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The Leaf and Stem Anatomy of Two Endemic *Salvia* (Section *Salvia*, Lamiaceae) from Turkey: *S. aucheri* subsp. *canascens* and *S. heldreichiana*

Gülnur EKŞİ*[1], Gülderen YILMAZ[2]

Abstract

Salvia L. has a wide variety of traditional usages as digestive, appetizer, carminative, antiseptic, stimulant, pain reliever, antipyretic, diuretic, antitussive and for bronchitis, asthma and cold. Anatomical features are extremely important for the taxonomy of *Salvia* species, especially leaf and stem represent many specific characteristics. The aim of this study is to determine the distinctive anatomical structures of *S. aucheri* subsp. *canascens* (Boiss. & Heldr.) Celep, Kahraman & Doğan and *S. heldreichiana* Boiss. comparatively. The transverse-sections were taken from the leaf and stem to determine the anatomical features of two *Salvia* species. They differ in many anatomical characters like leaf type, which is bifacial and hypostomatic for *S. aucheri* subsp. *canascens* and monofacial and amphistomatic for *S. heldreichiana*. Besides, the leaf surfaces of both species are covered by different types of glandular trichomes. The midrib contains a big vascular bundle in *S. aucheri* subsp. *canascens* and has three big vascular bundles in *S. heldreichiana*. Additionally, the stem surface of *S. aucheri* subsp. *canascens* is glabrous while the stem is pilose in *S. heldreichiana*. In conclusion, our anatomical results were contributed to the taxonomy of *S. aucheri* subsp. *canascens* and *S. heldreichiana* and confirmed the importance of anatomy in the species level for the genus.

Keywords: anatomy, Lamiaceae, *Salvia aucheri* subsp. *canascens*, *S. heldreichiana*.

1. INTRODUCTION

Salvia L. is the largest genus of Lamiaceae consisting of ca. 1000 species worldwide. The genus is distributed mainly in Central and South America and Asia [1–3]. It has more than 100 species in Turkey, half of which are endemic demonstrating that Turkey is a possible diversity centre for *Salvia* in Asia [4, 5]. The members of the genus are herbaceous, suffruticose or shrubby, generally perennials and often strongly aromatic. Stems erect or procumbent and glandular to glabrous. Leaves undivided and lyrate or pinnatisect. Inflorescence verticillaster. Calyx and corolla are bilabiate [6]. *Salvia* species have many usages worldwide, not only for medicinal purposes but also for food, in cosmetics, perfumery and pharmaceutical industry [7–9]. The genus is one of the most well-known medicinal plant group in Turkey since ancient times with its various traditional use such as diuretic, stimulant, laxative and for abdominal

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pain, cold, flatulence, rheumatism, stomach ache, wounds [1, 10–18]. Recent bioactivity studies proved that *Salvia* species contain bioactive secondary metabolites efficient as antioxidant, antimicrobial, antialzheimer, anticancer, antidiabetic, antitumor, antiplasmodial, antiinflammatory [19–25]. Besides, numerous anatomical studies have been conducted on *Salvia* species [2, 12, 26–29]. *Salvia aucheri* subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Doğan and *S. heldreichiana* Boiss. (Lamiaceae) are both belong to sect. *Salvia* (Figure 1).

In the present study, the anatomical characteristics of these two endemic species have been studied for the first time. The results are presented by photographs. The characteristic elements of anatomical structures have been determined and these results compared to other studies in the literature. This study aims to contribute to the taxonomy of examined *Salvia* species and the genus.

2. MATERIALS AND METHODS

Aerial parts of *S. aucheri* subsp. *canascens* and *S. heldreichiana* were collected from Karaman province at the flowering time on 27th of June 2019 from Yeşildere Village at an elevation of 1300 m by the second author and the specimens were stored in Ankara University, Pharmacy Faculty Herbarium with the numbers of AEF 28920 and AEF 28919, respectively. For anatomical studies, living materials were kept in 70% alcohol. Transverse-sections of leaf and stem were taken by hand and examined using Sartur and chloral hydrate reagents. The measurements (Table 1) and photos (Figures 2, 3, 4, 5) of characteristic elements were photographed by a binocular light microscope with a Leica DFC280 camera at 4x, 10x and 40x magnifications.

3. RESULTS

3.1. S. aucheri subsp. canescens

S. aucheri subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Doğan, Distribution: Southern Turkey. Habitat: Stony slopes, rocky areas. Flowering: July to October. Phytogeographical Region: East Mediterranean element.

3.1.1. Stem

The stem is quadrangular in transverse-section (Figure 2. A, C). The epidermis is covered by a cuticle layer and consists of a single layer of elliptical to rectangular cells (Figure 2. F, I). The collenchyma tissue is in one or two rows, thicker at the edges (Figure 2. A, C, F, I). Parenchyma is three to five layered in the cortex. Starches are visible in parenchymatic cells (Figure 2. G). Sclerenchyma cell groups in the cortex are above the phloem. The cambium is distinctive (Figure 2. A, C). The pith consists of lignified and unlignified parenchymatic cells which are large, polygonal or orbicular in shape (Figure 2.E, H).

3.1.2. Leaf

In the transverse-section of the leaf lamina including the midrib, the epidermis is covered by a cuticle layer on both sides of the leaf (Figure 3. B, C). The epidermis consists of a single layer of elliptical to rectangular cells which are larger at upper surface. The multicellular non-glandular and glandular trichomes are present on both sides of the leaves (Figure 3. B, C). The non-glandular multicellular trichome consists of two or three cells (Figure 3. F, G, I). Two types of glandular trichome are present. The first one is a larger glandular trichome that has two or three cells including a neck cell in the stalk with unicellular heads (Figure 3. G, H). The second one is a small glandular trichome containing one or two cells including a neck cell in the stalk with one or two celled heads (Figure 3. F, H, I). The midrib has a big vascular bundle covered with 5-6 layers of polygonal to circular collenchyma cells from the adaxial and 3-4 layers from the abaxial (Figure 3. D, E). The stomata type is diacytic and they present on the lower epidermis (Figure 3. K, L). The leaf is hypostomatic and bifacial (Figure 3. B, C). The palisade parenchyma cells are rectangular elongated, 4-6 layered and 1–3 layers of intercellular spaced spongy parenchyma cells (Figure 3. B, C). The vascular bundle is the collateral type (Figure 3. D, E).

3.2. *Salvia heldreichiana*

Salvia heldreichiana Boiss., Prodr. 17: 320, (1873). Distribution: Southern Turkey. Habitat: Stony slopes, rocky places. Flowering: July-October. Phytogeographical Region: East Mediterranean element.

3.2.1. Stem

The stem is quadrangular to circular in transverse-section (Figure 4. A, B). The epidermis is covered by a cuticle layer and non-glandular trichomes consisted of one to three cells (Figure 4. A, B, H, I). The epidermis is composed of uniseriate, thick walled, elliptical to rectangular cells (Figure 4. G, H, I). Cortex exhibits three different layers. The first layer has thick walled, flattened, collenchyma cells in two to six rows under the epidermis. The second layer is three to five rows of bigger and ovoid parenchyma cells. At the third layer, cells are smaller and without intercellular spaces cells (Figure 4. G, H, I). Sclerenchyma cells are in the third layer of the cortex above the phloem cells (Figure 4. E, G). The cambium is distinctive cells (Figure 4. A, B). The pith consists of lignified parenchyma cells, which are larger and polygonal or orbicular in shape (Figure 4. D).

3.2.2. Leaf

In the transverse-section of the leaf lamina, the upper and the lower epidermis are covered with a cuticle layer cells. The epidermis is composed of a single layer of elliptical to rectangular cells which are thick walled (Figure 5. E, F, G, H, I). The multicellular non-glandular and glandular trichome are on both sides of the leaves. The non-glandular multicellular trichome consist of two or three cells. Two types of glandular trichome are distinguished. One has a unicellular stipe with one or two celled heads, second has a unicellular stipe with multicellular heads (Figure 5. F, G, I). The midrib contains three big vascular bundles covered with four to six layers of collenchyma cells from the adaxial and six to nine layers of collenchyma cells from the abaxial (Figure 5. A, B, C, D, E). The collenchyma cells are polygonal to circular in shape. The vascular bundle is the collateral type (Figure 5. D). The leaf is monofacial (Figure 5. A, B, C, H). The palisade parenchyma cells are elongated rectangular as 6-8 layers in the mesophyll. The spongy parenchyma cells are one or two layers on both sides of the palisade parenchyma (Figure 5. H, I). On the surface, the stomata cells are located on both sides of the leaf The leaf is amphistomatic and the stomata type is diacytic (Figure 5. J, K, L).

4. DISCUSSION

Salvia species have non-glandular and glandular trichomes characteristic to Lamiaceae [2, 4, 12, 30–34] (Table 3). In the leaf transverse and the leaf superficial sections, upper and lower epidermis of *S. aucheri* subsp. *canascens* and *S. heldreichiana* were both covered by non-glandular and glandular trichomes. The trichome characteristics are very important for the classification of *Salvia* species [2, 4, 32, 33]. The non-glandular trichome in Lamiaceae could be erect, triangular, unicellular to multicellular and variable in shape and length [30, 35]. *S. heldreichiana* has long and non-glandular multicellular trichome on the stem surface in contrast to *S. aucheri* subsp. *canascens* (Figure 2. A, C, G, I; Figure 4. A, B, H, I; Table 3). Glandular trichome in *Salvia* displays variability among species [32, 33]. Capitate and peltate are the two main types of glandular trichome based on the shape of secretory heads [2, 4, 35]. The capitate trichome contains short or long stalks with unicellular heads. The peltate trichome is formed of short stalks with a broad multicellular secretory head. *S. heldreichiana* has both. The peltate trichome in *S. heldreichiana* comprises of a glandular head with eight cells in a row (Figure 5. L). The capitate trichome in *S. heldreichiana* has one to two celled stalks with spherical secretory heads (Figure 5. F, G, I,). *S. aucheri* subsp. *canascens* has two main types of capitate trichome, one is short capitate (the short stalk consists of one or two cells-if two cells, one of them is neck cell-) and the other is long capitate (the long stalk contains two or more cells including neck cell). *S. aucheri* subsp. *canascens* has the long capitate trichome with two to three cells in stalk and the short capitate trichome with a round unicellular secretory head and a stalk with

two cells including a neck cell. The leaf epidermis of both *S. aucheri* subsp. *canascens* and *S. heldreichiana* consist of a single layer of the oval to rectangular thick-walled epidermis cells covered with a cuticle. The leaf of *S. aucheri* subsp. *canascens* is bifacial like *S. potentillifolia* Boiss. & Heldr. ex Benth., *S. nydeggeri* Hub.-Mor. [12], *S. quezelii* Hedge & Afzal-Rafii[2] and *S. divaricata* Montbret & Aucher ex Benth. [28] (Table 2). The leaf of *S. heldreichiana* is monofacial like *S. macrochlamys* Boiss. & Kotschy [36], *S. ballsiana* (Rech.f.) Hedge [37] (Table 2). In both *S. aucheri* subsp. *canascens* and *S. heldreichiana*, mesophyll consists of elongated rectangular palisade parenchyma and nearly isodiametric spongy parenchyma cells. In *S. aucheri* subsp. *canascens*, the midrib has a single large vascular bundle in the middle (Figure 3. A, D) like in *S. macrochlamys* [36], *S. ballsiana* [37], *S. potentillifolia*, *S. nydeggeri* [12] and *S. divaricata* [28]. In *S. heldreichiana* three large vascular bundles were in the midrib (Figure 5. D, E) like in *S. indica* L. [38] and *S. vermifolia* Hedge & Hub.-Mor. [39]. The midrib comprises multilayers of collenchyma cells around vascular bundles in both species. *S. aucheri* subsp. *canascens* and *S. heldreichiana* are both have diacytic stoma type like the many others members of *Salvia* [27, 38, 39]. *S. heldreichiana* has amphistomatic leaves (number of stomata on the lower surface is equal to stomata number on the upper surface) (Figure 5. J, K, L) and *S. aucheri* subsp. *canascens* has hypostomatic leaves (stomata are just located on the lower surface) (Figure 3. J, K, L). The stems of both *S. aucheri* subsp. *canascens* and *S. heldreichiana* were quadrangular and cortex was composed of one to two layers of collenchyma, which is multilayer at the corners making them quadrangular and several layers of parenchymatic cells. In both species, sclerenchyma cells were visible above the phloem (Figure 2. B, C, D, E, F, I; Figure 4. A, B, E, G) similar to other *Salvia* species [30, 40]. In both examined species, large trachea and smaller tracheid were observed in the xylem (Figure 2. A, C, D, F, I; Figure 4. A, B, C, E, F). Medullary rays in stems of *S. aucheri* subsp. *canascens* and *S. heldreichiana* were visible and the cambium was well distinguished (Figure 2. A, C, D; Figure 4. A, B, C, F) The stem anatomies of both species were more or less similar in previously examined *Salvia* species [1, 2, 12, 26, 27, 37, 40].

5. CONCLUSION

In this study, the stem and leaf anatomy of *Salvia aucheri* subsp. *canascens* and *S. heldreichiana* were studied for the first time. These two endemic species were found to be closely related to each other but *S. aucheri* subsp. *canascens* and *S. heldreichiana* has exhibited many different anatomical characters such as indumentum of stem, glandular trichome, vascular bundle numbers and orientations. Our results provide significant data to the taxonomy of two examined endemic species and the genus *Salvia* and confirm the importance of anatomical structures in the species level.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

The first author contributed 60%, the second author 40%.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial

board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

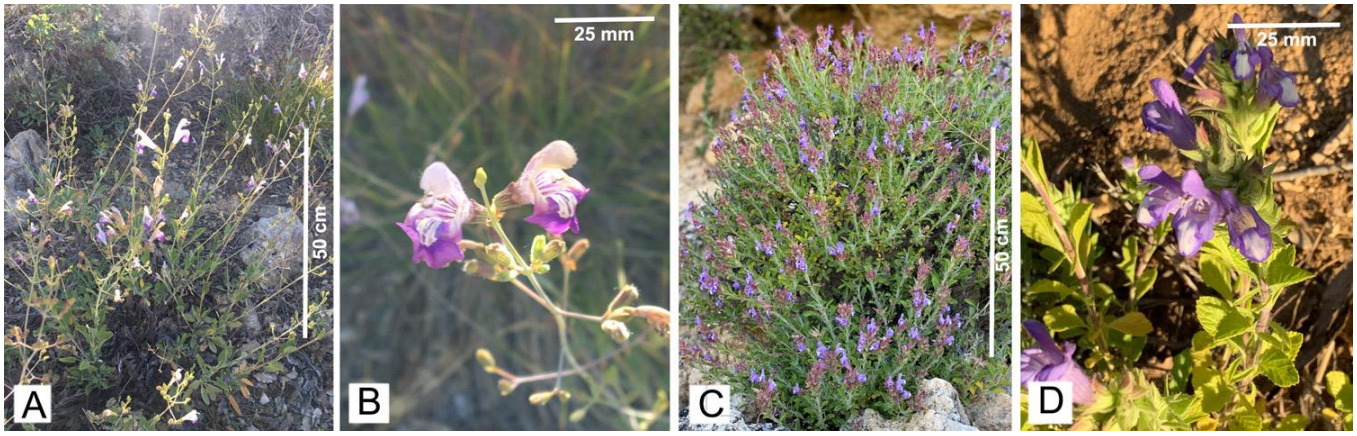

Figure 1 A-B *Salvia aucheri* subsp. *canascens*, C-D *Salvia heldreichiana*.

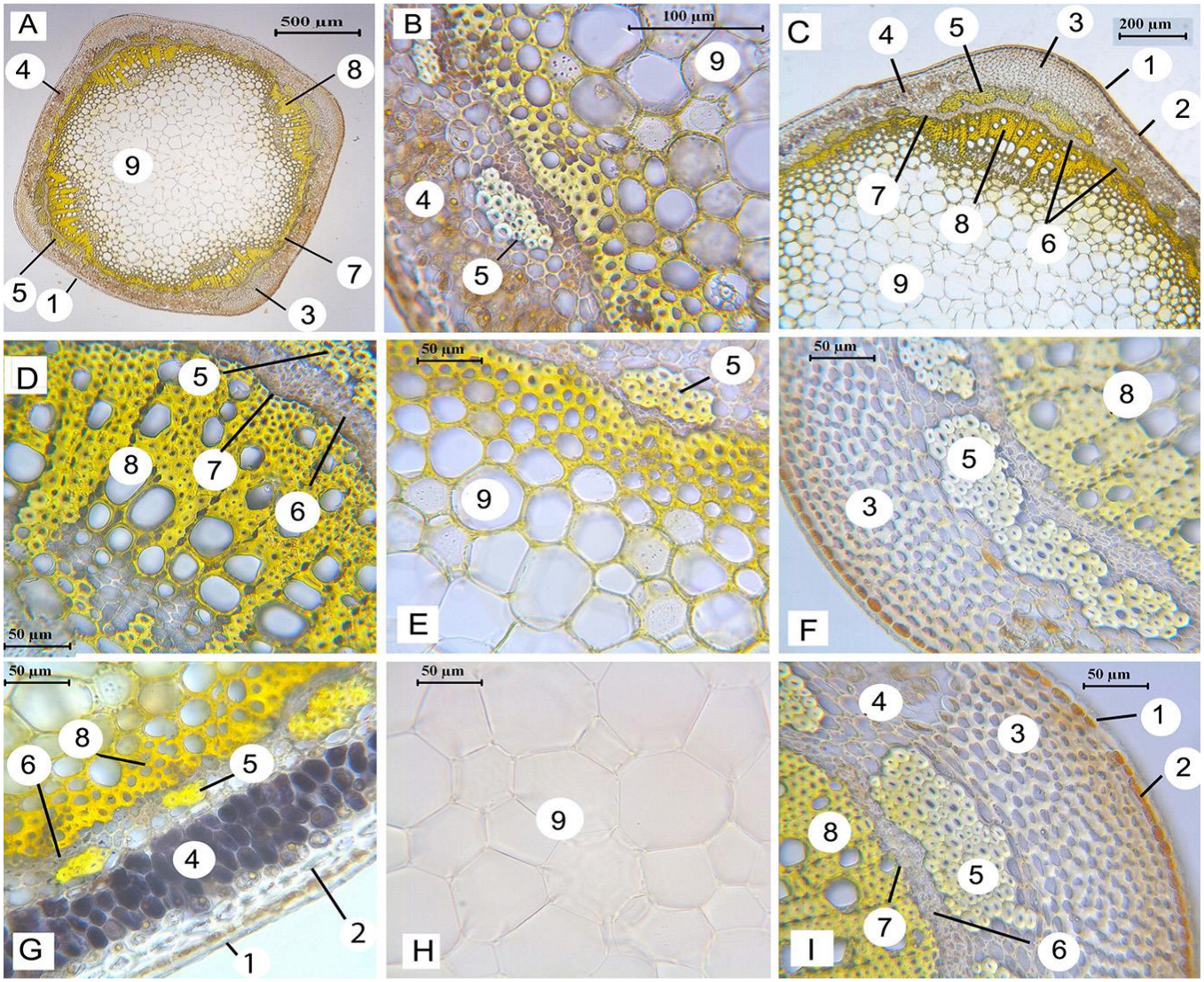

Figure 2 *Salvia aucheri* subsp. *canascens*. (A-I) Stem transverse-section, (1) cuticle, (2) epidermis, (3) collenchyma, (4) parenchyma, (5) sclerenchyma, (6) phloem, (7) cambium, (8) xylem, (9) pith.

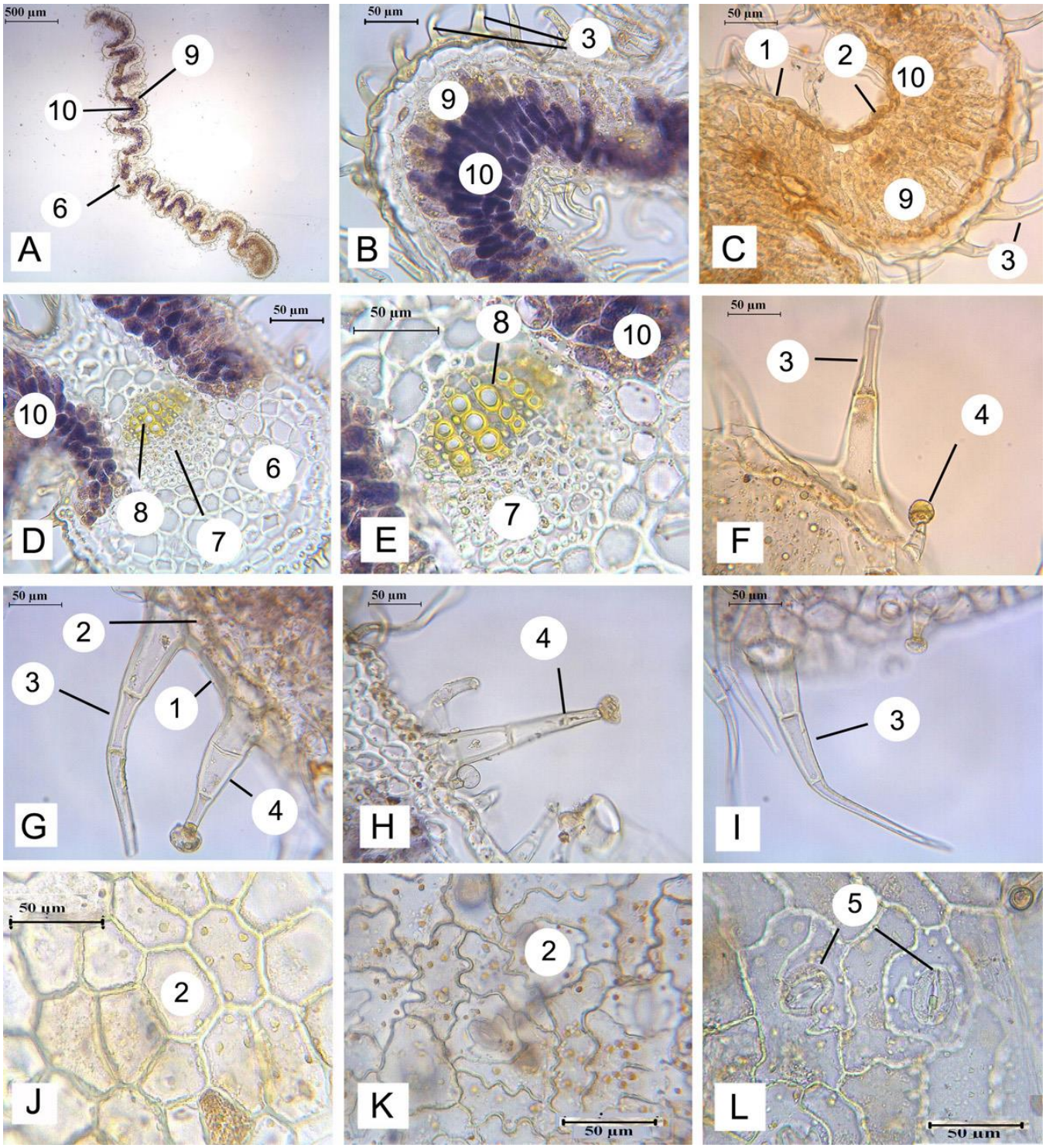

Figure 3 *Salvia aucheri* subsp. *canascens*. (A-I) Leaf transverse-section, (J-L) leaf surface, (1) cuticle, (2) epidermis, (3) non-glandular trichome, (4) glandular trichome, (5) stomata, (6) collenchyma, (7), phloem, (8) xylem, (9) palisade parenchyma, (10) spongy parenchyma.

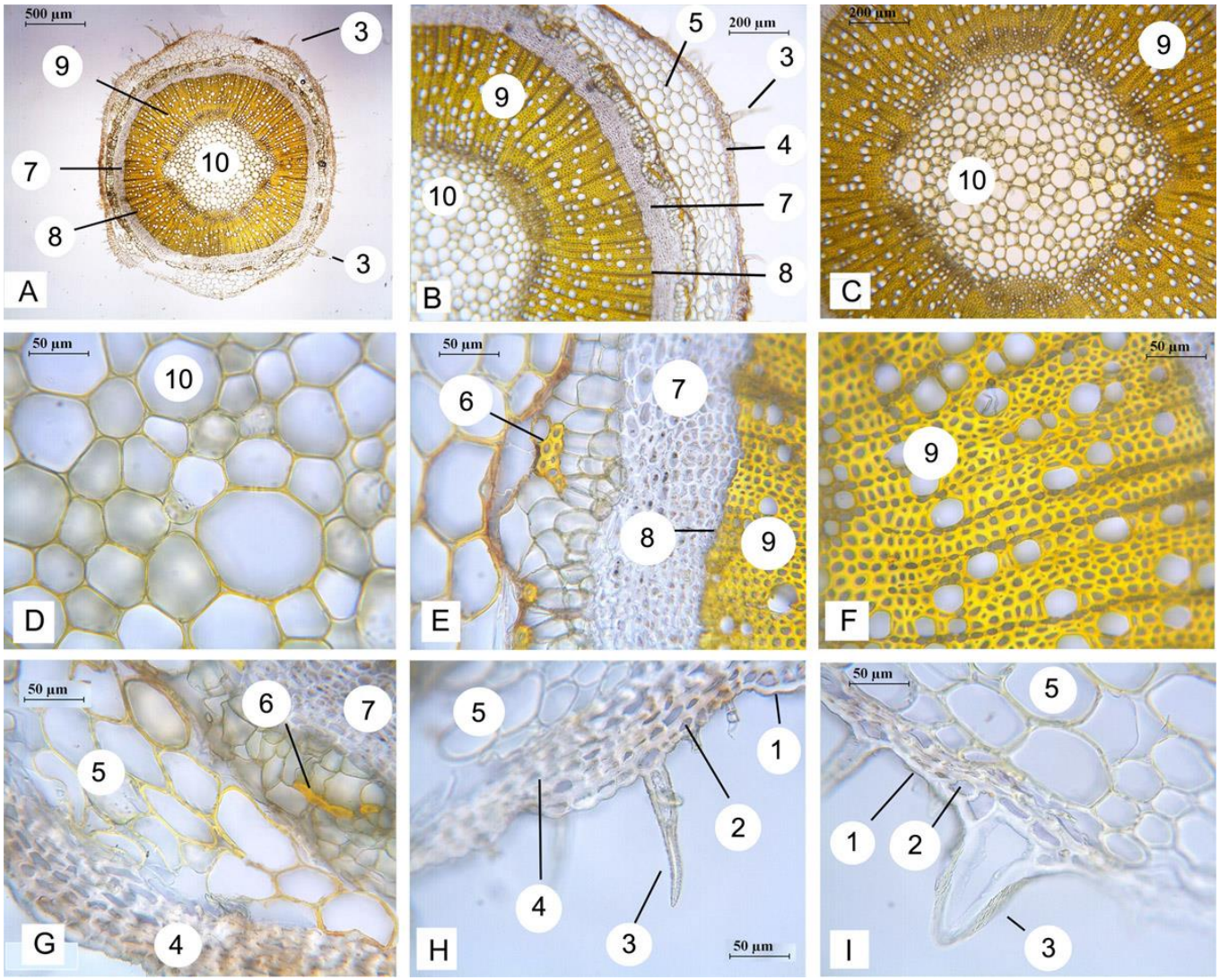

Figure 4 *Salvia heldreichiana*. **(**A-I) Stem transverse-section, (1) cuticle, (2) epidermis, (3) non-glandular trichome, (4) collenchyma, (5) parenchyma, (6) sclerenchyma, (7) phloem, (8) cambium, (9) xylem, (10) pith.

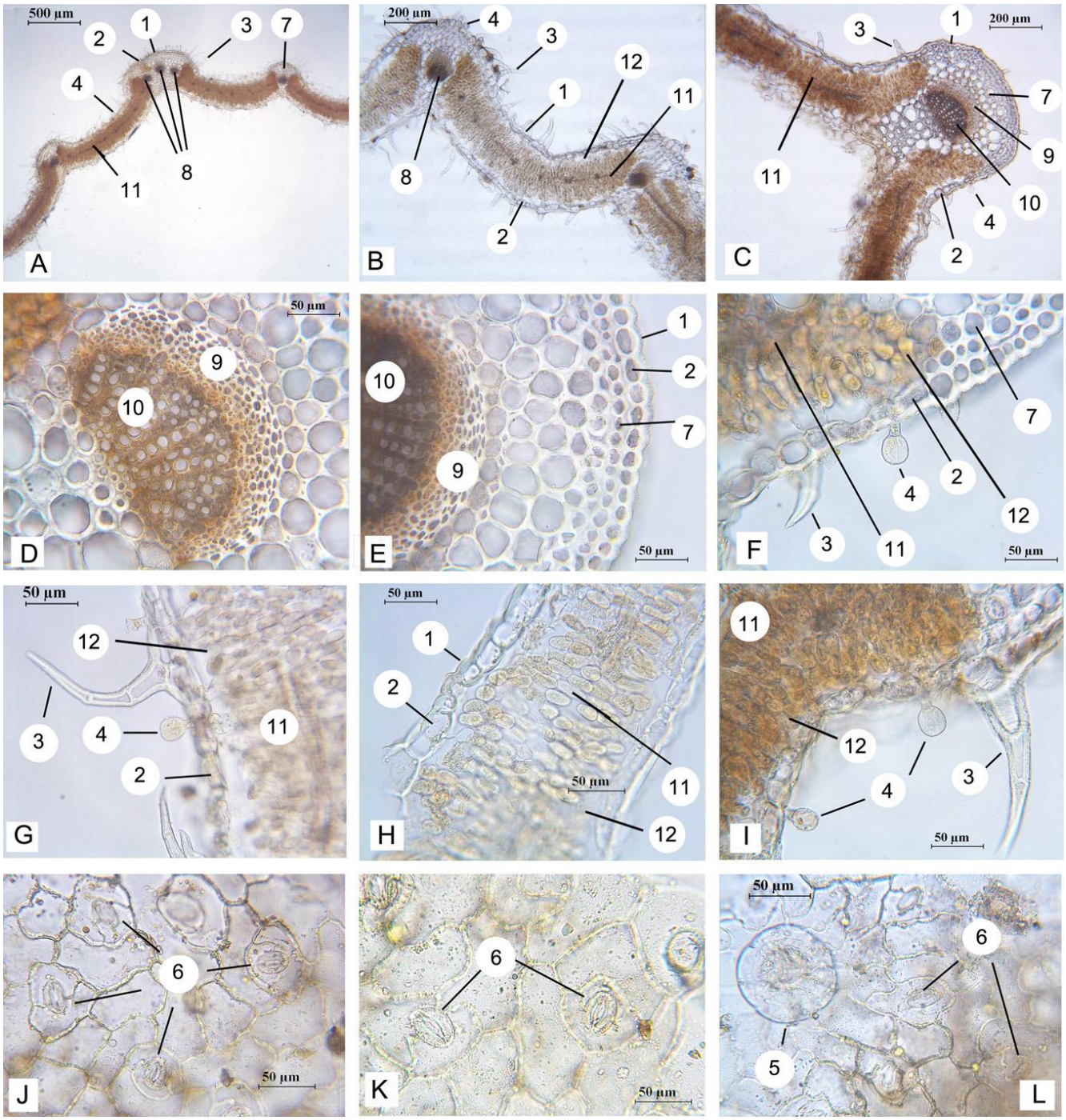

Figure 5 *Salvia heldreichiana*. (A-I) Leaf transverse-section, (J-L) leaf surface, (1) cuticle, (2) epidermis, (3) non-glandular trichome, (4) glandular trichome, (5) Lamiaceae type glandular trichome, (6) stomata, (7) collenchyma, (8) vascular bundle, (9) phloem, (10) xylem, (11) palisade parenchyma, (12) spongy parenchyma.

Table 1 Anatomical measurement of the stem and the leaf characteristic elements of *S. aucheri* subsp. *canascensa* and *S. heldreichiana*.

			Width (µm)		Length (µm)	
			Min.–Max.	**Mean ± S.D.**	**Min.–Max.**	**Mean ± S.D.**
S. *aucheri subsp. canascens*	Stem	Cuticle	1.33-7.00	3.83 ± 2.53	2.67-5.00	3.92 ± 0.10
		Epidermis Cell	14.67-23.33	19.00 ± 3.78	18.33-30.00	26.30 ± 5.10
		Trachea Cell	5.35-40.33	17.99 ± 9.48	6.67-33.00	18.12 ± 8.55
		Pith Cell	38.00-86.00	55.524 ± 16.71	30.00-78.67	55.90 ± 18.25
	Leaf	Cuticle	3.75-12.19	8.19 ± 3.20	2.81-9.06	5.88 ± 2.77
		Upper Epidermis Cell	10.94-27.81	18.39 ± 6.84	13.44-20.63	16.88 ± 2.60
		Lower Epidermis Cell	5.63-18.12	12.37 ± 4.87	4.38-10.00	7.99 ± 2.10
		Collenchyma Cell	16.00-36.4	23.54 ± 7.39	12.80-33.20	22.53 ± 7.08
		Mesophyll Region	55.94-110	78.94 ± 20.23	42.81-85	64.56 ± 17.05
		Palisade Parenchyma Cell	3.13-11.56	8.29 ± 2.51	7.50-25.94	16.36 ± 6.10
		Non-glandular Trichome	22.00-29.33	26.67 ± 4.05	127.33-227.63	193.21 ± 57.07
		Glandular Trichome	15.33-27.33	20.666 ± 4.83	19.33-147.33	74.532 ± 55.95
S. heldreichiana	Stem	Cuticle	3.67-1.67	2.75 ± 0.92	1.33-2.67	2.08 ± 0.57
		Epidermis Cell	5.67-17.00	11.22 ± 4.97	6.67-15.33	11.56 ± 3.79
		Trachea Cell	2.67-10.33	5.33 ± 2.43	2.67-9.33	5. 00 ± 2.12
		Pith Cell	20.00-80.00	42.23 ± 19.69	20.67-73.67	41.33 ± 17.99
		Non-glandular Trichome	7.33-74.13	25.18 ± 24.93	23.8-185.71	84.435 ± 61.10
	Leaf	Cuticle	2.14-3.43	2.71 ± 0.66	1.43-2.14	1.81 ± 0.36
		Upper Epidermis Cell	15.00-25.00	20.30 ± 4.05	10.71-26.43	18.88 ± 6.06
		Lower Epidermis Cell	13.57-42.14	26.78 ± 12.04	12.86-19.64	16.29 ± 2.85
		Collenchyma Cell	7.86-39.29	23.04 ± 14.33	11.07-40.71	24. 42 ± 13. 54
		Mesophyll Region	140.71-246.43	191.19 ± 53.02	124.29-216.43	155.80 ± 52.52
		Palisade Parenchyma Cell	5.71-11.79	8.97 ± 2.39	17.5-25.71	22.68 ± 2.79
		Spongy Parenchyma Cell	11.43-14.29	12.45 ± 1.11	12.14-18.57	15.86 ± 2.86
		Non-glandular Trichome	17.5-35.36	24.88 ± 9.32	72.5-128.57	100.54 ± 39.65
		Glandular Trichome	18.57-25	21.70 ± 2.66	31.07-42.5	36.695 ± 5.75
		Lamiaceae Type Glandular Trichome	57.86-98.21	80.89 ± 17.48	64.64-96.07	82.14 ± 13.08

S.D.: Standard Deviation.

Table 2 The leaf anatomical features of *S. aucheri* subsp. *canascens*, *S. heldreichiana* and related taxa.

	Leaf type	Stoma type	Large vascular bundle numbers in the midrib	References
Sect. *Salvia*				
S. aucheri subsp. *canascens* (Boiss. & Heldr.) Celep, Kahraman & Doğan	bifacial	hypostomatic	3	
S. heldreichiana Boiss.	monofacial	amphistomatic	1	
S. macrochlamys Boiss. & Kotschy	monofacial	amphistomatic	1	36
S. ballsiana (Rech.f.) Hedge	monofacial	amphistomatic	1	37
S. potentillifolia Boiss. & Heldr. ex Benth.	bifacial	amphistomatic	1	12
S. nydeggeri Hub.-Mor.	bifacial	amphistomatic	1	12
S. divaricata	bifacial	amphistomatic	1	27
Sect. *Hymenosphace*				
S. euphratica Montbret & Aucher ex Benth.	bifacial	amphistomatic	4	27
Sect. *Aethiopis*				
S. hypargeia Fisch. & C.A.Mey.	bifacial	amphistomatic	4-5	27
S. indica L.	bifacial	amphistomatic	3	38
S. vermifolia Hedge & Hub.-Mor.	bifacial	amphistomatic	3	39
S. argentea L.	bifacial	amphistomatic	1	30
Sect. *Horminum*				
S. viridis L.	bifacial	amphistomatic	1	28
Sect. *Drymosphace*				
S. glutinosa L.	bifacial	hypostomatic	1	26

Tablo 3 The trichome features of *S. aucheri* subsp. *canascens*, *S. heldreichiana* and related taxa.

	Stem Indumentum	**Leaf Indumentum**	**References**
Sect. *Salvia*			
S. aucheri subsp. *canascens* (Boiss. & Heldr.) Celep, Kahraman & Doğan	glabrous	glandular and non-glandular trichomes	
S. heldreichiana Boiss.	non-glandular trichomes	glandular and non-glandular trichomes	
S. macrochlamys Boiss. & Kotschy	glandular and non-glandular trichomes	glandular and non-glandular trichomes	36
S. ballsiana (Rech.f.) Hedge	glabrous	glandular and non-glandular trichomes	37
S. potentillifolia Boiss. & Heldr. ex Benth.	glandular and non-glandular trichomes	glandular and non-glandular trichomes	12
S. nydeggeri Hub.-Mor.	glandular and non-glandular trichomes	glandular and non-glandular trichomes	12
S. divaricata	glandular and non-glandular trichomes	glandular and non-glandular trichomes	27
Sect. *Hymenosphace*			
S. euphratica Montbret & Aucher ex Benth.	glabrous	glandular trichomes	27
Sect. *Aethiopis*			
S. hypargeia Fisch. & C.A.Mey.	glandular and non-glandular trichomes	glandular and non-glandular trichomes	27
S. indica L.	glandular and non-glandular trichomes	non-glandular trichomes	38
S. vermifolia Hedge & Hub.-Mor.	glandular and non-glandular trichomes	non-glandular trichomes	39
S. argentea L.	glandular trichomes	glandular trichomes	30
Sect. *Horminum*			
S. viridis L.	non-glandular trichomes	glandular trichomes	28
Sect. *Drymosphace*			
S. glutinosa L.	glandular and non-glandular trichomes	glandular and non-glandular trichomes	26

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