	Not specified
	Collenchyma layer under the epidermis	Not available	1–3	Not specified	1–3	1-3	1-3	1
	Cortex cell row number	6–8	6–7	7–8	7–8	6–7	7–8	6–7
	Cortex cell shape	Circular or Oval	Oval	Oval	Oval	Oval	Oval	Oval
	Cambium	Uncertain	Uncertain	Uncertain	Not specified	Uncertain	Uncertain	Uncertain
	Self cell shape	Circular or oval	Cylindric	Cylindric	Cylindric	Cylindric	Cylindric	Cylindric
Le	· · · · · · · · · · · · · · · · · · ·	Oval-Circular	Triangle	Triangle	Not specified	Triangle	Triangle	Triangle
	Size of lower epidermis cells relative to upper epidermis cells	It's big	It's big	It's big	It's big	It's big	It's big	It's big
	Leaf type according to mesophyll	Ecuifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial
	Palisate parenchyma cell row number	3-4 cells below the upper epidermis; 3- 4 cells above the lower epidermis		1–3	2–3	3–4	5–6	3–4
	Sponge parenchyma cell line number	2–4	3–4	2–3	2–3	4–5	5–6	5–6
	Presence of stoma	Amphistomatics	Amphistoma tics	Amphistomatics	Amphistomatics	Amphistomati cs	Not specified	Not specified
	Presence of idioblast embedded in the palisate parenchyma	Specified	Specified	Specified	Not specified	Specified	Not specified	Not specified

Anatomical studies have been carried out on some species of the genus Scrophularia (S. chrysantha Jaub. & Spach, S. scopolii Hoppe ex Pers. var. adenocalyx Somm. & Lev. and S. olympica Boiss., S. sosnowskyi Kem.-Nath., S. canina L.) which are naturally distributed in Turkey. Makbul and Beyazoğlu (2009) studied the stem and leaf anatomy of these species[8]. The stem and leaf anatomy of S. ilwensis C. Koch, S. capillaris Boiss. & Ball., S. nodosa L., S. libanotica Boiss. var. pontica R. Mill, S. lucida L. and S. cinerascens Boiss. were also studied by Makbul et al. (2006) [16]. The results of these two studies and the anatomical features of S. lepidota species that we have revealed in this study are compared in Table 1 and Table 2 [8, 16]. When the anatomical characteristics of S. lepidota determined in our study are compared with these studies, it is seen that some characters show significant differences. For example, in the studies of Makbul et al. (2006) and Makbul and Beyazoğlu (2009), the epidermis cell shape of 9 Scrophularia taxa were reported to be rectangular or circular, whereas S. lepidota has oval, rectangular-oval, rarely square shaped stem epidermis cells (Table 1 and 2) (Figure 3A, B, and C) [8, 16].

Another important difference in terms of the stem is the presence of collenchyma under the epidermis. In the 10 species shown in Table 1 and Table 2, where the presence of collenchyma is indicated, collenchyma layers ranging from 1 to 3 cell rows were found, whereas no collenchyma was found under the epidermis in *S. lepidota* (Figure 3A, B, C). The stem pith cells were cylindrical in all 10 species studied and characterized by Makbul et al. (2006) and Makbul and Beyazoğlu (2009). However, in *S.* *lepidota*, the pith cells appear circular or oval in crosssection (Figure 3A, B, C). In terms of leaves, the most important differences between *S. lepidota* and the species in Table 1 and Table 2 are midrib shape and leaf type relative to the mesophyll. Accordingly, Makbul et al. (2006) and Makbul and Beyazoğlu (2009) reported that the midrib was triangular, kidney or semicircular in all *Scrophularia* taxa studied (Table 1; Table 2). In *S. lepidota*, the midrib was found to be oval circular (Figure 4D). The most significant difference in terms of leaf shape is the leaf shape compared to the mesophyll. While the leaf type of all species studied by Makbul et al. (2006) and Makbul and Beyazoğlu (2009) was bifacial according to mesophyll, *S. lepidota* leaves were found to be equifacial (Figure 4A) [8, 16].

In another study conducted by Shields (1950), he stated that plants having developed palisate parenchyma in their leaves would be directly proportional to their arid character [22]. This is also in agreement with our study. *S. lepidota* is an arid plant and has equifacial type leaves with two different palisate parenchyma (Figure 4A, B, C).

In this study, idioblast cells were observed just below the upper epidermis on *S. lepidota* leaves and again below the epidermis on leaf margins (Fig 4F). Previously, Volkens (1887) observed such idioblasts in species growing in the Middle East [7]. Furthermore, Solereder (1908) reported that "the tannin-bearing idioblasts of the mesophyll of *Scrophularia deserti* are large ellipsoidal cells, the lower end of which is attached to an epidermal cell which penetrates the mesophyll at a very small distance; these idioblasts extend to the middle of the leaf where they are embedded in the ends of the vessels" [23].

Table 2. Comparison of the results of the stem and leaf anatomy of <i>Scrophularia lepidota</i> species with the results of the
stem and leaf anatomy of five <i>Scrophularia</i> species studied by Makbul and Beyazoğlu (2009)

	This study			Makbul and Beyazoğlu (2009)			
	Features/Species	S. lepidota	S. chrysantha	S. scopolii var. adenocalyx	S. olympica	S. sosnowskyi	S. canina
Stem	Epidermis cell shape	Oval, oblong-oval, rarely square	Rectangle	Rectangle or circular	Rectangle or circular	Rectangle or circular	Rectangle or circular
	Epidermis altında kollenkima tabakası	Not available	1–3	1	1	1	1–3
	Cortex cell row number	6–8	5–6	4–5	5–6	6–7	5–6
	Cortex cell shape	Circular or oval	Oval	Oval	Oval	Oval	Oval
	Cambium	Uncertain	Uncertain	Not specified	Not specified	Not specified	Uncertain
	Self cell shape	Circular or oval	Cylindric	Not specified	Cylindric	Cylindric	Cylindric
Leaf	Midrib shape	Oval-circular	Triangular or kidney- shaped	Triangular	Semicircle	kidney- shaped	Triangular
	Size of lower epidermis cells relative to upper epidermis cells	It's big	It's big	It's big	It's big	It's big	It's big
	Leaf type according to mesophyll	Ecuifacial	Bifacial	Bifacial	Bifacial	Bifacial	Bifacial
	Palisate parenchyma cell row number	3-4 cells below the upper epidermis; 3-4 cells above the lower epidermis	1–2	1	2–3	3	4–5
	Sponge parenchyma cell line number	2–4	4–5	2–3	3–5	3–4	2–3
	Presence of stoma	Amphistomatics	Amphistomati cs	Amphistomatics	Amphistomatics	Amphistomati cs	Amphistomati cs
	Presence of idioblast embedded in palisate parenchyma	Specified	Not specified	Specified	Not specified	Not specified	Not specified

After these studies, the most comprehensive study on leaf idioblasts of Scrophulariaceae family and *Scrophularia* genus species was conducted by Lersten and Curtis (1997) [6]. As a result of this study, it was reported that 62 of 89 *Scrophularia* species had idioblasts in their leaves, which corresponded to 69.7%. In the aforementioned study, it was also reported that the presence of idioblasts may vary in samples taken from different localities of the same species, and as an example, while there were no idioblasts in samples of *Scrophularia libanotica* taken from one locality, large idioblast cells were found in another sample.

In our study, since *S. lepidota* species is not widespread, the sample taken from a single locality was studied and idioblast cells with primary cell wall and seemingly empty lumen were found. In the study of Lersten and Curtis (1997), idioblasts in all specimens with idioblasts had only primary cell walls and their lumens appeared to be empty. In this respect, our study coincides with the results of Lersten and Curtis (1997) study.

Conclusion

In this study, the anatomical features of *S. lepidota*, which is endemic to our country, were revealed for the first time. The results obtained were compared with the species of the genus *Scrophularia* whose anatomy was previously studied and their similarities and differences were revealed. We believe that the results of this study will contribute to future anatomical studies on the other

members of the genus and to determine their place in the systematics.

Conflict of Interest

Authors declare that there are no conflict of interest between them. Also, we note that our manuscript contains original material.

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