

MORPHOLOGICAL, ANATOMICAL AND TRICHOMES PROPERTIES OF *SALVIA WIEDEMANNII* BOISS. ENDEMIC TO TURKEY

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Abstract

Salvia wiedemannii Boiss. (Lamiaceae) is an endemic plant of the Irano-Turanian phytogeographic region. Morphological, anatomical and trichomes properties of the plant were examined in this study. Scanning electron microscopy (SEM) was used to describe the micromorphology of trichomes. Two types of glandular trichome were found: peltate and capitate. Morphological properties of various organs of the plant such as stem, leaf and flower were described in detail. As a result, trichomes on stem, pinnatisect leaves, properties of calyx and corolla and type of stamens (A type) were found to be helpful to distinguish *Salvia* species. In anatomical studies, transverse sections of the stem and leaf were examined and supported by photographs. Anatomical characters such as mesophyll structure in leaves, vascular bundles in leaves and stems and trichome properties provide information of taxonomic significance.

Key words: Anatomy, Lamiaceae, Morphology, *Salvia wiedemannii*, SEM, Trichome.

Türkiye'ye Endemik *Salvia wiedemannii* Boiss.' nin Morfolojik, Anatomik ve Tüy Özellikleri

Salvia wiedemannii Boiss. (Lamiaceae) Iran-Turan fitocoğrafik bölgesinin endemik bir bitkisidir. Bu çalışmada bitkinin morfolojik, anatomik ve tüy özellikleri incelenmiştir. Tüylerin mikromorfolojik tanımlarında taramalı elektron mikroskobu (SEM) kullanılmıştır. İki tip salgı tüyü gözlenmiştir: Sapsız ve saplı. Bitkinin gövde, yaprak ve çiçek gibi çeşitli organlarının morfolojik özellikleri ayrıntılı olarak tanımlanmıştır. Sonuç olarak, gövde tüyleri, pinnatisek yapraklar, kaliks ve korolla özellikleri ve stamen tipi (A tipi) *Salvia* türlerinin tanınmasında kullanılacak özellikler olarak tespit edilmiştir. Anatomik çalışmalarda, bitkinin gövde ve yapraklarının enine kesitleri incelenmiş ve fotoğraflarla desteklenmiştir. Yapraklarda mezofil yapısı, yaprak ve gövdelerde iletim demetleri ve tüy özellikleri önemli taksonomik bilgi sağlamaktadır.

Anahtar kelimeler: Anatomi, Lamiaceae, Morfoloji, *Salvia wiedemannii*, SEM, Tüy örtüsü.

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INTRODUCTION

Anatolia is a major diversity centre for genus *Salvia* in Asia with 50.6 % endemics out of 89 species, or 94 taxa (1-4). Species of *Salvia* are known as “adaçayı” in Turkey and their leaves are used for herbal tea in the region. The name *salvia*, coming from the Latin word for health (*salvare* or heal). The use of *Salvia* species as a tonic, stimulant, carminative, antiseptic, antihydrotic and flavour food in cosmetics, perfumes and pharmaceutical industries were reported (5). The plants of this genus are rich in essential oils and among their constituents, 1,8-cineol and guaiene mono and sesquiterpenes are very common (6). 1,8-Cineol (eucalyptol), is also one of major component of *S. wiedemannii*. *Salvia* species including eucalyptol have been used in traditional medicine as a secretolytic remedy for bronchitis, sinusitis, and colds (7-9).

Salvia wiedemannii Boiss. is an endemic plant of the Irano-Turanian phytogeographic region. It grows in limestone slopes, roadsides and fieldsides of central Anatolia (3). Various morphological, anatomical (10-14) and glandular trichome (15-20) studies on *Salvia* species are present.

The morphological and anatomical structures of *Salvia wiedemannii* have not been studied before. Therefore, the aim of the present study is to give a detailed account of the morphological and anatomical properties and micromorphology and distribution of trichomes of *S. wiedemannii* which is endemic to Turkey.

EXPERIMENTAL

Plant material

Salvia wiedemannii samples were collected during the flowering period (May, 2004) in Eskişehir (near Oğlakçı village) province Turkey. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir, Turkey (ESSE 14357).

Light microscopy (LM)

Live material was stored in 70 % alcohol for anatomical studies. All sections were taken from median leaves of the plant. Transverse sections and surface preparations of leaves and stems were made manually. All sections were embedded in glycerin-gelatine and mounted on microscope slides with Canada Balsam and photographs were taken with light microscopy (Olympus SZX12 and Olympus BX51T).

Scanning electron microscopy (SEM)

Stems, leaves, calyces and corollas were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 for 4 h at 4°C. After washing the material was dehydrated by acetone critical point drying. The specimens were mounted onto SEM stubs using double-sided adhesive tape and coated with gold. Photographs were taken with a scanning electron microscope (Zeiss EVO 50).

RESULTS

Morphological properties

Salvia wiedemannii was found to be a perennial suffruticose herb, 10–20 (-25) cm, erect, stems generally branched, with eglandular scabridulous often antrorse white hairs and some sessile glands (Fig. 1 and Plate 1, 2). Leaves pinnatisect with two pairs of lateral segments, terminal segment linear-oblong to linear-obovate, 0.8–2.2 x 0.3–0.5 cm, terminal segment

longer than laterals, eglandular with short antrorse hairs on the veins, sessile glands on both surfaces, margins entire, apex acute. Petiol 0.2–0.5 cm, surrounded the stem and long ciliate towards to base (Fig. 1 and Plate 3-9). Verticillasters 2–6 flowered, 0.4–1.8 cm. Length of verticillasters 1–5.5 cm and verticil number 2–6. Bracts ovate, ovate-lanceolate, 2.5–10 x 2–4.5 mm longly ciliate (Fig. 1c). Bracteoles ovate to lanceolate, 1.5–2 mm, longly ciliate (Fig. 1d). Calyx reddish-purple, tubular campanulate, 6–12 mm long, 13-veined, bilabiate, upper lip tridentate 0.8–1.5 mm long, shortly mucronate, lower lip bidentate 2.5–4.5 mm long, lanceolate, inner surface of the calyx short haired, teeth ciliated, outer surface of the calyx villous on veins and with sessile glands between the veins, pedicel 1.5–3.5 mm (Fig 2 a,b,c and Plate 10-13). Corolla lilac-blue, 10–15 mm, tube 7–10 mm, bilabiate, annulate upper lip straight bifid, lower lip trilobed, middle lobe larger than laterals, densely covered by glandular and eglandular trichomes on the lips (Plate 14-18 and Fig. 2f). Style 9–15 mm long. Stamens two, with a short filament (2–3 mm) and short connective (1–1.5 mm) bearing a fertile and sterile theca (type A) (Fig. 2e). Nutlets rounded-trigonous, oblong 2.8 x 1.8 mm.

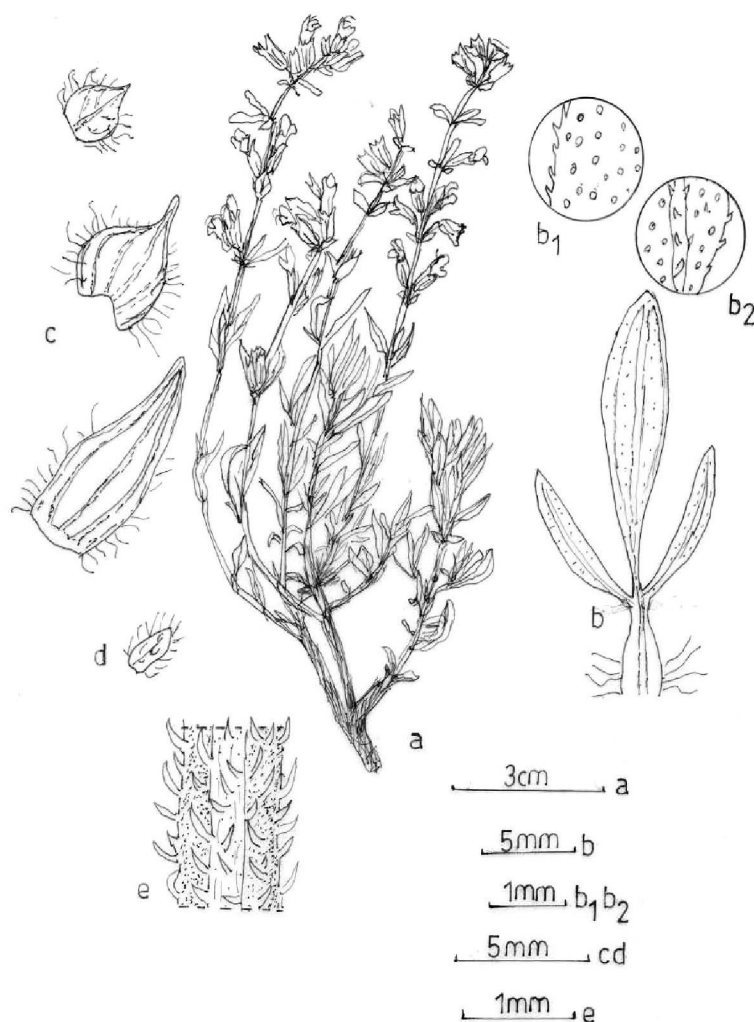


Figure 1. *Salvia wiedemannii*: a-habit b-leaves b₁-trichomes in upper surface of leaves b₂-trichomes in lower surface of leaves c-bracts d-bracteole e-trichomes of stem

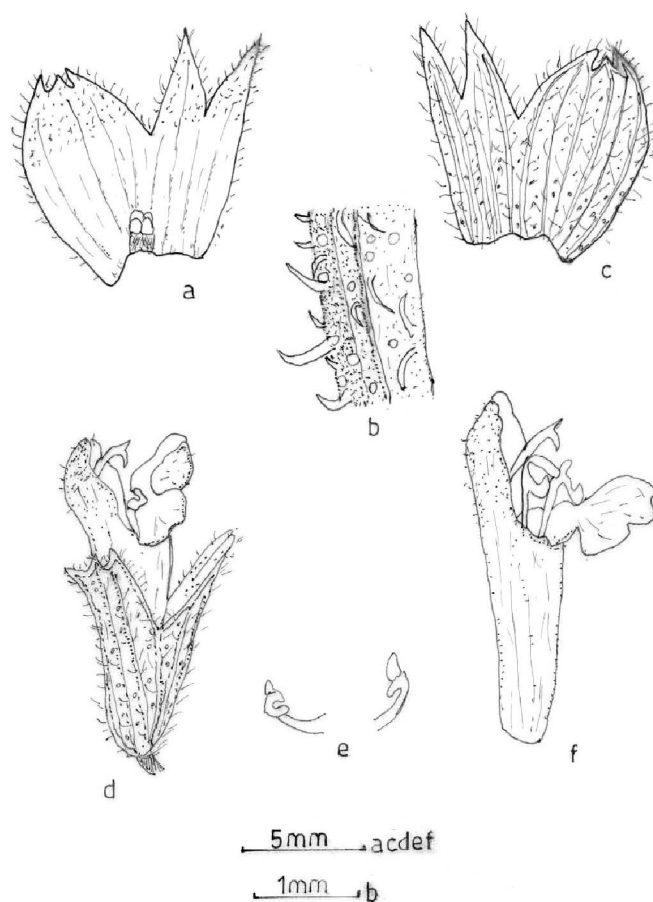


Figure 2. *Salvia wiedemannii*: a-inner surface of calyx b-trichomes of calyx c-outer surface of calyx d-flower e-stamen f-corolla

Anatomical properties

Stem

Transverse sections taken from the middle part of the stem were observed as follows; The stem is rectangular (Fig. 3). The epidermis is composed of a single layer of almost square-ovoid compactly arranged cells. The upper and lower walls of the epidermis are thicker than the lateral walls. The upper surface is covered with an almost thick cuticle and trichomes. Trichomes are both of glandular and eglandular types (Fig. 3, 4 and Plate 1, 2). Eglandular hairs are more common than the other. Collenchyma tissue, consisting of roundish-ovoid cells, is located immediately under the epidermis. There are 4–7 layers of collenchyma in the corners and 1 (-2) layers in between the corners of the stem sections (Fig. 4). The cortex tissue is 3–5 layered parenchymatous, and usually squashed in the corners. The cells are ovoid-orbicular in shape. The numerous (14-23) vascular bundles are arranged in a ring and they are well developed in the corners. Vascular bundles are either next to each other or in isolated bundles separated by sclerenchymatous cells. Endodermis is inconspicuous. The pericycle consists of sclerenchymatous cells which are well developed on the outside of the phloem. The phloem is 3–4 layered and consists of irregular cells. The cambium is usually squashed and 1 (-2) layered. The xylem consists of regular trachea and tracheids cells. Those cells underlying the xylem are narrower and thick walled. The pith is composed of large orbicular or polygonal parenchymatous cells (Fig. 4).

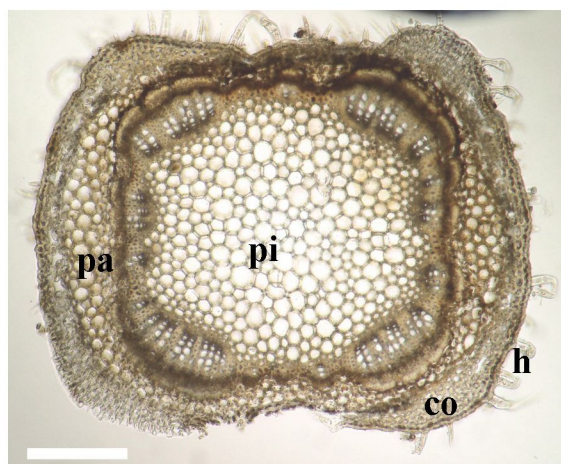


Figure 3. *Salvia wiedemannii*: stem cross section: co-collenchyma h-hairs pa-parenchyma pi-pith cross section: Scale: 200 μ m

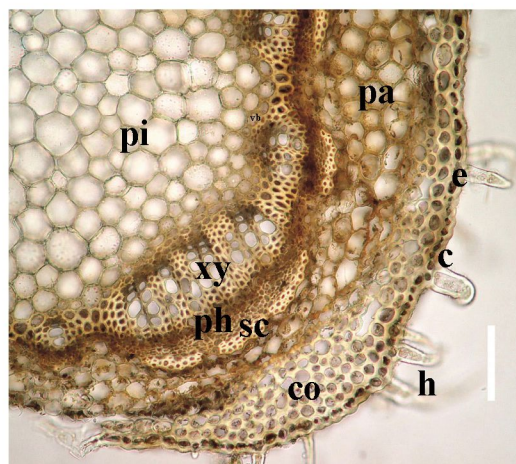


Figure 4. *S. wiedemannii*: corner anatomy in stem c-cambium co-collenchyma e-epiderma h-hairs p-parenchyma ph-phloem pi-pith sc-sclerenchyma xy-xylem Scale: 100 μ m

Leaf

Transverse sections of the lamina and the midrib and surface preparations of both epidermis revealed the following elements.

The upper and lower epidermis consist of uniseriate square and rectangular cells in transverse sections. The upper walls of the epidermis are thicker than lower and lateral walls. Both the adaxial and abaxial epidermis is covered with an almost thick cuticle. Covering trichomes are of both glandular and eglandular types on both surfaces. Most of them are glandular hairs. The leaf is of the bifacial type. Mesophyll tissue is differentiated into 3–4 seriate palisade and 2 (-3) seriate spongy tissue (Figs. 5, 6). The shape of the cells in the palisade and spongy parenchyma in surface preparations is circular or ovoid. The vascular bundle is collateral type. The xylem faces towards the upper (adaxial) surface and the phloem faces the lower (abaxial) epidermis (Fig. 7). Uniseriate collenchymatous cell layer and 3–4 seriate parenchymatous cell layers are located below the lower epidermis while several-seriate

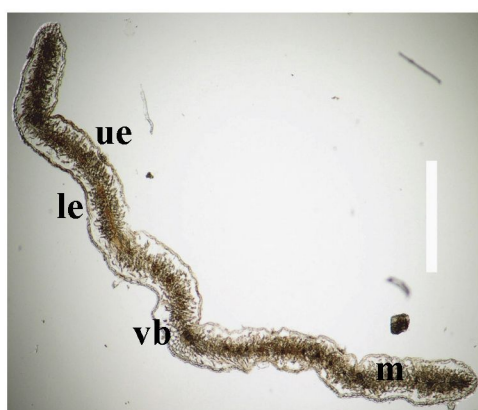


Figure 5. *Salvia wiedemannii*: leaf cross section: le-lower epiderma ue-upper epiderma m-mezophyll vb-vascular bundle Scale: 0.5 mm

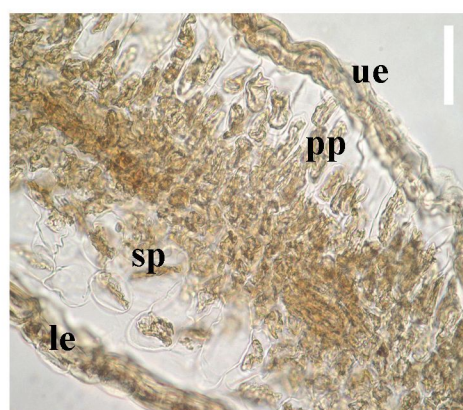


Figure 6. *S. wiedemannii*: mesophyll tissue cross section: le-lower epiderma pp-palisade parenchyma sp-spongy parenchyma ue-upper epiderma Scale: 50 μ m.

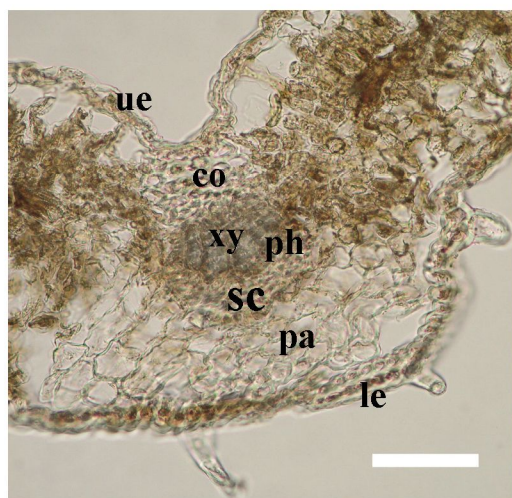


Figure 7. *S. wiedemannii*: vascular bundle co-collenchyma le-lower epiderma pa-parenchyma ph-phloem sc-sclerenchyma ue-upper epiderma Scale: 50 μ m

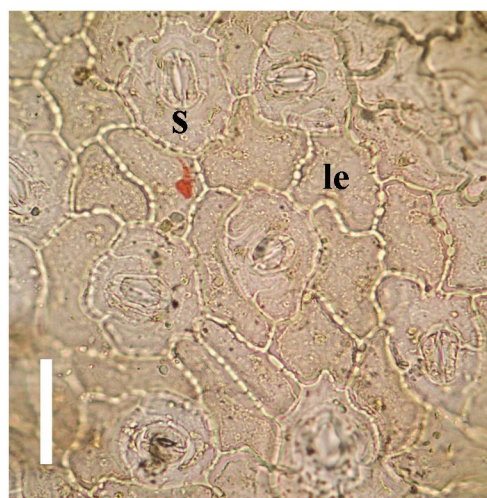


Figure 8. *S. wiedemannii*: surface preparation of lower epidermis s-stomata le-lower epiderma Scale: 50 μ m

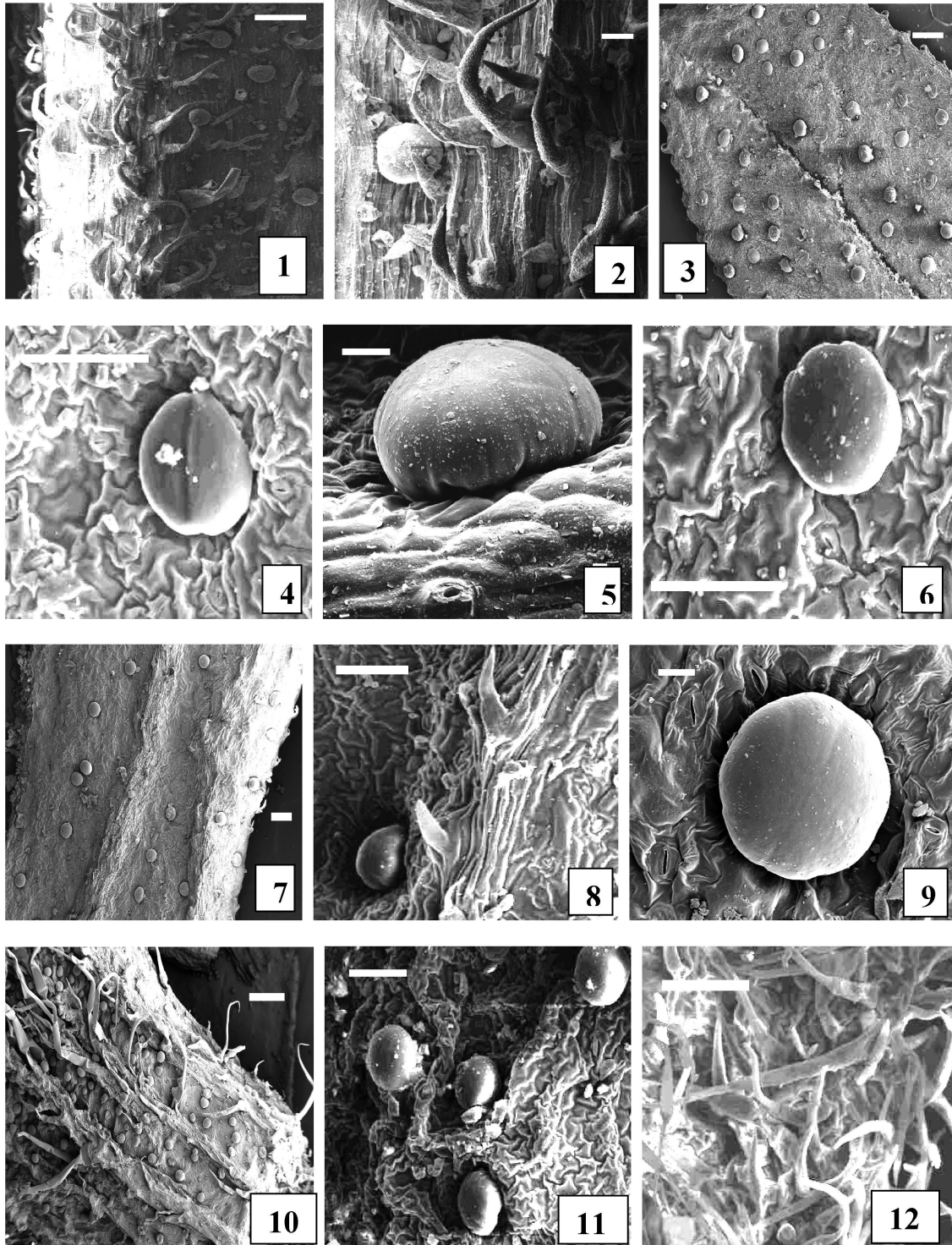
collenchymatous cells located below the upper epidermis in the midrib region. The phloem are surrounded by several-seriate sclerenchymatous tissue. The stomata are diacytic and present on the surfaces of both epidermis (amphistomatic). The shape of the stomata is ovoid in surface preparations (Fig. 8). The stomata are located at almost the same level as the epidermal cells (mesomorphic type).

Trichome properties

These can be divided into two main types: eglandular and glandular hairs. Two types of glandular hairs, peltate and capitate, are encountered in *S. wiedemannii*.

The eglandular trichomes consist of one to four cells. They are present on the stems, leaves, calyces and corollas. They are dense, three-four cells with cuticular micropapillae and often antrorse in the stem (Plate 1, 2). Similar trichomes are located on the margins of the leaves (Plate 3, 7) and on the ribs of the abaxial surface of the leaves (Plate 8) but, they are more scattered and usually short, one or two celled. Long, three-four celled, villous trichomes are situated mainly on the veins of the calyx (Plate 10, 12) and on outer surface of corolla (Plate 14). Many short antrorse hairs are present inner surface of the calyx and they usually consist of one or two cells (Plate 13).

Peltate hairs are situated on the vegetative and reproductive organs, mainly stems, leaves, calyces and corollas, respectively. They are observed in large numbers on the stem (Plate 1) and on the both surface of the leaves (Plate 3, 7) of *S. wiedemannii*. Similar trichomes present abundantly in the grooves of the calyces (Plate 10) and located on outer surface of corolla lips (Plate 14). Peltate hairs have a characteristic colourness to pale-yellow colour and a balloon shape and they consist of a basal cell, a short stalk and a broad head of (two-) four to eight secretory cells. The anticlinal wall of the stalk cell and the cells of the secretory head are completely cutinized. As the peltate trichomes matured, their shape changed from the two-celled to the eight-celled head stage (Plate 4-6). Droplets of secreted material can be seen on the peltate head (Plate 18). In SEM micrographs, peltate glandular heads show a wrinkled or smooth surface (Plate 9, 15). Peltate hairs are more common than capitate ones.



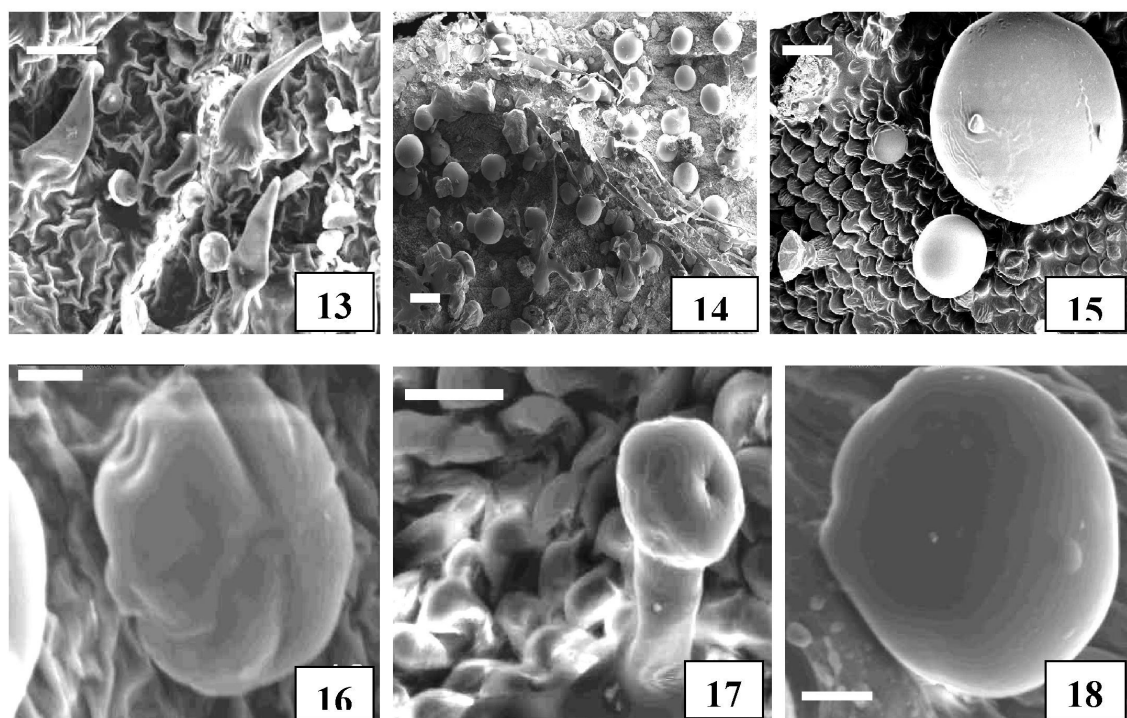


Plate 1–18. Scanning electron micrographs of *S. wiedemannii* trichomes. 1-2 Stem, 3-6 leaf adaxial surface, 7-9 leaf abaxial surface 10-12 outer surface of calyx, 13 inner surface of calyx, 14-18, outer surface of corolla. Scale bars: 1, 4, 6, 7, 8, 11, 12, 15 = 100 μm 2, 5, 9, 13, 16, 17, 18 = 20 μm , 3 = 200 μm , 10 = 400 μm , 14 = 50 μm

Capitate hairs are few in number and they are observed only on calyx and corolla, especially on inner surface of calyx (Plate 13) and on outer surface of corolla lips (Plate 15, 17). Capitate hairs are two types which consisted of a short unicellular or bicellular stalk and unicellular secretory head. The secretory material in glandular hairs probably exuded through a pore (Plate 17).

DISCUSSION

As can be seen from the results presented here, the morphological properties of *S. wiedemannii* show some differences compared with those reported in the flora of Turkey (3). The dimensions of the plant, leaves, flower number in the verticil, calyx, corolla and nutlet were found to be lower than previously reported (Table 1). Some morphological characters of this species are absent in the Flora of Turkey, such as number and distant of verticil, lengths of verticil, bracteole and calyx lips. These unknown characters were provided in detail in this study and the description of species is widened. These findings are expected to contributed to the Flora of Turkey to separate *S. wiedemannii* from similar to *Salvia* species. *S. wiedemannii* is closely similar to *S. pisidica* Boiss. & Heldr. ex Bentham and *S. freyniana* Bornm. according to Flora of Turkey. The morphological differences between these species are presented in Table 2. The present study showed that morphological characters such as trichomes types, inflorescens, shape of the terminal segment in leaves and lengths of the calyces and corollas are of diagnostic value in this group.

Table 1. The comparison of morphological characteristics between this study and Flora of Turkey (3), related with of *Salvia wiedemannii*.

| | The findings of this study | Flora of Turkey |
|------------------------|----------------------------|--------------------------|
| Stem | 10–20 (-25) cm | 15–30 cm |
| Leaves | | |
| terminal segment | 0.8–2.2 x 0.3–0.5 cm | 1.2–3(-4)x0.2–0.6(-1) cm |
| petiole | 0.2–0.5 cm | 0.2–1 cm |
| Verticillastrum | 2–6 flowered | 2–8 (-10) flowered |
| verticil number | 2–6 | - |
| verticil distance | 0.4–1.8 cm | - |
| verticil length | 1–5.5 cm | - |
| Bract | 2.5–10 x 2–4.5 mm | 6–8 x 3–4.5 mm |
| Bracteole | 1.5–2 mm | - |
| Calyx | 6–12 mm | 8–14 mm |
| lower lip | 2.5–4.5 mm | - |
| upper lip | 0.8–1.5 mm | - |
| pedicel | 1.5–3.5 mm | 3–5 mm |
| Corolla | 10–15 mm | 16–20 mm |
| tube | 7–10 mm | c. 12 mm |
| Nutlet | 2.8 x 1.8 mm | 3 x 2 mm |

Within *Salvia* three major types of stamens, which are taxonomically important (2), recognised: small fertile or subfertile theca in stamens of type A, variously shaped sterile tissue in stamens of type B, and usually articulating at junction of filament and connective for stamens of type C. According to this classification *S. wiedemannii* has stamens of type A (3).

Trichomes on stem, pinnatisect leaves, properties of calyx and corolla and type of stamens (A type) may also be diagnostic for *S. wiedemannii* according to morphological results.

Anatomical features typical of the Lamiaceae (21) such as the rectangular cross-section of the stem, well-developed collenchyma in each corner and diacytic stomata were also observed. The endodermis in Lamiaceae species is either or not well-defined according to Metcalfe and Chalk (21). However, it is inconspicuous in *S. wiedemannii*. Pericycle usually containing little sclerenchymatous cells, which are observed as a group on the phloem. The cambium is distinguishable. Results show that *S. wiedemannii*, in many respects, is similar to *Salvia hypargeia* Fisch. & Mey, previously described by Kandemir (12). Bifacial leaves and the occurrence of collenchyma located on both below and above the midrib, as encountered in the present study, has previously been reported in species of *Salvia* (12-14).

As in most Lamiaceae species, *S. wiedemannii* have glandular and eglandular trichomes and glandular hairs consist of peltate and capitate types (22). In *S. wiedemannii*, peltate hairs are more common than capitate hairs and they are found on the vegetative and reproductive organs. The peltate trichomes consist of a short unicellular stalk and a secretory head composed of four to eight cells in a single circle. The peltate hairs of *S. wiedemannii* are similar to those reported for *Salvia halophila* Hedge (11), *Salvia blepharophylla* Brandegees ex Epling (16), *Salvia glutinosa* L. (17), *Salvia divinorum* Epling & Jativa (18), *Salvia aurea* L (19), *Plectranthus ornatus* Codd (23), *Nepeta racemosa* L. (24), *Nepeta congesta* Fisch.& Mey. var. *congesta* (25).

Table 2. Comparative morphology of closely related species to *S. wiedemannii* according to Flora of Turkey (3)

| | <i>S. wiedemannii</i> | <i>S. pisidica</i> | <i>S. freyniana</i> |
|-------------------------|---|--|--|
| Trichome on stem | scabridulous, antrorse sessile glands <i>*scabridulous, often antrorse</i> | retorse, pilose to villous with or without capitate glandular hairs | antrorse, villous with capitate glandular hairs |
| Leaves | pinnatisect, terminal segment linear oblong to linear obovate margins always entire 1.2-3 (-4) x 0.2-0.6 (-1) cm <i>*0.8-2.2 x 0.3-0.5 cm</i> | triset or rarely pinnatisect, ter. seg. obovate-cuneate to linear-obovate margins apically crenulate 0.7-2.4 x 0.3-1 cm | pinnatisect, terminal segment linear oblong margins crenulate towards apex 2.5-5 x 0.4-1 cm |
| Verticillasters | 2-8 (10) flowered, \pm approximating <i>*2-6 flowered</i> | 2-8 flowered, usually distant | 4-10 flowered, distant |
| Calyx | reddish-purple, 8-14 mm tubular-campanulate <i>*6-12 mm</i> | purplish, 10-12 mm \pm tubular-campanulate | purplish, c. 7 mm campanulate |
| Corolla | lilac-blue, 16-20 mm <i>*10-15 mm</i> | violet-blue, 18-22 mm | lilac-blue, 12-16 mm |
| Nutlet | c. 3 x 2 mm, oblong <i>*2.8 x 1.8 mm</i> | c. 5 x 3 mm, ovoid | c. 4 x 3 mm, ovoid |

* findings

Capitate hairs are more common in Lamiaceae species. Furthermore they have vary greatly in structure and size. The capitate trichomes of *S. wiedemannii* consisting of short unicellular or bicellular stalk and unicellular secretory head. This types of capitate hairs are similar to those previously described by Kaya et al. (11, 17, 25), Kandemir (12), Ascensao et al. (23), Bosabalidis (26), Bisio et al. (16), Corsi & Bottega (20) and Satil & Kaya (27).

The material secreted by the peltate glandular hairs accumulated in the subcuticular space, and sometimes formed a bulge, as in the peltate glands of all the Lamiaceae species that have been examined so far (16, 19, 24). The secreted material can be released by breakage of the cuticle along equatorial lines of weakness, or via pores in the cuticular structure as *S. wiedemannii* and *S. blepharophylla* (16). While the first mode is common to the species examined (16,19,28), the second appears to have been described generally in capitate trichomes of members of the Lamiaceae (16, 29). Pore formation in *S. wiedemannii* has been observed by SEM (Plate 17).

Mesophyll structure, vascular bundles in the leaf and stem and structure and density of trichome may also be diagnostic for *S. wiedemannii* according to anatomical and SEM results.

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