# Full Length Research Paper

# Anatomical and palynological characteristics of *Salvia* willeana (Holmboe) Hedge and *Salvia veneris* Hedge endemic to Cyprus

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Accepted 18 March, 2010

In this study, anatomical and palynological features of the roots, stems, petiole and leaves of *Salvia willeana* (Holmboe) Hedge and *Salvia veneris* Hedge, *Salvia* species endemic to Cyprus, were investigated. In the anatomical characteristics of stem structures, it was found that the chlorenchyma composed of 6 or 7 rows of cells which was beneath the epidermis of *S. veneris*, whereas *S. willeana* did not have such a tissue. While the transverse section of the leaf of *S. veneris* had either a single large vascular bundle or a two-lobe vascular bundle in the midrib, there was only a single large vascular bundle in that of *S. willeana*. While there were 2-or-3-piece vascular bundles in the center and three-piece vascular bundles at the corners of the petiole of *S. veneris*, there was a single large vascular bundle in the center and two vascular bundles at the corners of the petiole of *S. willeana*. It was determined that pollens belonging to both taxa had hexazonocolpate, subprolate, semitectate structure and showed bireticulate ornamentation. However, the number of lumina in the secondary reticulum of *S. willeana* was fewer than ten while it was more than ten in *S. veneris*.

Key words: Salvia willeana, Salvia veneris, anatomy, palynology, endemic.

# INTRODUCTION

Labiatae with its more than 250 genera and approximately 7000 species has a cosmopolitan distribution (Thorne, 1992). *Salvia* L., the largest genus of this family, spreads in the warm and temperate regions of both North and South hemisphere. Of the 900 species of *Salvia* L., 500 are located in Central and South America, 200 in Western Asia and 200 in Eastern Asia (Walker and Systma, 2007).

Salvia species are used in medicine, perfumery and cosmetics industry as tonic, antibacterial, carminative, antiseptic and antihidrotic agents. In addition, since they contain essential oils, they are used in food industry as flavoring and aromatic agents (Kintzios 2000; Demirci et al., 2003; Kesercioğlu and Nakipoğlu, 1992). Recently, it has

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**Abbreviations:** P, Polar axis; E, equatorial axis; P/E, ratio of polar axis to equatorial axis.

also been found that *Salvia* species have an antioxidant effect by several researchers (Cuppett and Hall, 1998; Avato et al., 2005).

In Cyprus, there exist ten *Salvia* species and of them, only *Salvia willeana* (Holmboe) Hedge and *Salvia veneris* Hedge are endemic.

S. willeana (Holmboe) Hedge and S. veneris Hedge are endemic to Cyprus, S. veneris is listed in the Red Data Book of Cyprus. It is restricted to the Kythrea area, Confined to an area north and west of Kythrea (12 km²), in sparse pine forest, maquis and garigue, with about 4,000 individuals at an altitude of 150-300 m; 5 locations, 2 on state forest land. It is threatened by land development, afforestations, road construction, grazing and dust from nearby quarries (Tsintides et al., 2007).

S. veneris; perennial tufted herb, stems c. 20-40 cm high from a woody rootstock, erect, tetragonal, lanate at base of stem, above with a dense indumentum of capitate glandular and eglandular multicellular scabridulous hairs; leaves almost confined to basal rosettes, very broadly ovate to suborbicular, 2 - 5 - 8 cm and 1 -7-8 cm; petiole

lanate to pannose, 1 - 5 - 5 - 2 cm.

Salvia willeana; low-growing, strongly aromatic suffruticose herb, sometimes carpeting the ground; stems ascending to erect, c. 25-55 cm, tetragonal, below with mostly pilose to villous eglandular hairs, above with a dense indumentum of short capitate glandular hairs of varied length, sessile glands and sometimes also with eglandular hairs; leaves distributed over stem, ovate to elliptic, 1 - 5 - 6 cm long and 0 - 8 - 3 - 2 cm; petiole 0 - 8 - 4 - 2 cm long below, decreasing in length upwards (Meikle, 1985).

Although there are several floristic studies performed on plants in Cyprus (Meikle 1977, 1985; Viney 1994, 1996; Snogerop et al., 1990; Stephenson, 1993), the number of biologic studies is not many. One of these studies was conducted to investigate the pollen features of *S. veneris* and Palynological characteristics of taxa endemic to Cyprus (Yildiz et al., 2009). *S. veneris* was also investigated in terms of essential oils and terpenoids (Bellomaria et al., 1992; De la Torre et al., 1991). However, there are no studies conducted on anatomical characteristics of *S. willeana* and *S. veneris* and palynological characteristics of *S. willeana*.

In this study, of the ten species of *Salvia* found in Cyprus, the two endemic ones, *S. willeana* and *S. veneris*, were investigated in order to identify their anatomical and palynological characteristics.

### **MATERIALS AND METHODS**

# Plant materials

Samples of *S. willeana* taxon investigated in this study were picked up in Prodromos Forest College (Trodos Mountain) under *Pints brutia* and samples of *S. veneris* were picked up in Lefkoşa (Nicosia), above Değirmenlik Lake, sandstone hills, 200 m.

# **Anatomical studies**

In order to conduct anatomical studies, live plant materials were fixed in 70% ethyl alcohol. Cross sections of a root, stem, leave and petiole were taken from ten specimens of *S. veneris* and from ten specimens of *S. willeana* and 50 measurements were conducted for each parameter. Transverse sections from leaves, roots, trunks and petioles were taken with hands and stained with Sartur reactive (Baytop, 1972). Measurements in the sections were performed under PRIOR binocular light microscope by using micrometric ocular and the objectives used were x10 and x40. Photographs were taken with Jena NF-binocular microscope and Olympus BX51-Altra 20 Soft Imaging System camera.

# Palynological observations

Pollen preparates of both taxa were prepared in accordance with Wodehouse (1935) method. Measurements in the sections were performed under PRIOR binocular light microscope by using micrometric ocular and the objectives used were x10 and x40. Polar axis (P), Equatorial axis (E), Colpus length (Clg), Colpus width (Clt), exine and intine thickness in the pollens were measured and P/E ratio was assessed. All the measurements were conducted in 50

pollens. Pollen materials necessary for palynological observations were obtained from herbarium samples. JEOL JSN 6060 Scanning Electron Microscope was used to observe exine ornamentation of the pollens and to take the photographs. Pollen terminology of Hesse et al. (2009) was used.

# **RESULTS**

# **Anatomic properties**

### Stem

S. willeana has a 4-angle stem. Its epidermis usually has one layer and is composed of hexagonal or ovoidal cells. The upper surface is covered with a rather thick cuticle (2.5-7.5 um) and glandular or eglandular hairs. The cuticle has an undulate structure. Just beneath the epidermis is collenchyma, composed of regular cells. Transverse section of the stem reveals a 3-or 4-rowed lamellar collenchyma placed on the corners and just beneath it lies the angular collenchyma composed of 13 -14 layers of cells. The cortex, composed of 5 - 6 rows of parenchymatous cells, is located beneath the collenchyma. Cortex cells are of ovoidal or polygonal shapes (13.7 -35.0 x 15.0 - 57.5 µm) (Table 1). In the area located between the corners beneath the epidermis lies the lamellar collenchyma. There are well-developed islands of sclerenchyma in the outer side of the phloem (Figure 1-6). The phloem consists of 4 - 5 rowed irregular cells. Right beneath the phloem, there is the 2 -3 rowed cambium layer which is not very distinct. The xylem tissue existing beneath the cambium is composed of regular trachea and tracheid cells. The pith is wide and consist of hexagonal or orbicular parenchymatous cells with intercellular spaces. There are also starch particles (granules) in the parenchymatous pith.

The stem of S. veneris is four angled in the transverse sections. The epidermis is usually composed of onerowed flat rectangular cells (12.0 - 24.0 x 14.4 - 43.2 µm). The epidermis is covered with guite a thin cuticle (1.2 - 2.4 µm) (Table 2). In the transverse sections of the stem, there are large vascular bundles. In the area located between the corners, there are smaller vascular bundles. There is the 10-12 layered lamellar collenchyma in the corners. Beneath the collenchyma lies the parenchymatous cortex composed of 3 - 4 rows of cells consisting of lots of chloroplasts. In the areas located between the corners, there is the chlorenchyma composed of 6 - 7 rows of cells (Figure 3). Between the xylem and the phloem, there is the cambium which is not very noticeable. Just above the phloem, there are sclerenchymatic groups. The parenchymatous pith covers quite a large area. There are also starch particles in the pith.

# Petiole

The outermost layer of the petiole of the S. willeana is

| <b>Table 1.</b> Anatomical measurement of some chara | cters the of the stem, leaf, root and petiole of Salvia willeana. |
|--|---|
|--|---|

| Parameters             | Wid            | Width (μm)      |                | Length (µm)     |  |
|------------------------|----------------|-----------------|----------------|-----------------|--|
|                        | Min - Max      | Mean ± S.E.     | Min - Max      | Mean ± S.E.     |  |
| Stem                   |                |                 |                |                 |  |
| Cuticle                | 2.50 - 7.50    | 4.925 ± 0.889   |                |                 |  |
| Epidermis cell         | 7.50 - 17.50   | 13.50 ± 2.172   | 10.00 - 25.00  | 18.375 ± 3.979  |  |
| Parenchyma             | 13.75 - 35.00  | 22.525 ± 5.903  | 15.00 - 57.50  | 34,925 ± 9.531  |  |
| Trachea cell           | 8.75 - 28.75   | 18.300 ± 5.403  | 12.50 - 35.00  | 23.350 ± 5.716  |  |
| Pith cell              | 16.25 - 115.00 | 44.425 ± 3.584  | 17.50 - 110.00 | 52.425 ± 3.517  |  |
| Root                   |                |                 |                |                 |  |
| Peridermis cell        | 10.00 - 30.00  | 19.30 ± 4.46    | 25.00 - 65.00  | 39.825 ± 9.303  |  |
| Parenchyma             | 6.25 - 26.25   | 12.750 ± 4.510  | 12.50 - 55.00  | 28.225 ± 9.378  |  |
| Pith cell              | 6.25 - 28.75   | 11.050 ± 3.765  | 12.50 - 37.50  | 21.600 ± 6.201  |  |
| Trachea cell           | 7.50 - 50.00   | 20.050 ± 9.333  | 12.50 - 62.50  | 28.875 ± 9.147  |  |
| Leaf                   |                |                 |                |                 |  |
| Cuticle                | 2.50 - 6.25    | 4.625 ± 0.884   |                |                 |  |
| Adaxial epidermis cell | 10.00 - 22.50  | 13.600 ± 3.110  | 12.50 - 32.50  | 21.675 ± 5.259  |  |
| Abaxial epidermis cell | 17.50 - 37.50  | 29.050 ± 4.514  | 20.00 - 57.50  | 36.300 ± 6.551  |  |
| Mesophyll region       | 107.50 -195.00 | 152.850 ± 2.969 |                |                 |  |
| Palisade region        | 82.50 - 150.00 | 115.400 ± 2.395 |                |                 |  |
| Spongy region          | 20.00 - 50.00  | 35.600 ± 7.619  |                |                 |  |
| Palisade cell          | 7.50 - 15.00   | 11.100 ± 1.866  | 22.50 - 52.50  | 37.100 ± 8.102  |  |
| Spongy cell            | 7.50 - 22.50   | 13.725 ± 3.199  |                |                 |  |
| Petiole                |                |                 |                |                 |  |
| Adaxial epidermis cell | 7.30 - 33.75   | 17.775 ± 3.722  | 12.50 - 35.00  | 20.800 ± 5.497  |  |
| Abaxial epidermis cell | 12.50 - 26.25  | 17.225 ± 3.012  | 13.75 - 28.75  | 21.025 ± 3.691  |  |
| Parenchyma cell        | 13.75 - 70.00  | 31.950 ± 12.354 | 10.50 - 82.50  | 39.785 ± 13.084 |  |
| Trachea cell           | 7.50 - 27.50   | 14.620 ± 4.546  | 10.00 - 41.25  | 18.375 ± 6.503  |  |

composed of ovoidal and hexagonal epidermal cells. Over the one-layered epidermis, there is quite a thick and undulate cuticle. The epidermis consists of glandular and eglandular hairs. Epidermal cells are 7.3 - 33.7  $\times$  12.5 - 35.0  $\mu m$  in the upper epidermis and 12.5 - 26.2 x 13.7 - 28.7 µm in the lower epidermis (Table 1). There is a 2 - 3-layered lamellar collenchyma in the area between the corners. There is an 11 - 14layered parenchymatous cortex beneath the collenchymatic tissue. Cortex cells (13.7 - 70.0 x 10.5 - 82.5 µm) are ovoidal. At the two corners of the transverse section of the petiole just beneath the epidermis, the one-layered lamellar collenchyma and the 6-7-layered lacunar collenchyma exist. There are two small vascular bundles at each corner and a single vascular bundle in the center. The cambium is located between the xylem and the phloem. There are sclerenchyma groups on the phloem in the vascular bundles. The vascular bundle is collateral (Figure 9).

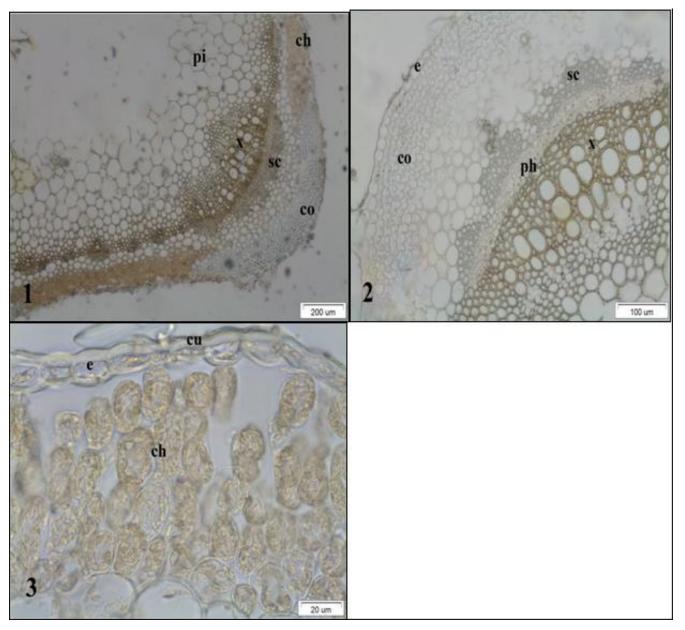
The outermost layer of the petiole of the *S. veneris* is composed of ovoidal and hexagonal epidermal cells. Over the one-layered epidermis, there is the cuticle. The epidermis consists of glandular and eglan-

dular hairs. Epidermal cells are 12.5 - 31.2 x 18.7 - 47.5  $\mu$ m in the upper epidermis and 12.5 - 21.2 x 17.5-40.0  $\mu$ m in the lower epidermis (Table 2). In the area between the corners, there is the 2 - 3-layered lamellar collenchyma and beneath it the 3 - 4-layered angular collenchyma. Beneath the collenchymic tissue lies the 10 - 15-layered parenchymatic cortex. Cortex cells (10.0 - 82.5 x 13.7 - 90.0  $\mu$ m) are ovoidal (Table 2). There are lots of starch particles in the cortex cells and the collenchyma. At the two corners of the transverse section of the petiole just beneath the epidermis, the one-layered lamellar collenchyma and the 2 - 3-layered lacunar collenchyma exist (Figure 10).

There are 2 - 3 vascular bundles and three smaller vascular bundles at each corner. The cambium is located between the xylem and the phloem. There are sclerenchyma groups on the phloem in the vascular bundles. The vascular bundle is collateral.

### Root

Periderm, the outermost layer of the root of *S. willeana*,

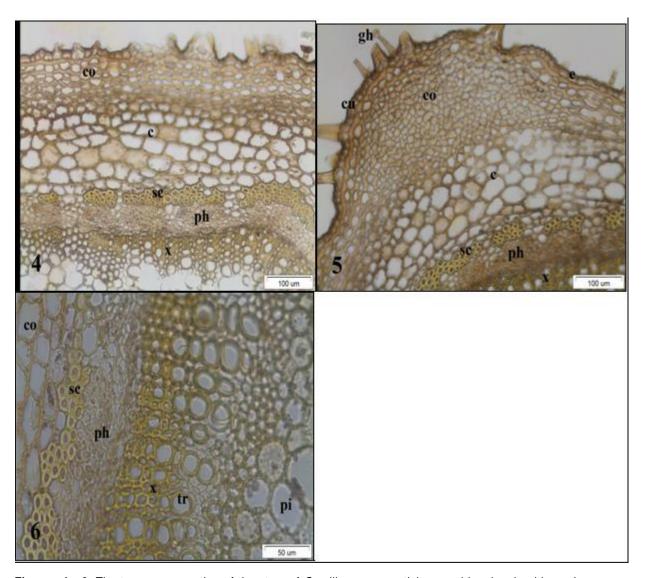


**Figures 1 - 3.** The transverse section of the stem of *S. veneris.* cu, cuticle; e, epidermis; ch, chlorenchyma; co, collenchyma; sc, sclerenchyma; ph, phloem; x, xylem; pi, pith region; and c, cortex.

has 6 - 18 layers. The dimensions of periderm cells are 10.0 - 30.0 x 25.0 - 65.0 µm. Beneath the periderm, there is the multi-layered cortex, composed of flat cells. The dimensions of parenchymatous cells are 6.2 - 26.2 x 12.5 - 55.0 µm (Table 1). Cortex cells are flatter than those of *S. veneris*. The pith region consists of the primary xylem tissue. The pith rays are composed of 1 - 2-layered parenchymatous cells. The cambium, composed of 2 - 3-layered irregular cells, is located between the xylem and the phloem. The ridge border of the xylem layer has an undulat structure. The cambium layer projects into the trachea in some places and is located over parenchymatous cells in other places. Over the cambium is

located the phloem layer (Figure 12).

The periderm on the root of *Salvia veneris* is 4 - 8 layered. The dimensions of the periderm cells are 8.4 - 33.6 x 21.6 - 57.6  $\mu$ m. Placed beneath the periderm is the cortex layer composed of flattened parenchymatous cells. The dimensions of the periderm cells are 8.4 - 25.2 x 13.2 - 52.8  $\mu$ m (Table 2). The primary xylem is located in the pith region. Pith rays are composed of 2 - 3 layers of cells. There are groups of stone cells in the periderm and the cortex. The cambium layer composed of 3 - 4-layered flat cells exist between the xylem and the phloem. Parenchyma cells are encountered beneath the cambium at intervals. The phloem is



**Figures 4 - 6.** The transverse section of the stem of *S. willeana.* cu, cuticle; e, epidermis; ch, chlorenchyma; co, collenchyma; sc, sclerenchyma; ph, phloem; x, xylem; pi, pith region; c, cortex.

located above the cambium (Figures 11 - 14).

# Leaves

The 2.50 - 6.25 µm-thick cuticle layer is the outermost layer in the transverse section of the leaf of S. willeana. There is a one-rowed epidermis layer on the upper and the lower surfaces of the leaf. There are glandular and eglandular hairs in the epidermis. Epidermis cells have irregular shapes. The dimensions of the upper epidermis cells are 10.0 - 22.5 x 12.5 - 32.5 µm while those of the lower epidermis cells are 7.5 - 37.5 x 20.0 - 57.5 µm. Located beneath the epidermis are the 2 - 3-rowed palisade parenchyma cells. The 3-4-rowed spongy parenchyma exists beneath the palisade (Figure 17 - 19). The spongy parenchyma

covers less space than does the palisade parenchyma. The dimensions of the palisade parenchyma cells are 7.5 - 15.0 x 22.5 - 52.5  $\mu$ m while those of the sponge parenchyma cells are 7.5 - 22.5  $\mu$ m (Table 1). The collateral vascular bundle is located in the midrib region. The 2 - 3-rowed lamellar collenchyma is seen in the midrib beneath the epidermis. Located beneath the lamellar collenchyma is the parenchyma composed of elliptical flat cells surrounding the vascular bundle. The stoma is diastic and the leaf is bifacial (Figure 19).

The outermost layer of the leaf of S. veneris is the 1.2 - 2.4  $\mu m$  thick cuticle layer. There is a one-rowed epidermis layer on the upper and the lower surfaces of the leaf. There are glandular and eglandular hairs in the epidermis (Figure 15). The dimensions of the upper epidermis cells are 12.0 - 28.8 x 13.2 - 55.2  $\mu m$  while those of the lower epidermis cells are 10.8 - 33.6

| <b>Parameters</b>      | Widt            | h (µm)          | Leng          | th (µm)        |
|------------------------|-----------------|-----------------|---------------|----------------|
|                        | Min - Max       | Mean ± S.E.     | Min - Max     | Mean ± S.E.    |
| Stem                   |                 |                 |               |                |
| Cuticle                | 1.20 - 2.40     | 1.884 ± 0.499   |               |                |
| Epidermis cell         | 12.00 - 24.00   | 19.104 ± 2.907  | 14.40 - 43.20 | 24.840 ± 5.069 |
| Parenchyma             | 12.00 - 48.00   | 25.056 ± 8.546  | 13.20 - 55.20 | 33,024 ± 1.464 |
| Trachea cell           | 9.60 -48.00     | 23.904 ± 8.260  | 15.60 - 51.60 | 31.872 ± 9.324 |
| Pith cell              | 12.00 - 129.60  | 48.048 ± 3.893  | 18.00 -144.00 | 59.160 ± 4.227 |
| Root                   |                 |                 |               |                |
| Peridermis cell        | 8.40 - 33.60    | 17.760 ± 5.737  | 21.60 - 57.60 | 35.520 ± 8.000 |
| Parenchyma             | 8.40 - 25.20    | 15.480 ± 4.073  | 13.20 - 52.80 | 28.204 ± 8.459 |
| Pith cell              | 8.40 -18.00     | 13.032 ± 2.195  | 14.40 - 48.00 | 29.952 ± 7.761 |
| Trachea cell           | 7.20 - 88.80    | 29.880 ± 3.130  | 9.60 - 124.80 | 3.308 ± 3.994  |
| Leaf                   |                 |                 |               |                |
| Cuticle                | 1.20 - 2.40     | 2.052 ± 0.470   |               |                |
| Adaxial epidermis cell | 12.00 - 28.80   | 18.936 ± 3.850  | 13.20 - 55.20 | 25.632 ± 7.772 |
| Abaxial epidermis cell | 10.80 - 33.60   | 18.552 ± 5.535  | 14.40 - 50.40 | 28.632 ± 8.851 |
| Mesophyll region       | 175.20 - 264.00 | 212.160 ± 3.200 |               |                |
| Palisade region        | 96.00 - 170.40  | 132.672 ± 2.495 |               |                |
| Spongy region          | 57.60 - 100.80  | 79.540 ± 1.952  |               |                |
| Palisade cell          | 12.00 - 24.00   | 17.160 ± 2.669  | 31.20 - 76.80 | 51.792 ± 9.098 |
| Spongy cell            | 15.60 - 36.00   | 24.072 ± 4.110  |               |                |
| Petiole                |                 |                 |               |                |
| Adaxial epidermis cell | 12.50 - 31.25   | 19.749 ± 4.297  | 18.75 - 47.50 | 27.150 ± 6.082 |
| Abaxial epidermis cell | 12.50 - 21.25   | 16.500 ± 2.187  | 17.50 - 40.00 | 27.400 ± 4.896 |
| Parenchyma cell        | 10.00 - 82.50   | 34.650 ± 2.714  | 13.75 - 90.00 | 47.725 ± 3.233 |
| Trachea cell           | 5.00 - 21.25    | 11.575 ± 3.959  | 7.50 - 35.00  | 15.500 ± 5.769 |

**Table 2.** Anatomical measurement of some characters of the stem, leaf, root and petiole of Salvia veneris.

x 14.4 - 50.4  $\mu$ m (Table 2). The dimensions of the palisade parenchyma cells are 12.0 - 24.0 x 31.2 - 76.8  $\mu$ m while those of the spongy cells are 15.6 - 36.0  $\mu$ m (Table 2). There is a collateral vascular bundle. There are sclerenchyma bundles over the phloem (Figure 16).

# **Palynology**

The pollen of *S. willeana* is hexazonocolpate, subprolate, semitectate and the ornamentation is bireticulate (Figure 24). When the anatomical features of the pollens are observed, it is seen that the dimensions of polar axis and equatorial axis are 37.5 - 52.5  $\mu m$  and 30 - 40  $\mu m$ , respectively. The ratio of P/E is 1.06 - 1.5  $\mu m$ . Colpus length is 25.0 - 47.5  $\mu m$ . Colpus width is 7.5 - 12.5  $\mu m$ . The exine thickness is 1.5 - 3.5  $\mu m$  and the intine thickness is 0.75 - 1.0  $\mu m$  (Table 3).

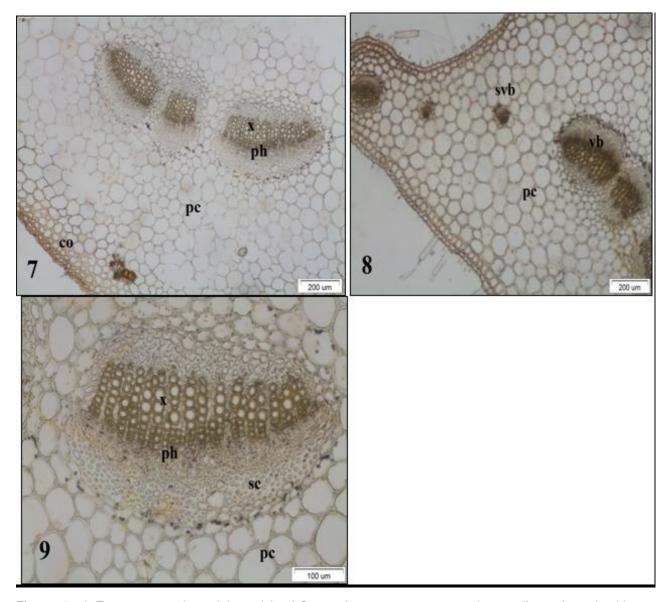
The pollen of *Salvia veneris* is hexazonocolpate, subprolate, semitectate and the ornamentation is bireticulate (Figures 20 - 25). The dimensions of polar axis and equatorial axis are 42.5 - 52.5 and 35 - 42.5  $\mu$ m, respectively. The ratio of P/E is 1.18 - 1.42  $\mu$ m.

Colpus length is 30.0 - 37.5 µm while its width is 7.5 - 12.5 µm. The exine thickness is 2.0 - 3.5 µm and the intine thickness is 0.75 - 1.0 µm (Table 3). In addition, the luminas of the secondary reticulum in the pollen samples of both taxa were counted and it was found that the number of the luminas of the secondary reticulum was fewer than 10 in S. willeana and more than 10 in S. veneris.

# DISCUSSION

Anatomical and palynological characteristics of *Salvia willeana* and *Salvia veneris* taxa endemic to Cyprus were investigated in this study and the findings obtained were compared with other studies conducted on *Salvia* genus.

Metcalfe and Chalk (1950) found the data on the anatomical characteristics of *S.* species. These researchers revealed that the species belonging to Labiatae family usually have rectangle or square stems. They also reported that there were collenchymatic tissues at the corners of the transverse section of the stem and that the



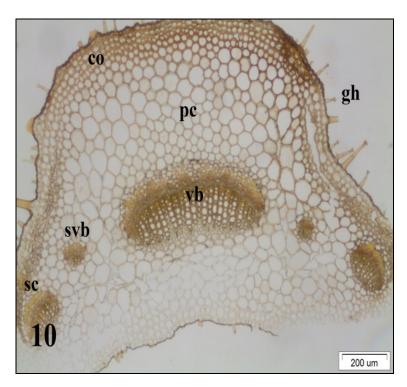
**Figures 7 - 9.** Transverse sections of the petiole of *S. veneris.* co, cortex; pc, parenchyma cell; x, xylem; ph, phloem; sbv, subsidiary vascular bundle; vb, vascular bundle; gh, glandular hair; sc, sclerenchyma.

pith rays in the root had 2 - 12 or more cell layers.

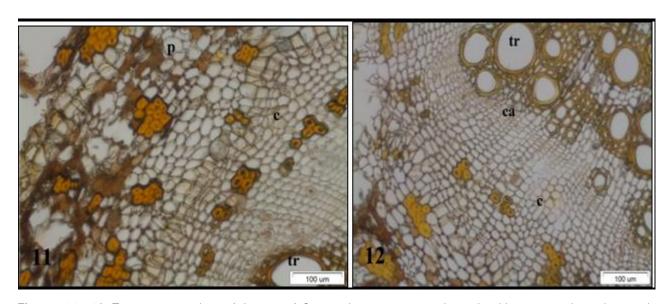
It has been found that pith rays of *S. willeana* and *S. veneris* are composed of 2 - 3 layers of cells. In a study in which anatomical features of *Salvia blepharoclaena* were investigated (Ozkan and Soy, 2007), it was found that the pith rays contained 1-2 layers of cells. In another study on *Salvia napifolia*, it was found that the pith rays were 2-6 layered (Baran and Ozdemir, 2006).

In the transverse sections of the stems of *S. willeana* and *S. veneris*, there was an undulate cuticle over the epidermis. Kahraman et al. (2009a) determined an undulate cuticle in *Salvia staminea*. It has also been determined that the stems of *S. willeana* and *S. veneris* are four angled and there are

well-developed lamellar collenchyma and angular collenchyma placed at the corners. Moreover, there are large vascular bundles at the corners and smaller ones in the space between the corners. In another study, it was reported that there was a well-developed collenchymatic tissue at the corners of the stem of *S. napifolia* (Baran and Ozdemir, 2006). Just beneath the epidermis between the corners in the transverse sections of the stems of *S. veneris*, a 6 - 7-rowed clorenchyma tissue containing lots of chloroplast was found (Figures 1 - 6). Kahraman et al. (2009b) performed a study on *Salvia indica* and found 3 - 5-rowed clorenchyma cells beneath the epidermis cells between the corners in the stem. In another study, it was found that there were 1 - 3 layered clorenchyma cells in *Salvia glutinosa* and 2 - 4-



**Figure 10.** Transverse sections of the petiole of *S. willeana*. co, cortex; pc, parenchyma cell; x, xylem; ph, phloem; sbv, subsidiary vascular bundle; vb, vascular bundle; gh, glandular hair; sc, sclerenchyma.

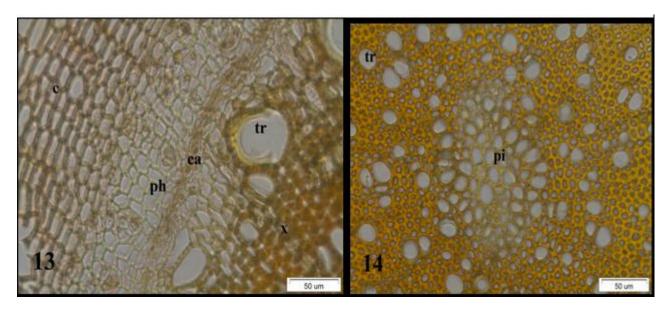


**Figures 11 - 12.** Transverse sections of the root of *S. veneris.* c, cortex; x, xylem; ph, phloem; sc, sclerenchyma; pi, pith region; ca, cambium; tr, trachea.

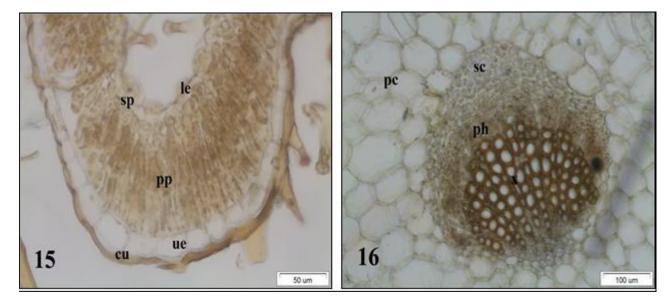
layered clorenchyma cells in *S. staminea* (Kahraman et al., 2009a). The findings of our study are consistent with those of the literature. However, this layer was not observed in *S. willeana*.

There are more groups of sclerenchyma above the

phloem in the transverse sections of the stems of *S. veneris* than in those of *S. willeana*. There are larger sclerenchyma groups above the phloem in the stem of *S. napifolia* (Baran and Ozdemir, 2006). In another study, it was reported that there were sclerenchyma groups



**Figures 13 - 14.** Transverse sections of the root of *S. willeana*. c, cortex; x, xylem; ph, phloem; sc, sclerenchyma; pi, pith region; ca, cambium; tr, trachea.



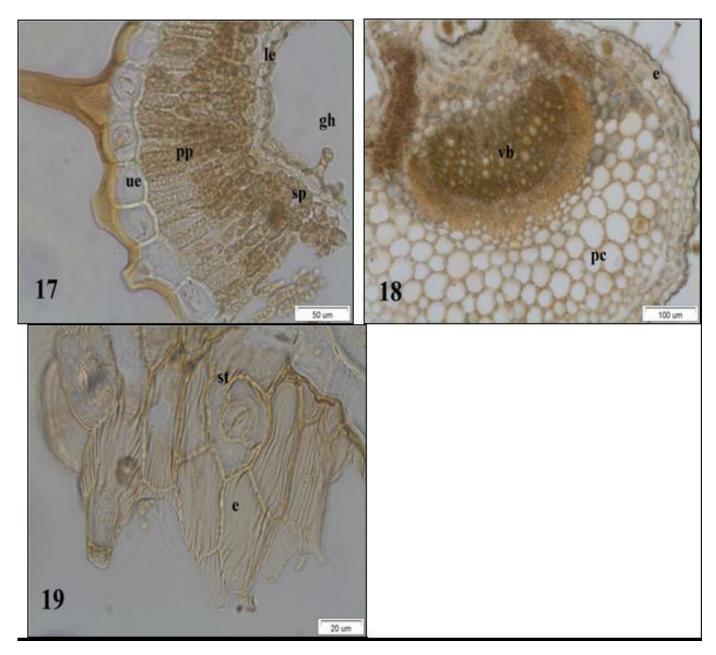
**Figures 15 - 16.** Transverse and surface sections of the leaf of *S. veneris*. cu, cuticle; ue, upper epidermis; le, lower epidermis; pp, palisade parenchyma; sp, spongy parenchyma; e, epidermis; gh, glandular hair; st, stomata; vb, vascular bundle; pc, parenchyma cell; sc, sclerenchyma; ph, phloem; x, xylem.

and one sclerenchyma ring above the phloem in the herbaceous stem of *S. hypargeia* (Kandemir 2003).

The cambium located between the phloem and the xylem was 2 - 3 layered and distinct in some Salvia species, but indistinct in some other Salvia species (Çobanoğlu 1988; Özdemir and Şenel 2001). There is a 2 or 3-layered cambium between the phloem and the xylem in the stems of S. willeana and S. veneris (Figure 6). Baran and Ozdemir, (2006) reported that the cambium layer was not distinct in Salvia

napifolia'da. Kahraman et al. (2009a) reported that the cambium layer was not distinct in *S. glutinosa and S. staminea*.

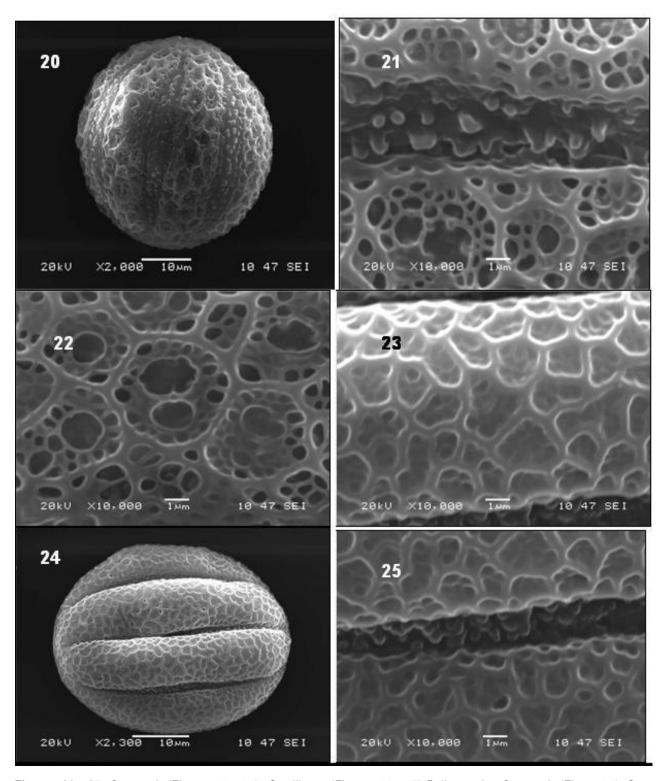
The arrangement of vascular bundles in the petioles of Labiatae species is of great importance (Metcalfe and Chalk 1950; Özdemir and Şenel, 1999; Kandemir 2003). In several studies conducted on the anatomical structure of *Salvia*, it has been determined that vascular bundles in the petiole differ from one species to another (Cirig and Seçmen, 1990). In a study, it was



**Figures 17 - 19.** Transverse and surface sections of the leaf of *S.willeana*. cu, cuticle; ue, upper epidermis; le, lower epidermis; pp, palisade parenchyma; sp, spongy parenchyma; e, epidermis; gh, glandular hair; st, stomata; vb, vascular bundle; pc, parenchyma cell; sc, sclerenchyma; ph, phloem; x, xylem.

Table 3. Palynological parameters of Salvia willeana and Salvia veneris.

| Damanatana           | Salvia willeana |                | Savia veneris |                |
|----------------------|-----------------|----------------|---------------|----------------|
| Parameters           | Min – Max       | Mean ± S.E     | Min – Max     | Mean ± S.E.    |
| Exine thicknes (µm)  | 1.50 - 3.50     | 2.430 ± 0.076  | 2.00 - 3.50   | 2.840 ± 0.054  |
| Intine thicknes (µm) | 0.75 - 1.00     | 0.925 ± 0.016  | 0.75 - 1.00   | 0.915 ± 0.017  |
| Colpus length (µm)   | 25.00 - 47.50   | 31.050 ± 0.471 | 30.00 - 37.50 | 33.750 ± 0.274 |
| Colpus width (µm)    | 7.50 - 12.50    | 10.175 ± 0.208 | 7.50 -12.50   | 9.525 ± 0.137  |
| Equatorial axis      | 30.00 - 40.00   | 34.975 ± 0.316 | 35.00 - 42.50 | 37.200 ± 0.263 |
| Polar axis (P)       | 37.50 - 52.50   | 45.850 ± 0.492 | 42.50 - 52.50 | 48.200 ± 0.364 |
| P/E ratio            | 1.06 - 1.50     | 1.314 ± 0.014  | 1.18 - 1.42   | 1.296 ± 0.008  |



**Figures 20 - 25.** *S. veneris* (Figures 20 - 21), *S. willeana* (Figures 24 - 25) Pollen grains. *S. veneris* (Figure 22), *S. willeana* (Figure 23) Sculpturing of pollen grain. SEM.

observed that there were 1, 2 or 3 central vascular bundles in the  $S.\ napifolia$  and 1 - 3 smaller bundles at the edge of the petiole (Baran and Ozdemir, 2006). In

another study conducted on *Salvia halophila*, it was found that six vascular bundles were located in the center of the petiole and 2 - 3 bundles at the left and

right corners (Kaya et al., 2007).

Kahraman et al. (2009a) reported that there was one vascular bundle in the center of the petiole of the *S. glutinosa* and 3 - 4 smaller bundles at the corners *and* one large vascular bundle in the center of the petiole of the *S. staminea* and 2-3 smaller bundles at one corner and two smaller bundles at another corner. In our observations, we found one large vascular bundle in the center of the petiole of the *S. willeana* and 2 smaller bundles at each corner (Figure 10). The arrangement of vascular bundles in the petiole of the *S. veneris* is completely different: a 2-3 piece vascular bundle in the center and three smaller vascular bundles at each corner (Figures 7 - 9).

Salvia has bifacial leaves and the stomas are diacytic (Metcalfe and Chalk 1972). S. willeana and S. veneris species have bifacial leaves too and the stomas are diacytic in both taxa (Figure 17). Salvia sclarea species also has bifacial leaves and the stoma is diacytic (Özdemir and Şenel, 1999). In another study, it was found that S. blepharoclaena has isolateral leaves and the stoma is diacytic (Özkan and Soy, 2007).

The mesophyll region in the Salvia species is completely composed of parancimatic cells and there are collenchymatic cells both beneath and above the midrib (Metcalfe and Chalk, 1972). The mesophyll tissue of S. willeana and S. veneris is composed of palisade and sponge layers. There is a collenchymatic tissue in the midrib too. The structure of the vascular bundles in the leaf anatomy of Salvia species can play a key role in distinguishing the species. There is one large, lobed bundle in the midrib of S. indica. Ozkan and Soy (2007) investigated S. blepharoclaena and reported that there was a single bundle in the center of the midrib and two smaller bundles at the sides. Kaya et al. (2007) observed two large bundles in the center in S. halophila. In the transverse section of the leaf of S. veneris, sometimes a large one-lobed vascular bundle and sometimes two-lobed vascular bundles were observed. In the midrib of the S. willeana, there is one large vascular bundle. The bundles are collateral in both species and the cells beneath the xylem have narrow and thick walls. There are thick-walled cells beneath the xylem in *S. halophila* too.

Pollen characteristics are of taxonomical importance. These characteristics are used for the classification of Labiatae family (Erdtman, 1945). In general, pollen characteristics of Labiatae family, like pollen grains, are generally radially symmetric, isopolar, prolate-spheroidal, oblate-spheroidal or suboblate, subprolate. Most of them have 3 - 8 colpus. Tektum is generally reticulate (Perveen and Qaiser, 2003). The measurements performed on the different palynological characteristics of *S. veneris* and *S. willeana* are compared in details in Table 3. In the light of these measurements, when the pollens of *S. veneris* are investigated, it is seen

that the pollens are hexazonocolpate, subprolate, semitectate and the ornamentation is bireticulate. Palynological characteristics of *S. veneris* were identified by Yildiz et al. (2009) and it was found that their findings were consistent with those of ours. It was also observed that the pollens of *S. willeana* were hexazonocolpate, subprolate, semitectate and the ornamentation was bireticulate. However, the number of the lumina of the secondary reticule of *S. willeana* was fewer than ten whereas it was more than ten in *S. veneris*.

The ornamentation in *S. halophila* was bireticulateperforate (Kaya et al., 2007). It was reported that the pollens of *S. indica* were hexacolpate, radially symmetric and isopolar. The ornamentation was bireticulateperforate (Kahraman et. al., 2009b).

The measurements in *S. veneris* were as follows: Polar axis (P):  $48.200 \pm 0.364 \mu m$ , Equatorial axis (E):  $37.200 \pm 0.264 \mu m$ , P/E:  $1.296 \pm 0.008$ , Exine thickness: 2.840 ± 0.054 um. Intine thickness: 0.915 ± 0.016  $\mu$ m, Colpus length (Clg): 33.75  $\pm$  0.274  $\mu$ m, and Colpus width (Clt):  $9.525 \pm 0.138 \mu m$ . In a study conducted by Yildiz et al. (2009), the measurements of the pollens of S. veneris were found as follows: P:  $56.83 \pm 3.27 \,\mu\text{m}$ , and E:  $45.70 \pm 4.01 \,\mu\text{m}$ . In the same study, other measurements were as follows: exine thickness: 1.89 ± 0.21 µm, Colpus length (Clg): 39.41  $\pm$  3.28  $\mu$ m, Colpus width (Clt): 3.52  $\pm$  0.93  $\mu$ m. However, the measurements of *S. willeana*'da were as follows: Polar axis (P): 45.850 ± 0.493 µm, Equatorial axis (E):  $34.975 \pm 0.316 \, \mu m$ , P/E:  $1.314 \pm 0.015$ , Exine thickness:  $2.430 \pm 0.077 \mu m$ , Intine thickness:  $0.925 \pm$ 0.016  $\mu$ m, Colpus length (Clg): 31.050  $\pm$  0.471  $\mu$ m, and Colpus width (Clt):  $10.175 \pm 0.208 \mu m$ .

In a study by Kahraman et al (2009b), pollen characteristics of *S. indica* were researched and the measurements were reported as follows: P: 44.98  $\pm$  4.22 µm, E: 52.24  $\pm$  4.41 µm, P/E: 0.86, Colpus length (Clg): 39.02  $\pm$  3.49 µm ve Colpus width (Clt): 8.64  $\pm$  1.17 µm, Exine thickness: 1.49  $\pm$  0.10 µm and Intine thickness: 0.52  $\pm$  0.05 µm.

As a result, it is assumed that the findings of this study performed on the anatomical and palynological characteristics of *S.willeana* and *S. veneris* endemic to Cyprus will shed light to similar future studies and revision studies.

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