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# Anatomical, Micromorphological, Karyological and Biochemical study of *Scutellaria orientalis* subsp. *virens* and *Scutellaria salviifolia*

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## ABSTRACT

In this study, the anatomical and micromorphological structure, karyological characteristics and biochemical content of *Scutellaria orientalis* subsp. *virens* and endemic *Scutellaria salviifolia*, whose distributions areas overlap, were compared. Some anatomical and micromorphological differences were observed on the taxa; scleranchymatic pericycle layer on the stem, stomata density, distribution of trichomes, as well as the main vascular bundle and general shape of the petiole. The chromosome numbers of both taxa were determined as 2n = 22. However, there was a difference between chromosome length range and total chromosome length. The chromosome numbers and chromosome morphologies of these species have been defined for the first time in this paper. Differences in biochemical content were observed between species. Chlorophyll a (Chl a), total chlorophyll (Total Chl), total carbohydrate and malondialdehyde (MDA) contents were determined higher in leaf and stem samples of *S. orientalis* subsp. *virens* than *S. salviifolia*. There was no significant difference between the two taxa in terms of chlorophyll b (Chl b) content. Carotenoid (Car) content was detected higher in leaves samples of *S. orientalis* subsp. *virens*, but no significant difference was found between stems samples. Also, the effect of taxa on biochemical contents in relation to the habitat they live in is given in this study.

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# *Scutellaria orientalis* subsp. *virens* ve *Scutellaria salviifolia* üzerinde Anatomik, Mikromorfolojik, Karyolojik ve Biyokimyasal bir çalışma

## ABSTRACT

Bu çalışmada, yayılış alanları örtüşen *Scutellaria orientalis* subsp. *virens* ve endemik *Scutellaria salviifolia*'nın anatomik ve mikromorfolojik yapısı, karyolojik özellikleri ve biyokimyasal içeriği karşılaştırılmıştır. Taksonlarda bazı anatomik ve mikromorfolojik farklılıklar gözlenmiştir. Bunlar; gövdede skleranchymatik periskl tabakası, stoma yoğunluğu, trikomların dağılımı, petiyolün genel şekli ve ayrıca ana iletim demetinde birtakım farklılıklar şeklindedir. Her iki taksonun kromozom sayıları 2n=22 olarak belirlenmesine rağmen kromozom uzunluk aralığı ile toplam kromozom uzunluğu arasında fark görülmüştür. Bu türlerin kromozom sayıları ve kromozom morfolojileri ilk kez bu çalışmada tanımlanmıştır. Türler arasında biyokimyasal içerik farklılıkları da gözlenmiştir. *S. orientalis* subsp. *virens*'in yaprak ve gövde örneklerinde klorofil a (Chl a), toplam klorofil (Toplam Chl), toplam karbonhidrat ve malondialdehit (MDA) içeriğinin *S. salviifolia*'ya göre daha yüksek olduğu belirlenmiştir. Klorofil b (Chl b) içeriği açısından iki takson arasında önemli bir fark tespit edilmemiştir. *S. orientalis* subsp. *virens*'in yaprak örneklerinde karotenoid (Car) içeriği daha yüksek saptanmış, ancak gövde örnekleri arasında önemli bir fark bulunmamıştır. Çalışmada taksonların yaşadıkları habitata göre biyokimyasal içerikleri üzerindeki etkisi de verilmiştir.

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## INTRODUCTION

Lamiaceae is the third largest family in terms of number of taxa in Turkey. Lamiaceae family is represented by 48 genera and 782 taxa (603 species, 178 subspecies and varieties) in Türkiye. 44% of these taxa are endemic. In addition, the family has 23 hybrid species and 19 of them are endemic (%83). Studies of Turkey's Lamiaceae result has proven to be one of the centers of diversity in the Old World. In addition, in Turkey, there are about 10% of all species of Lamiaceae (Celep and Dirmenci, 2017). Being from the Lamiaceae family, the *Scutellaria* L. genus is one of the semi-cosmopolitan genera of the Lamiaceae family with its 471 species identified (Yilmaz, et al., 2020). Due to the suspicious species status of some taxa defined from the Soviet Union and China, in reality this number is close to 360 (Paton, 1990). The *Scutellaria* species usually grow in stony and rocky slopes in Turkey. The genus *Scutellaria* has 39 taxa (17 species, 23 subspecies, 2 varieties and 1 hybrid) were represented in Turkey (Güner et al., 2012, Yilmaz et al., 2020). 17 taxa are endemic (44%) in Turkey (Davis, 1988; Duman, 2000; Güner et al., 2012; Celep and Dirmenci, 2017).

Chlorophylls are the main pigments that drive photosynthesis by absorbing light and converting it into chemical energy (Agathokleous et al., 2020). Carotenoids absorb certain wavelengths of light energy in the photosynthetic system and then transfer it to the chlorophyll molecule and thus contribute to the photosynthesis process. In addition, according to some researchers, carotenoids prevent the breakdown of chlorophyll (photo oxidation) in an environment with excessive light and oxygen and protect the plant against physiological tissue injuries caused by excessive light (Leiva-Ampuero et al., 2020; Zhang et al., 2020).

Carbohydrates are direct photosynthetic activity products and are structural building blocks as well as energy sources and metabolites. They act as a source of energy and provide carbon necessary to produce new tissue (Trouvelot et al., 2014; Homayoonzadeh et al., 2020). Lipid peroxidation is the reaction that occurs in unsaturated fatty acids of cell membrane phospholipids. Products such as malondialdehyde resulting from lipid peroxidation event determine the severity of peroxidation (Abdelrahim et al., 2020; Álvarez-Robles et al., 2020).

The genus *Scutellaria* is taxonomically complex because it has many species and especially some taxa closely in morphologically. Therefore, anatomical findings, karyo-morphological analysis of taxa are important for the systematics. Also, the effect of taxa on biochemical contents in relation to the habitat they live in is given in this study. This study aims to reveal the anatomical structure and karyomorphological features of *S. orientalis* L. subsp. *virens* (Boiss. & Kotschy) J.R. Edm. and *S. salviifolia* Benth. taxa whose overlapping areas of distribution, and to improve the knowledge on their biochemical content. Thus, the results obtained from this study will provide data for future studies.

## MATERIAL and METHOD

*Scutellaria* taxa, the study material, were collected from their natural habitats in the field. The localities of taxa are given in Table 1 and Figure 1. The collected samples have been turned into herbarium material and are kept in the herbarium of Munzur University.

Table 1. The localities and collector number of taxa
*Çizelge 1. Taksonların lokaliteleri ve numaraları*

| Taxon | Locality |
|---|---|
| *S. orientalis* subsp. *virens* | B7 Tunceli: Between Tunceli center and Ovacık, roadsides, 1000 m, May 2020, MA 2000 |
| *S. salviifolia* | B7 Tunceli: Between Tunceli center and Ovacık, Aktuluk neighborhood, the roadsides May 2020, 950 m, MA 2001 |

Anatomical studies was made on samples kept in 70% ethyl alcohol. And performed on at least two individuals to represent the population for each taxon. Anatomical sections were carried out manually. In order to better distinguish the tissues and cells were stained with safranin-fastgreen. After, it was made into a permanent preparation with entellan (Tardif and Conciatori, 2015). Anatomical examinations were made under an Olympus BX53 microscope. Nutlet was examined in SEM (JCM-5000) and microphotographs were taken.

Nutlets of the studied taxa were collected from their

natural habitats for karyological analysis. In order to obtain somatic chromosomes, the germination environment was provided by sowing in an oven at 22 ° C. After the seed germinated, the cut root tips were kept in colchicine solution for 2 hours at room temperature (Elçi, 1982; Gedik et al., 2014). Root tips kept in colchicine solution for 2 hours were taken from this solution and placed in Farmer's solution (3: 1). Root tips were fixed in acetic alcohol at +4 ° C in a refrigerator for 24 hours. At the end of 24 hours, the root tips were hydrolyzed in 1N HCl for 8-10 minutes in an oven set at 60 ° C. After the hydrolysis process was completed, the root tips were dyed with feulgen dye for 1 hour at 22 °C in a dark environment. The meristems taken for chromosome examinations were broken up in a drop of aceto orcein dye with a sharp razor blade and the coverslip was closed (Levan et al., 1964). Levan's naming system was used in determining the location of the centromere (Levan et al., 1964). Intra-chromosomal asymmetric index (A1) and inter-chromosomal asymmetric index (A2) were calculated according to Zarco (1986).


Figure 1. General view of taxa. A,B- *Scutellaria orientalis* subsp. *virens* C,D- *S. salviifolia*
*Şekil 1. Taksonların genel görünümü. A,B- Scutellaria orientalis subsp. virens C,D- S. salviifolia*

### Biochemical analysis

Plant parts were stored at -80° C until analysis. Pigment analysis, total carbohydrate and lipid peroxidation analysis were determined.

### Photosynthetic Pigment Analysis

Method suggested by De-Kok and Graham (1980) was used for pigment analysis. 0.5 gr of each leaf and stem samples ground in the blender were taken for extraction in 3 replicates for each sample and placed in a glass mortar and homogenized in 25 ml of acetone. They were homogenized in a shaking oven for 30 minutes. These samples were then stored for 24 hours at 4 ° C. Then samples were filtered and 1/5 water was added. Samples were centrifuged at 3000 rpm for 10 minutes. The absorbance values of samples were read at 470 nm, 645nm and 662 nm according to Lichtenthaler and Welburn (1983) and Chl-a, Chl-b, Car and Total Chl contents were determined.

### Total Carbohydrate Content

The total carbohydrate content was assayed according to Rosenberg (1980). Anthrone method was used for colorimetric method of determining the concentration of the total carbohydrate. Absorbance was measured at 620 nm (Shimadzu UV-1201V). Glucose values have been calculated corresponding to the standard values entered on the computer in the Slide program.

### Lipid Peroxidation Analysis

The method was done according to Heath and Packer (1968). 0.5 gram of leaf tissue was homogenized in 5 mL of 0.1% TCA. The homogenate was centrifuged at 10,000 g for 10 minutes. 2 mL of 0.5% TBA (prepared in 20% TCA) was added to 2 mL of this solution and kept in a water bath at 95 ° C for 30 min. than samples were centrifuged at 10,000 gram for 15 min again. MDA content was calculated at 532 nm and 600 nm.

### Statistical Analysis

Statistical evaluation of the obtained data was made using the SPSS 21.0 program. In this program, variance analysis was performed and Duncan test (Duncan, 1955) was applied within the scope of significance test.

## RESULTS AND DISCUSSION

### Stem anatomy and micromorphology

The stem anatomical structure of taxa shows the general characteristics of the family. The stem has 4 corners and vascular bundles at the corners are developed (Figure 2-3). In addition, developed collenchyma layer was seen in the corners. *S.*

*orientalis* subsp. *virens*, scleranchymatic pericycle surround partly the stem, whereas in *S. salviifolia* there is no scleranchymatic pericycle layer. Endodermis cannot be distinguished from parenchyma tissue completely. Between the corners, parenchyma tissue in *S. salviifolia* occupies relatively more area. Although the collenchymatic hypodermis layer is seen between the corners in both taxa, it was seen more in *S. orientalis* subsp. *virens*. In addition, although vascular cambium was observed in *S. salviifolia*, it is not obvious in *S. orientalis* subsp. *virens*. Non-glandular trichomes and glandular trichomes have seen in both taxa; Non-glandular trichomes have been observed as a multicellular with micropapillae and developed cell wall. Non-glandular trichomes cell wall thickness more in *S. orientalis* subsp. *virens*. The glandular trichomes are; both taxa were observed as Labiatae type (peltate) and capitate type glandular trichome. Capitate glandular trichomes are of three subtypes. Rounded head with a short stalk cell and a broad round head shape (A type) and with a neck or without neck structure and with 2 stalk cells and multicellular round head shape (B1 type) and long 2-3 cell stalk and with neck or without neck structure and rounded head shape (B2 type). Although these two types of glandular trichomes are observed in taxa, relatively long capitate (B1 type) is more dense in *S. salviifolia*. Short large headed capitate (A type) was more intense in *S. orientalis* subsp. *virens*. Besides, in the stem structure, B2 type only observed in *S. orientalis* subsp. *virens*. Only in *S. salviifolia,* a cup-shaped head structure was commonly seen after releasing secretion B1 type capitate trichomes. However, this head cell shape was not seen in *S. orientalis* subsp. *virens*.

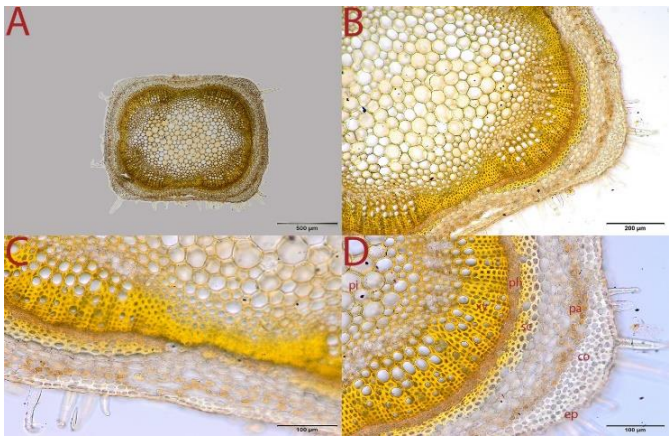


Figure 2. The anatomical structure of the stem *S. orientalis* subsp. *virens*. ep: epidermis, co: collenchyma, pa: parenchyma, sc: sclerenchyma, ph: phloem, xy: xylem, pi: pith

*Şekil 2. S. orientalis subsp. Virens' in kök anatomisi. co: kollenkima, pa: parankima, sc: skleranchima, ph: floem, xy: ksilem, pi: öz*

## Leaf anatomy and micromorphology

Leaf anatomical structure of taxa differs more than stem anatomical structure. Generally, in both taxa midrib has developed and swollen in abaxial direction


Figure 3. The anatomical structure of the stem *S. salviifolia* e: epidermis, co: collenchyma, pa: parenchyma, ph: phloem, xy: xylem

*Şekil 3. S. salviifolia' nın kök anatomisi. co: kollenkima, pa: parankima, sc: skleranchima, ph: floem, xy: ksilem, pi: öz*

(Figure 4-5). Midrib area; it is arranged as, below the upper epidermis, collenchyma, below it parenchyma, xylem, phloem, parenchyma, again collenchyma and lower epidermis. Collenchyma layer is less developed in *S. salviifolia*. In both taxa, the mesophyll consists of only the palisade parenchyma, while in *S. orientalis* subsp. *virens* it consists of 4-5 rows and the parenchyma cells in the abaxial direction can be a little more oval. In *S. salviifolia*, there are 4 rows of palisade parenchyma. While the epidermis is mostly rectangular shape in *S. orientalis* subsp. *virens*, it is more oval shape in *S. salviifolia*. In addition, stomata are located on both the upper and lower surfaces in both taxa, while it is dense on the upper surface in *S. orientalis* subsp. *virens*, while it is dense on the lower surface in *S. salviifolia* While in *S. orientalis* subsp. *virens* the leaf bottom surface is densely covered with non-glandular trichome, it is not so in *S. salviifolia*. Both non-glandular trichome and glandular trichome were observed in both taxa, and the non-glandular trichome is denser in *S. orientalis* subsp. *virens* and the glandular trichome is denser in *S. salviifolia*. In addition, B2 type has been observed only in *S. salviifolia* and P type is quite intense.In addition, while the non-glandular trichomes are curved in *S. orientalis* subsp. *virens*, they are relatively not curved in *S. salviifolia*.

## Petiole anatomy and micromorphology

The petiole is flattened in the adaxial direction in *S. orientalis* subsp. *virens*. In *S. salviifolia*, it is hollowed in the adaxial direction and swollen and ribbed from

the edges. There are 3 vascular bundles in both taxa. One is in the middle and the other two are on the edges. The middle vein is developed. Both taxa are in arc shape and consist of almost two lobes in *S. salviifolia*. Chlorenchymatic cells were seen in the corners (Figure 6-7).

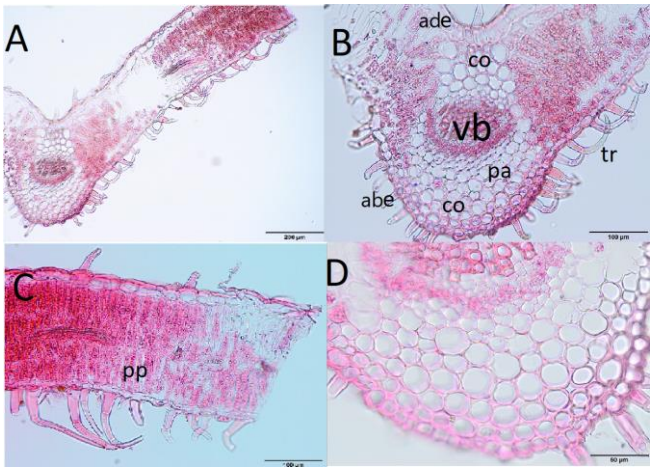


Figure 4. *S. orientalis* subsp. *virens*, anatomical structure of the leaf ade: adaxial epidermis, abe: abaxial epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

*Şekil 4. S. orientalis subsp. Virens yaprağının anatomik yapısı. ade: adaksiyel epidermis, abe: abaksiyel epidermis, co: kollenkima, vb: vasküler demet, pa: parankima, pp: palisat parankiması, tr: trikom*

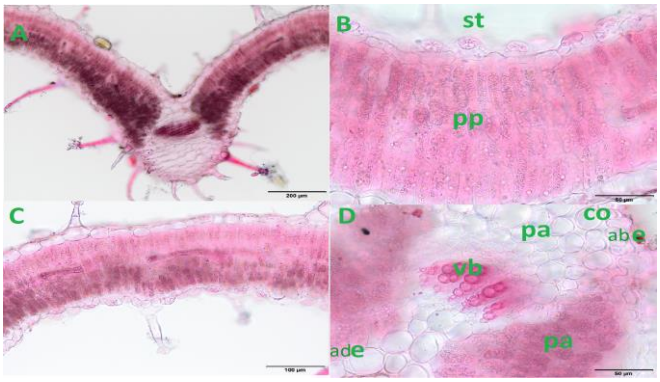


Figure 5. S.*salviifolia* , anatomical structure of the leaf ade: adaxial epidermis, abe: abaxial epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

*Şekil 5. S.salviifolia yaprağının anatomik yapısı. ade: adaksiyel epidermis, abe: abaksiyel epidermis, co: kollenkima, vb: vasküler demet, pa: parankima, pp: palisat parankiması, tr: trikom*

Vascular bundles in the corners are surrounded by a parenchymatic sheath. Both non-glandular and glandular trichomes were observed in both taxa, while A, P, B1 and B2 types were observed in *S. orientalis* subsp. *virens*; A, B1 and B2 types were observed in *S. salviifolia* and P type was not observed (Figure 8).

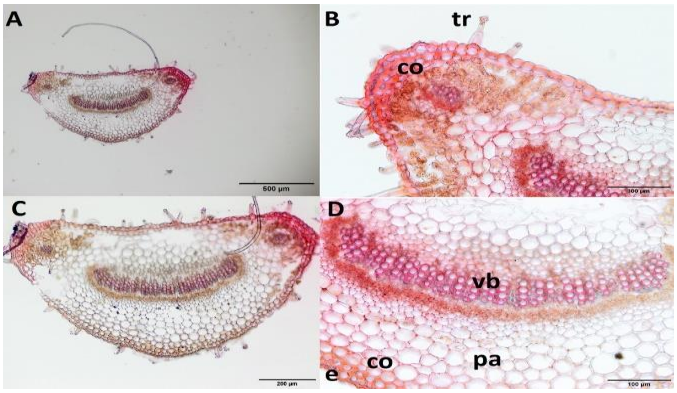


Figure 6. S. *orientalis* subsp. *virens,* anatomical structure of petiole, A and C- General view, B-, D- Middle vein, e: epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

*Şekil 6. S. orientalis subsp. Virens. Yaprark sapının anatomik yapısı. Ave C genel görünüş, B-, D* köşe görünümü

e: epidermis, co: kollenkima, vb: vasküler damar, pa: parankima, pp: palizet parankiması, tr: trikıom

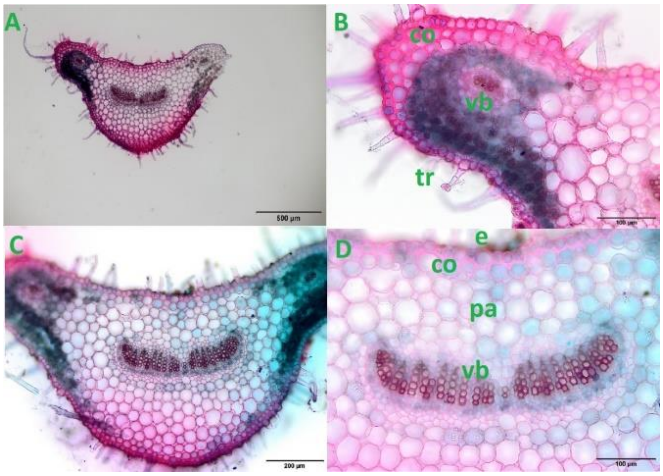


Figure 7. *S. salviifolia,* anatomical structure of petiole, A and C- General view, B- Corner view, D- Middle vein, e: epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

*Şekil 7. S. salviifolia. Yaprark sapının anatomik yapısı. Ave C genel görünüş, B-, D* köşe görünümü

e: epidermis, co: kollenkima, vb: vasküler damar, pa: parankima, pp: palizet parankiması, tr: trikom

## Nutlet Structure

The nutlet surface structure of taxa could not be observed since it is covered with dense trichomes. However, the non-glandular trichomes differ from each other. In *S. orientalis* subsp. *virens*, surface completely covered with ashy hairlets. In *S. orientalis* subsp *virens*, there are curved non-glandular trichomes; It is flat in *S. salviifolia*. Nutlet shape is obovate in both taxa.

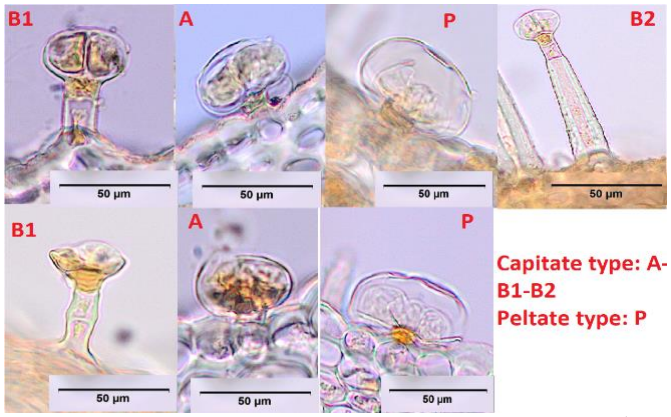


Figure 8. Glandular trichome structure of taxa (top; *S. orientalis* subsp. *virens,* under; *S. salviifolia*

*Şekil 8. Taksonların glandüler trikom yapısı (üstte; S. orientalis* subsp. *virens,* altta; *S. salviifolia)*

Karyomorphological finding are given in Figure 9-10 and Table 2-3.

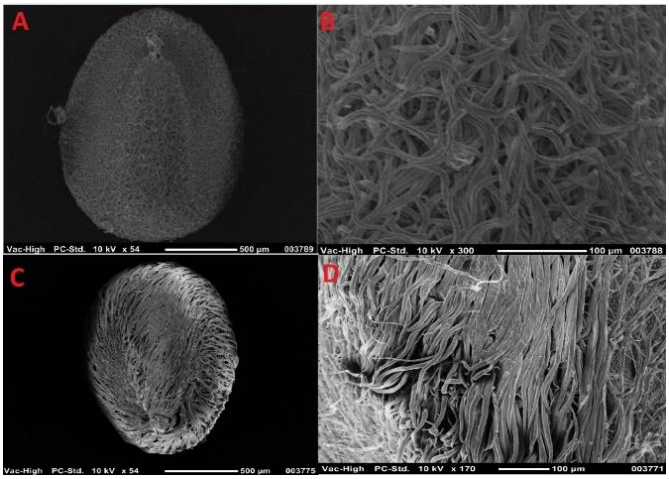


Figure 9. A and C- General view of *S. orientalis* subsp. *virens* and *S. salviifolia*, B and D- Nutlet surface of *S. orientalis* subsp. *virens* and *S. salviifolia*

*Şekil 9. A ve C- S. orientalis* subsp. *virens ve S. salviifolia' nın genel görünüşü. B ve D S. orientalis* subsp. *virens ve S. salviifolia' nın nutlet yüzeyi*

## Karyomorphological Findings

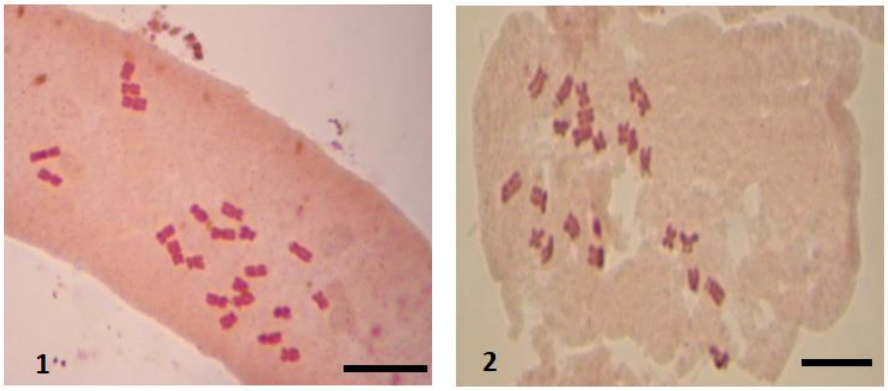


Figure 11. Metaphase chromosomes belonging to the taxa studied; 1. S. orientalis subsp. virens 2. S. salviifolia (scale bar 10 µm)

*Şekil 11. Incelenen taksonlara ait metafaz kromozomları; 1. S. orientalis subsp. virens 2. S. salviifolia* (scale bar 10 µm)

Table 2. Somatic chromosome number, polyploid level, karyotype formula, chromosome length range, total chromosome length (TKL) and asymmetric index (A1, A2) of the examined taxa.

*Çizelge 2. İncelenen taksonların somatik kromozom sayısı, poliploid düzeyi, karyotip formülü, kromozom uzunluk aralığı, toplam kromozom uzunluğu ve asimetrik indeks (A1, A2)*

| Taxon | 2n | Polyploid level | Karyotype formula | Chromosome length range (µm) | TKL (µm) | A1 | A2 |
|---|---|---|---|---|---|---|---|
| *S. orientalis* subsp. *virens* | 22 | 2x | 1M+10m | 2.18-3.72 | 30.50 | 3.6 | 2.77 |
| *S. salviifolia* | 22 | 2x | 2M+ 2sm+8m | 2.38-3.65 | 34.24 | 15.8 | 3.11 |

## Pigmentation Results

As analysis of pigment was evaluated in *S. salviifolia*, Chl a content in leaves and stems samples were determined respectively 6.45 µg $g^{-1}$ and 2.32 µg $g^{-1}$.

Chl b content was found 2.60 µg $g^{-1}$ in the leaves and 0.45 µg $g^{-1}$ in the stems. Total Chl content in leaves and stems samples were measured as 9.05 µg $g^{-1}$ and 2.76 µg $g^{-1}$ respectively. Car content was found as 0.71 µg $g^{-1}$ in leaves and 0.44 µg $g^{-1}$ in stems samples. When the Chl a content in leaves and stem samples in *S. orientalis* subsp. *virens* were determined respectively 7.37 µg $g^{-1}$ and 2.54 µg $g^{-1}$. The concentration of Chl b was contained in leaves at 2.58 µg $g^{-1}$ and stems at 0.48 µg $g^{-1}$. Total Chl content was calculated in leaves and stems samples as 9.95 µg $g^{-}$ and 3.01 µg $g^{-1}$ respectively. Car concentrations were detected on the leaves as 0.82 µg $g^{-1}$ and on the stems as 0.48 µg $g^{-1}$. When we compared the Chl a content in *S. salviifolia* and *S. orientalis* subsp. *virens*, the Chl a and Total Chl contents were determined higher in the leaves and stems samples of *S. orientalis* subsp. *virens*. There was no significant difference between the two plants in terms of Chl b content. When the Car content was evaluated, it was determined higher in leaves samples of *S. orientalis* subsp. *virens*, but no significant difference was found between stems patterns ($p < 0.05$) (Figure 12).

Table 3. Karyomorphological parameters of the examined taxa: (NB: Relative length, L / S: arm ratio, CI: centromere index, SD: Centromere status,M:median point, m: median, sm: submedian)

*Çizelge 3. Incelenen taksonların karyomorfolojik parametreleri: (NB: nispi boy, L / S: kol oranı, CI: sentromer indeksi, , SD: sentromer durumu, M:noktalı medyan, m: medyan, sm: submedyan)*

| ***S. orintalis* subsp. *virens*** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Haploid** | **Total Lenght** | **Long Arm L** | **Small Arm S** | **Arm Ratio (AR)** | **Centromere İndex İ=100*(S/C)** | **Relative Length N.P.** | **Centromere Status S.D** |
| 1 | 3.72 | 1.90 | 1.82 | 1.04 | 48.92 | 12.20 | m |
| 2 | 3.38 | 1.78 | 1.60 | 1.11 | 47.34 | 11.08 | m |
| 3 | 3.12 | 1.60 | 1.52 | 1.05 | 48.72 | 10.23 | m |
| 4 | 3.05 | 1.58 | 1.47 | 1.07 | 48.20 | 10.00 | m |
| 5 | 2.91 | 1.50 | 1.41 | 1.06 | 48.45 | 9.54 | m |
| 6 | 2.85 | 1.47 | 1.38 | 1.07 | 48.42 | 9.34 | m |
| 7 | 2.75 | 1.40 | 1.35 | 1.04 | 49.09 | 9.02 | m |
| 8 | 2.47 | 1.25 | 1.22 | 1.02 | 49.39 | 8.10 | m |
| 9 | 2.18 | 1.05 | 1.13 | 0.93 | 51.83 | 7.15 | m |
| 10 | 2.04 | 1.02 | 1.02 | 1.00 | 50.00 | 6.69 | M |
| 11 | 2.03 | 1.03 | 1.00 | 1.03 | 49.26 | 6.66 | m |
| ***S. salviifolia*** | | | | | | | |
| **Haploid** | **Total Lenght** | **Long Arm L** | **Small Arm S** | **Arm Ratio (AR)** | **Centromere İndex İ=100*(S/C)** | **Relative Length N.P.** | **Centromere Status S.D** |
| 1 | 3.65 | 2.40 | 1.25 | 1.92 | 34.25 | 10.66 | sm |
| 2 | 3.50 | 2.28 | 1.22 | 1.87 | 34.86 | 10.22 | sm |
| 3 | 3.95 | 2.15 | 1.80 | 1.19 | 45.57 | 11.54 | m |
| 4 | 3.25 | 1.92 | 1.33 | 1.44 | 40.92 | 9.49 | m |
| 5 | 3.72 | 1.86 | 1.86 | 1.00 | 50.00 | 10.86 | M |
| 6 | 3.18 | 1.63 | 1.55 | 1.05 | 48.74 | 9.29 | m |
| 7 | 2.91 | 1.45 | 1.46 | 0.99 | 50.17 | 8.50 | m |
| 8 | 2.74 | 1.37 | 1.37 | 1.00 | 50.00 | 8.00 | M |
| 9 | 2.53 | 1.32 | 1.21 | 1.09 | 47.83 | 7.39 | m |
| 10 | 2.43 | 1.28 | 1.15 | 1.11 | 47.33 | 7.10 | m |
| 11 | 2.38 | 1.25 | 1.13 | 1.11 | 47.48 | 6.95 | m |

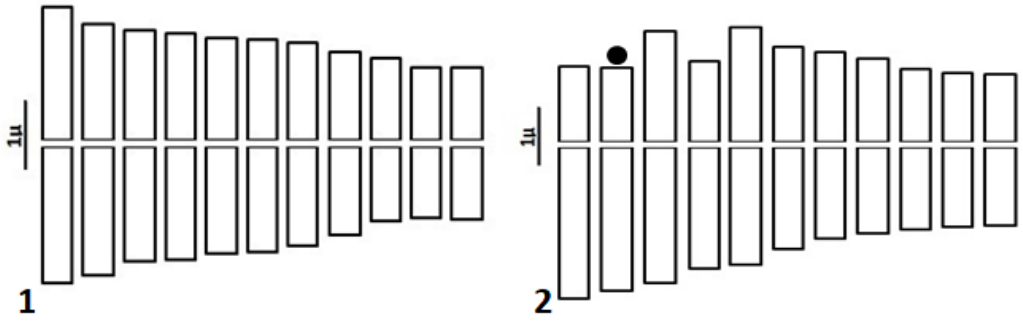


Figure 12. Haploid idiograms belonging to the taxa studied; 1. *S. orientalis* subsp. *virens* 2. *S. salviifolia*
*Şekil 12. Incelenen taksonlara ait haploid idiyogramlar; 1. S. orientalis subsp. virens 2. S. salviifolia*

## Total Carbohydrate Results

The total carbohydrate content of *S. salviifolia* plant was determined as 1.27 µg $g^{-1}$ and 0.55 µg $g^{-1}$, respectively, in leaves and stems samples. The total carbohydrate content of *S. orientalis* subsp. *virens* leaf and stem samples was found to be 1.46 µg $g^{-1}$ and 0.69 µg $g^{-1}$ respectively. When we compared the total carbohydrate content in *S. salviifolia* and *S. orientalis* subsp. *virens*. Total carbohydrate content in *S. orientalis* subsp *virens* was found higher in leaves and stems samples. Statistically, these changes were determined significant ($p < 0.05$) (Figure 13).

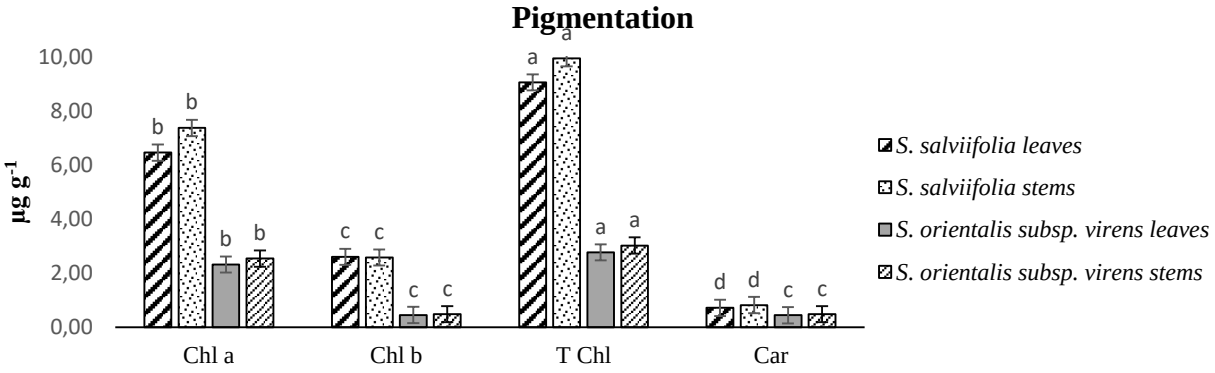


Figure 13. Changes in pigment contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

Şekil 13. *S. salviifolia* ve *S. orientalis* subsp. *virens*' in pigment içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı ($p < 0.05$),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)

## MDA Results

The MDA content of *S. salviifolia* plant was found as 2.55 µmol MDA/ g fresh weight (FW) and 1.07 µmol MDA/ g FW, respectively, in leaves and stem samples. The MDA content of *S. orientalis* subsp *virens* was determined as µmol MDA/ g FW in leaves and µmol MDA/ g fresh weight in stems samples. When we evaluated the MDA content in *S. salviifolia* and *S. orientalis* subsp. *virens*, MDAcontent in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples. Statistically, these changes were found to be significant ($p < 0.05$) (Figure 14).

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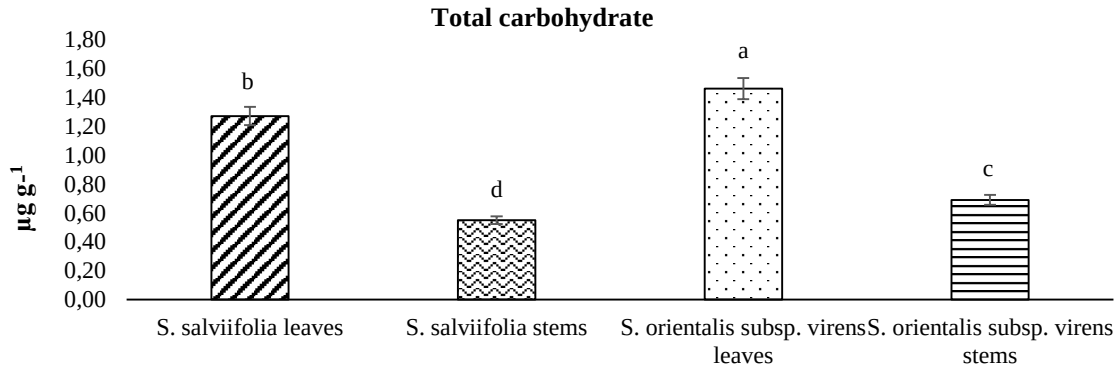


Figure 14. Changes in Total carbohydrate contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

*Şekil 14. S. salviifolia ve S. orientalis subsp. virens' deki toplam karbonhidrat içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı (p <0.05),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)*

In this study; the anatomical and karyological characteristics and biochemical content of the samples taken from *S. orientalis* subsp. *virens* and *S. salviifolia* belonging to the genus of *Scutellaria*, Lamiaceae family, which are frequently used in traditional medicine, pharmacology, cosmetics and food industry and are of great economic importance, were investigated.

Anatomical and micromorphological studies conducted on Lamiaceae family recently can provide useful characters in revealing their similarities and differences in distinguishing taxa (Açar and Satıl, 2019; Ecevit-Genç et al., 2018; Polat et al., 2017; Kaya et al., 2013; Selvi et al., 2013; Satıl and Kaya, 2007; Satıl et al., 2011).

The vascular structure of the petiole carries a taxonomic character. In the cross sections, the middle vein curves in the form of a half-moon or it creates an annular structure by further curling; the arrangement of small bundles of vascular vessel or circular structure of small bundles; The number and sequence of small vascular bundles at the ends of the petiole (wing) are systematically important characters in identifying genera and species (Metcalfe and Chalk, 1950).

According to the study by Akçın et al. (2011), the *S. salviifolia* petiole gave similar results to the research we conducted, but the vascular bundle in the middle part in this study differs in that it consists of almost two parts and the absence of peltate-type hair. Çalı (2017) stated in his study that there were sclerenchymatic cells in the stem of *S. salviifolia*, but sclerenchyma was not found in this study. The fact that the mesophyll is made entirely of palisade is similar to the stoma being on both surfaces. In addition, the fact that the petiole main vascular bundle consists of one piece reveals its difference from in this study. In addition, type II C glandular hair seen only in calyx in his study was not observed in this leaf stem and petiole. Özdemir and Altan (2005) stated in their study that the vascular bundles in *Scutellaria orientalis* subsp. *santolinoides* and *S. orientalis* subsp. *bicolor* petioles were surrounded by scleranchymatic cells, but in this study, these scleranchymatic cells were not observed in the other subspecies of this species*, S. orientalis* subsp *virens*. In the stem, it has been stated that the vascular bundles are surrounded by scleranchymatic cells, and in the case it is seen that it is in partly form. It is also stated that the leaf mesophyll is bifacial, in the case it is unifacial. In *S. orientalis* subsp. *virens*, the leaf consists only of the palisade parenchyma. However, in some observations, it was also observed that the palisades on the lower surface were slightly more oval.

Karyological studies have been carried out on some species belonging to the genus *Scutellaria*. The chromosome number of *S. tomentosa* Bertol., *S. theobromina* Rech.f., *S. araxensis* Grossh., *S. platystegia* Juz., *S. nepetifolia* Benth*., S., S. persica* Bornm. and *S. pinnatifida* has been reported as 2n = 2x = 22 (Ranjbar and Mahmoudi, 2013) In another study, the chromosome number of endemic *S. orientalis* subsp*. bicolor* species was determined as 2n = 22 (Gedik et al., 2016). However, there is no information about the chromosome number and structure of *S. salviifolia* species, which is an endemic taxon.

The chromosome number of *S. salviifolia* species, which is an endemic species was determined as 2n = 22. It is seen that the total chromosome lengths of

this species vary between 2.38-3.65 µm and arm ratios between 1.11-1.92 µm. The karyotype formula is 7m + 2sm + 2M. The total chromosome length is 34.24 um. It has been determined that this species has a satellite on its second chromosome. The karyology of the *S. salviifolia* species was first determined in this study (Figure 11-12).

The chromosome number of *S. orientalis* subsp. *virens* was determined as 2n = 22. It is seen that the total chromosome lengths vary between 2.08-3.72 µm and arm ratios between 1.03-1.04 µm. The karyotype formula is 10m + 1M. Total chromosome length is 30.50 µm. The *S. orintalis* subsp. *virens* taxon was first discussed in terms of chromosome number and chromosome morphology in this study (Table 2).

Chlorophylls absorb light energy of certain wavelengths and either convert this energy into another wavelength used in photosynthesis or transfer it directly to the compounds required for photosynthesis. Also they act like a catholyzer in the stages of photosynthesis. The spectral distribution of light sources in crop production plays an important role in finding photomorphogenic reactions. It has been noted that the plant grows in direct proportion as the pigment system absorbs the sunlight (Amoozgar et al., 2017; Izzo et al., 2019). Carotenoids are not only one of the plant pigments but also important antioxidants that play a role in oxidative stress tolerance. There are studies showing that the carotenoid oxidation products as stress signals for plants (Berru et al., 2021; Xia et al., 2021). In this study, the contents of Chl a and Total Chl were higher in the leaves and stem samples of *S. orientalis* subsp. *virens*, There was no significant difference in the content of Chl b between the two plants. Car content was dound higher in the leaf samples of *S. orientalis* subsp. *virens*, but no significant difference was found between the patterns of stems. Statistically, it was observed that these changing were substantial ($p < 0.05$) (Figure 13).

The most important product of lipid peroxidation is MDA. It leads to binding of ion permeability and enzyme it causes negative consequences such as change of activity. Due to this feature of MDA, it can be used to protect DNA with nitrogen bases. React and hence mutagenic, cell genotoxic and carcinogenic for cultures (Hafez et al., 2020; Khoubnasabjafari and Jouyban, 2020; Nilsson and Liu, 2020). MDA content in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples than *S. salviifolia*. Statistically, it was observed that these changing were substantial ($p < 0.05$) (Figure 14).

Carbohydrates are organic compounds containing carbon, oxygen and hydrogen atoms in their structures. It is also stated that carbohydrates act as signal molecules similar to hormones (Shah et al., 2019; Smeekens et al., 2000). Total carbohydrate content in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples than *S. salviifolia*. Statistically, these changes have been determined to be significant ($p < 0.05$) (Figure15). Pigment, total carbohydrate and MDA contents are used as important markers in plants under stress conditions. The biochemical characteristics of the plant are important in the response and adaptation to stress in ecological conditions. In this study, it was determined that the biochemical composition of *S. orientalis* subsp. *virens* was found higher than that of *S. salviifolia*.

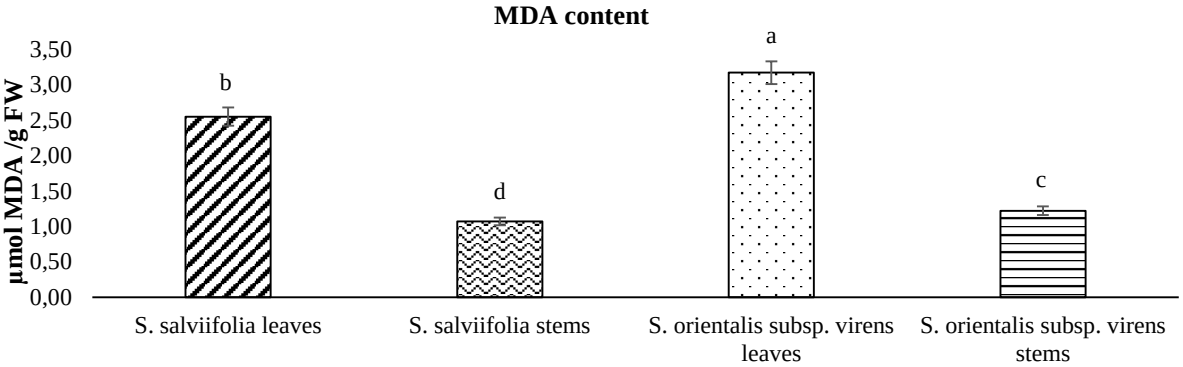


Figure 15. Changes in MDA contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

Şekil 15. *S. salviifolia* ve *S. orientalis* subsp. *virens*' de MDA içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı ($p < 0.05$),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)

## CONCLUSION

In this study, *S. orientalis* subsp. *virens* and endemic *S. salviifolia* species were investigated in terms of anatomical, karyological and biochemical content.

The anatomical structure of the species has been determined. Biochemical content has been described. The chromosome number and chromosome morphology of the species were first revealed. It was found that the chromosome number of both study samples was the same, but there was a difference in terms of chromosome structures and chromosome morphologies. The results increase the knowledge of these species. This information will contribute to the determination of the systematic location of the species and other biological researches.

## Author's Contributions

The contribution of the authors is equal.

## Statement of Conflict of Interest

Authors have declared no conflict of interest.

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