

Full Length Research Paper

An anatomical study of medicinal species *Ajuga orientalis* L. (Lamiaceae) from Turkey

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***Ajuga orientalis* (Lamiaceae), with a wide distribution area in Turkey, is a traditionally used medicine in the treatment of some skin diseases in Anatolia. The aim of this study was to determine the anatomical characteristics of the root, stem, leaf, petiole, calyx and corolla of medicinal species of *A. orientalis* in cross sections. As a result of the study, it was found out that the pith rays of the root composed 3 to 4 rowed cells and stem was quadrangular. The shape of pith cell in the stem was ovoidal-polygon. There were glandular and non-glandular hairs on the surface layers on stem, leaves, petiole, calyx and corolla. Starch particles were also detected in the cortex cells of stem. The stomata were diastatic and the leaf was bifacial. There were one big vascular bundle in the center and 4 to 5 small vascular bundles on each corner of the petiole. It was also determined that adaxial epidermis cell shapes of corolla are papillose type.**

Key words: *Ajuga orientalis*, anatomy, medicinal plant, Turkey.

INTRODUCTION

The Lamiaceae is a large family. Many species of Lamiaceae are shrubby and herbs (Heywood, 1978). The family has a cosmopolitan distribution. The Lamiaceae includes more than 250 genera and approximately 7000 species (Thorne, 1992). According to Başer (1993), Turkey is accepted as a gene center for this family. Many taxa of this family are aromatic and are often used as herb spices, folk medicines and fragrances (Werker et al., 1985). With their pleasant fragrance, many species of Lamiaceae have been used as herbal teas in Turkey. Many species are used as raw material in the cosmetic industry. Some species are traditionally used as medicinal plants (Baytop, 1984). It was reported that some *Ajuga* L. and *Salvia* L. species are cultivated as ornamental plants (Baytop, 1984; Özdemir and Şenel, 2001; Akçin et al., 2006). In addition to this, Lamiaceae has great importance due to its economical value and its variety of species.

The genus *Ajuga* belongs to Lamiaceae family and is

represented in Turkey by 13 species and 22 taxa, 6 species and 1 subspecies being endemic (Davis et al., 1982, (1982 - 1988)). *Ajuga* species are used in folk medicine in different parts of the world for the treatment of rheumatism, gout, asthma, diabetes, malaria, ulcers and diarrhea and have antibacterial, antitumor, anti-feedant, and vulnerary properties (Chen et al., 1996; Ben Jannet et al., 2000). Baytop (1999) reported that some *Ajuga* species generally known as "mayasıl otu" in Turkey have been widely used in the Turkish folk medicine for their aromatic, diuretic, antipyretic, tonic, diaphoretic, astringent, bitter and homeopathic properties. *Ajuga orientalis* one of the species of *Ajuga* genus is used against some skin diseases caused by hugging (Koyuncu et al., 2010).

Although many species of Lamiaceae family are investigated anatomically (Çobanoğlu, 1988; Uysal et al., 1991; Özdemir and Altan, 2005; Aktaş et al., 2009); there is no anatomical study of *A. orientalis* in literature. Due to its importance, the study was carried out to determine the

anatomical features of *A. orientalis*.

MATERIALS AND METHODS

A. orientalis were collected during the flowering period and natural populations in A5 Amasya (in the vicinity of Direkli village, open areas, at 1800 m, June 2012, İÖztürk Çalı - 461) which is a city in the Black Sea region of Turkey. Its taxonomical description was made according to the description given by Davis (1982).

Anatomical investigations were performed using an average of fresh specimens kept in 70% alcohol. Cross sections of root, stem, leaf, petiole, calyx and corolla were taken from 30 specimens of *A. orientalis* and 50 anatomical measurements (to determine the minimum and maximum values of width-height measurements in various tissues) were conducted for each parameter. Transverse sections were made by hand using commercial razor blades and stained with Sartur reactive (Çelebioğlu and Baytop, 1949). Measurements in the sections were taken under a Leica ICC₅₀ HD binocular light microscope by using a Leica Digital Camera and objectives used were $\times 10$ and $\times 40$. Pictures were taken with a Leica ICC₅₀ HD binocular light microscope and a Leica Digital Camera.

RESULTS

In cross-sections taken from the root, stem, leaf, petiole, calyx and corolla of *A. orientalis*, the following significant properties were observed.

In the transverse section of the root, there was a periderm which made up the outermost layer of the root of *A. orientalis* (Figure 1). The periderm had 8 to 9 layers. The dimensions of periderm cells were 15 to 60 \times 15 to 57.5 μm (Table 1). Beneath the periderm, there was the multi-layered cortex, composed ovoidal and paranchymatic cells. The cortex was 9 to 10 layers. The cambium, that composed 3 to 4 layers, was located between the xylem and the phloem. Beneath the cambium, there were xylem tissue (7.5 to 15 \times 7.5 to 20 μm) composed regular trachea and tracheid cells. Xylem cells were also presented in the center, therefore the pith is not seen in the center. There were 3 to 4 layers primary pith rays between the secondary xylem cells.

A. orientalis, as a typical characteristic of the Lamiaceae, had a 4-angle stem. Its epidermis usually had one layer composed of ovoidal cells (12.5 to 42.5 \times 17.5 to 50 μm). The upper surface was covered with a cuticle (1.25 to 2.5 μm). There were glandular and non-glandular hairs on the epidermis (Figure 2). Transverse section of the stem revealed 9 to 10 layered collenchyma placed on the corners. The cortex was composed of 6 to 7 layered paranchymatous cell. The dimensions of paranchymatous cells were 27.5 to 75 \times 15 to 55 μm (Table 1). Starch particles were also observed in the paranchymatous cells of stem (Figure 3). There were 2 to 3 layers of sclerenchyma in the outer side of phloem. Right beneath the phloem, there was a 1 to 2 rowed cambium layer. The xylem tissue existing beneath the cambium was composed of regular trachea and tracheid

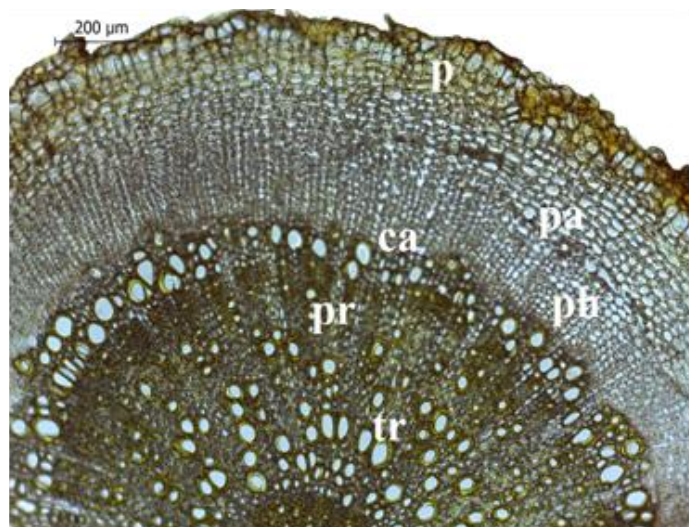


Figure 1. The cross section of root of *A. orientalis* **p**: periderm, **pa**: parenchyma cell, **ph**: phloem, **ca**: cambium, **pr**: pith rays, **tr**: trachea cell.

cells. The vascular bundles were bigger on the corner than other parts of stem. Between the corners, there were also numerous small bundles in the *A. orientalis* stem. The vascular bundles were collateral (Figure 2). The pith was wide and consist of flat cells with intercellular spaces. There was a cavity in the centre of the pith.

In the leaves, the epidermis was single layered on upper and lower surface. There were glandular and non-glandular hairs on epidermis. The cuticle was 2.5 to 5 μm thick. Just beneath the upper epidermis cells, there were 2 to 3 rowed palisade parenchyma cells (Figure 5). The dimensions of the palisade parenchyma cells were 12.5 to 25 \times 25 to 50 μm (Table 1). The 2 to 3 rowed spongy parenchyma existed beneath the palisade. The spongy parenchyma covered less space than the palisade parenchyma. The collateral vascular bundle was located in the midrib region (Figure 5). There were sclerenchyma cells in the outer side of phloem. All vascular bundles in the leaves were surrounded by bundle sheet cells. The stoma was diastic and the leaf was bifacial (Figures 4 and 5). The stoma was presented on upper and lower surfaces of the leaf (Figure 4).

Both adaxial and abaxial epidermis cells were single layered in the petiole (Figure 6). The dimensions of the adaxial epidermis cell were 15 to 45 \times 17.5 to 27.5 μm , while those of the abaxial epidermis were 12.5 to 27.5 \times 15 to 17.5 μm (Table 1). There were a lot of glandular and non-glandular hairs on epidermal cells which were ovoidal-rectangular shapes. Parenchymatic cortex cells were 10 to 11 layered. There was one big vascular bundle in the center and 4 to 5 small vascular bundles at each corners of petiole. The vascular bundles were surrounded by sclerenchymatic cells (Figure 6). There were

Table 1. Anatomical measurements of various tissues of *A. Orientalis*.

| Parameter | Width (µm) Min.-Max. | Height (µm) Min.-Max. |
|------------------------|----------------------|-----------------------|
| Root | | |
| Peridermis cell | 15 - 60 | 15 - 57.5 |
| Parenchyma cell | 12.5 - 42.5 | 12.5 - 22.5 |
| Trachea cell | 7.5 - 15 | 7.5 - 20 |
| Stem | | |
| Cuticle | 1.25 - 2.5 | - |
| Epidermis cell | 12.5 - 42.5 | 17.5 - 50 |
| Parenchyma cell | 27.5 - 75 | 15 - 55 |
| Trachea cell | 10 - 25 | 10 - 20 |
| Leaf | | |
| Cuticle | 2.5 - 5 | - |
| Adaxial epidermis cell | 12.5 - 50 | 15 - 37.5 |
| Abaxial epidermis cell | 12.5 - 40 | 15 - 32.5 |
| Palisade cell | 12.5 - 25 | 25 - 50 |
| Spongy cell | 15 - 62.5 | - |
| Mesophyll region | 170 - 300 | - |
| Palisade region | 70 - 162.5 | - |
| Spongy region | 100 - 137.5 | - |
| Petiole | | |
| Adaxial epidermis cell | 15 - 45 | 17.5 - 27.5 |
| Abaxial epidermis cell | 12.5 - 27.5 | 15 - 17.5 |
| Parenchyma cell | 20 - 85 | 17.5 - 70 |
| Trachea cell | 10 - 17.5 | 7.5 - 17.5 |
| Calyx | | |
| Adaxial cuticle | 1.25 - 2.5 | - |
| Adaxial epidermis cell | 7.5 - 17.5 | 7.5 - 15 |
| Abaxial cuticle | 2.5 - 3.75 | - |
| Abaxial epidermis cell | 12.5 - 40 | 12.5 - 40 |
| Parenchyma cell | 12.5 - 27.5 | 12.5 - 25 |
| Corolla | | |
| Adaxial cuticle | 1.25 - 2.5 | - |
| Adaxial epidermis cell | 7.5 - 25 | 10 - 37.5 |
| Abaxial cuticle | 2.5 - 3.75 | - |
| Abaxial epidermis cell | 7.5 - 32.5 | 12.5 - 42.5 |
| Parenchyma cell | 10 - 30 | 10 - 25 |

parenchymatic bundle sheets on the all vascular bundles. The type of vascular bundle was collateral (Figure 6). There were 2 to 3 layered collenchyma in the area between the corners.

In the calyx, the adaxial epidermis cells were smaller than abaxial epidermis cells. The dimensions of adaxial cuticle were 1.25 to 2.5 µm, whereas those of the abaxial cuticle is 2.5 to 3.75 µm (Table 1). Parenchymatic cells

were flat ovoidal. There were glandular and non glandular hairs on the epidermis (Figure 7a).

In the cross-section of corolla, cuticle was present on both abaxial and adaxial epidermis cells covered by glandular and non-glandular hairs. The shapes of adaxial epidermis cells were papillose type (Figure 8). Beneath the adaxial epidermis, there were the parenchyma cells with intercellular spaces (Figure 7b). There was a vascular

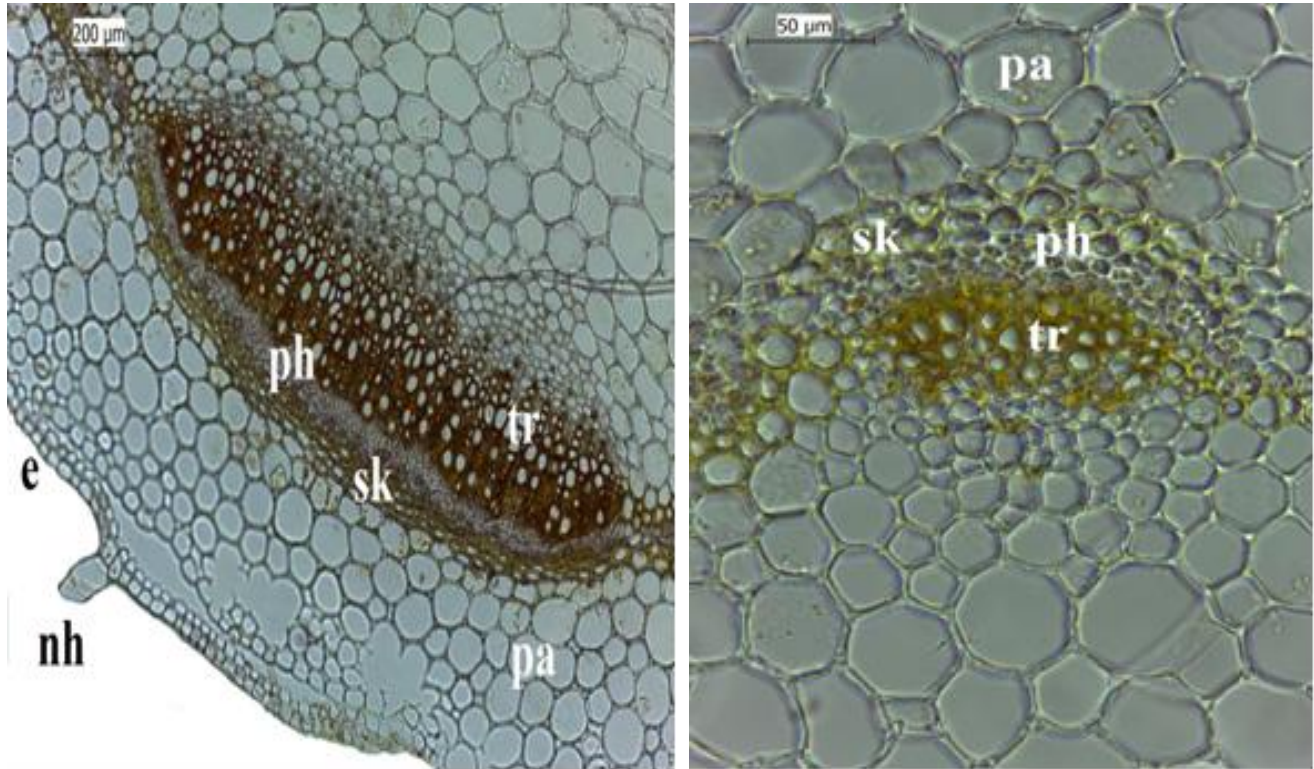


Figure 2. The cross sections of stem of *A. Orientalis*. nh: Non-glandular hair, e: epidermis, pa: parenchyma cell, sk: sclerenchyma, ph: phloem, tr: trachea cell.

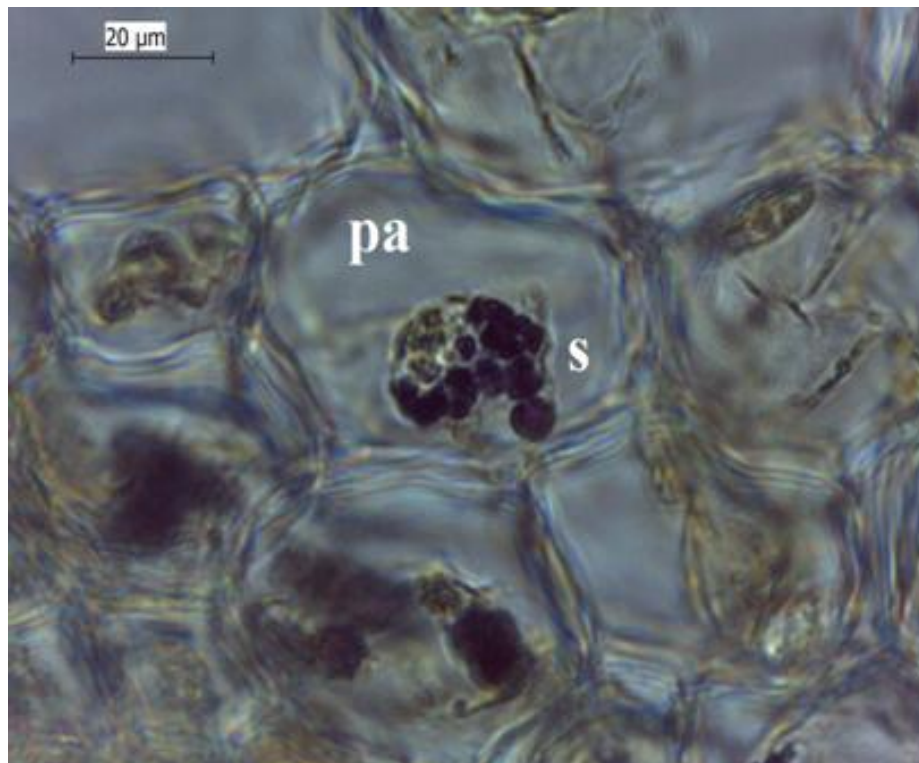


Figure 3. Starch in the parenchyma cell of stem. s: Starch, pa: parenchyma.

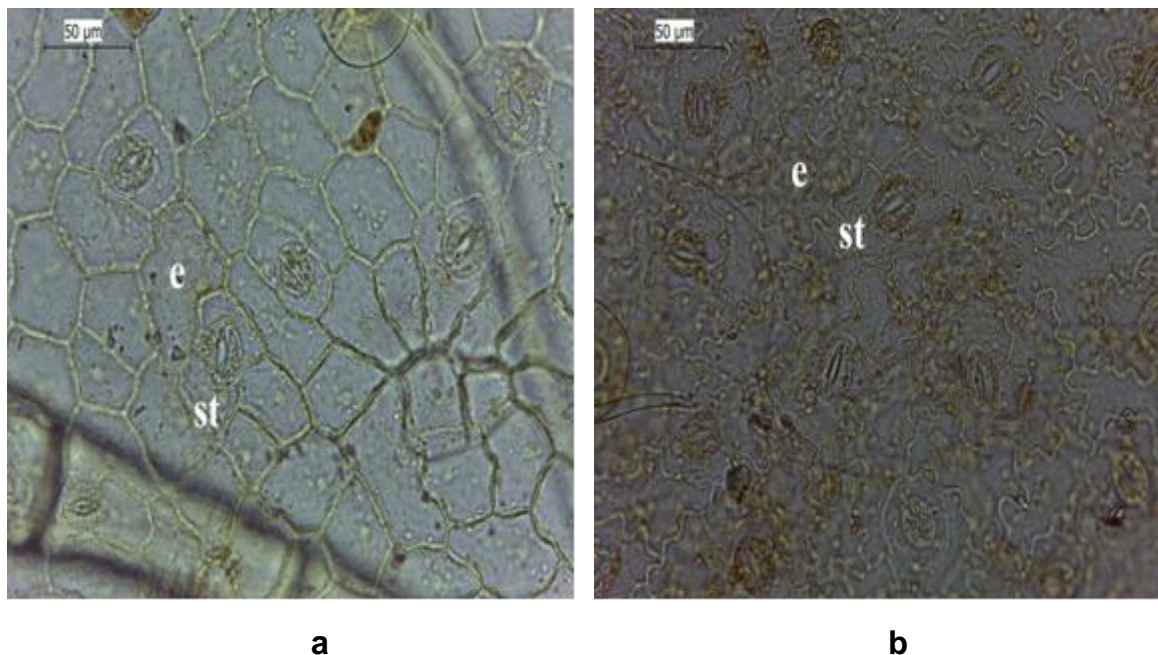


Figure 4. The surface sections of leaf of *A. Orientalis*. (a) Adaxial surface of leaf. (b) Abaxial surface of leaf. e: Epidermis cell st: stoma cell.



Figure 5. The cross sections of leaf of *A. orientalis*. gh: Glandular hair, nh: non-glandular hair, ad: adaxial epidermis cell, ab: abaxial epidermis cell, pa: parenchyma pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle.

bundle in the midrib.

DISCUSSION

The present study provided useful information on the

anatomy of *A. orientalis*. Few studies on species *A. orientalis* had been found in literature (Sajjadi and Ghannadi, 2004; Koyuncu et al., 2010). But, the measurements and observation of anatomical characters belonging to the medicinal taxon *A. orientalis* were reported

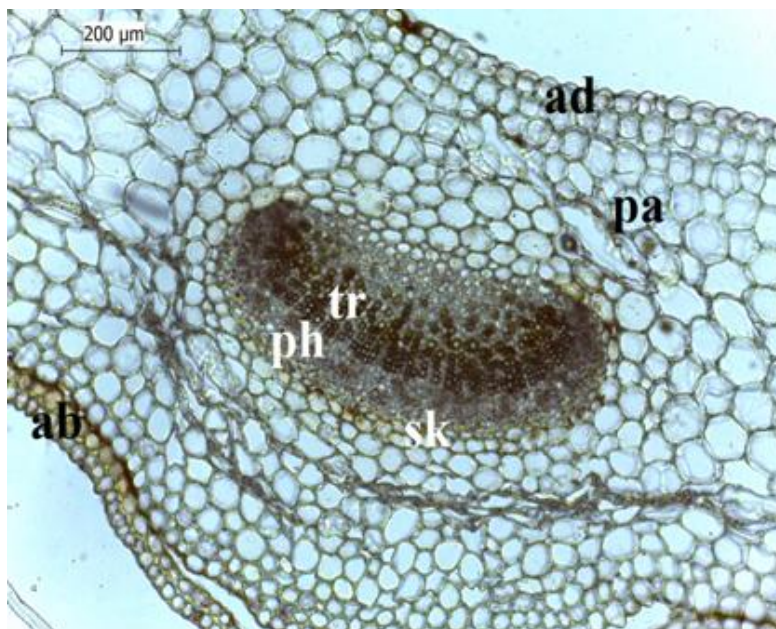


Figure 6. The cross section of petiole of *A. orientalis* ad: Adaxial epidermis cell, ab: abaxial epidermis cell, pa: parenchyma, sk: sclerenchyma, ph: phloem, tr: trachea cell.

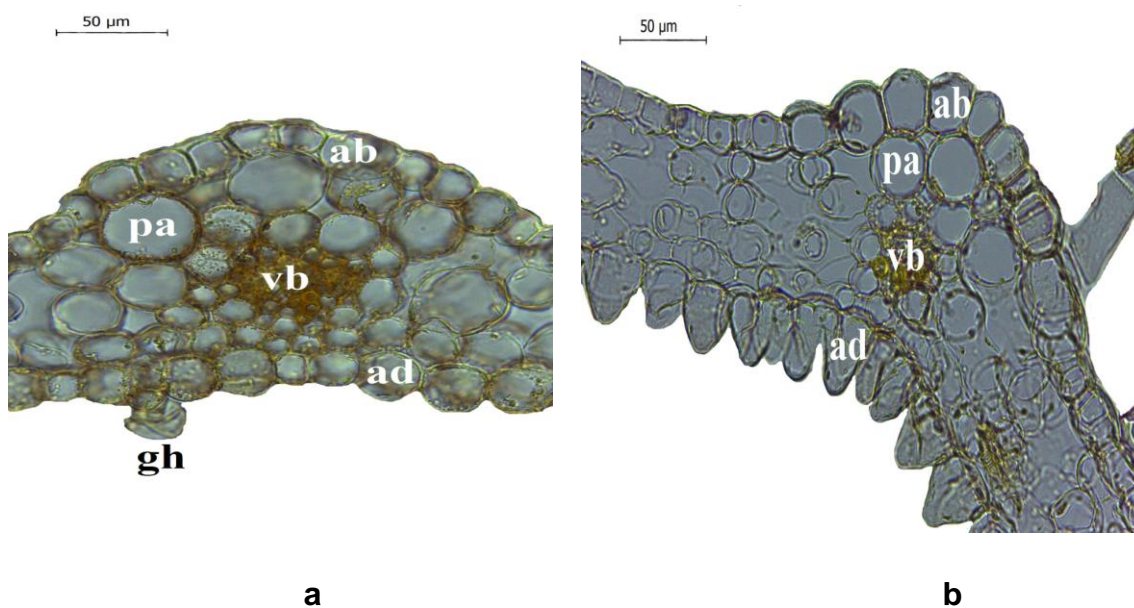


Figure 7. The cross sections of calyx and corolla of *A. orientalis*. (a) Calyx, (b) Corolla, gh: glandular hair, ad: adaxial epidermis cell, ab: abaxial epidermis cell, pa: parenchyma, vb: vascular bundle.

anatomical information about root anatomy of Lamiaceae family. They stated that the pith rays of roots of the family are 2 to 12 or more rowed cells. It was found that the pith rays of *A. orientalis* were composed 3 to 4 rowed cells. These findings were consistent with those of Metcalfe

and Chalk (1972) and those of some studied species of Lamiaceae (Baran and Özdemir, 2006; Özkan and Soy, 2007; Baran and Özdemir, 2009). Metcalfe and Chalk (1972) also stated that the members of Lamiaceae family have quadrangular with well-defined collenchyma in the

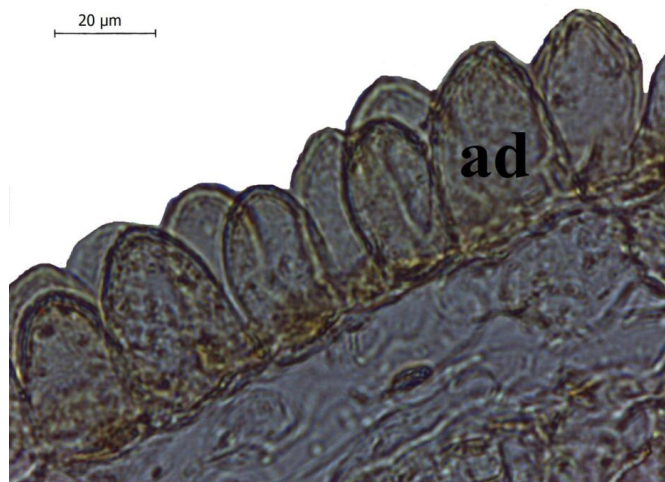


Figure 8. Cross section of corolla of *A. orientalis*; the papillose type of adaxial epidermis cells is seen. **ad**: adaxial epidermis cell.

for the first time in this paper.

Metcalf and Chalk (1972) determined some imported four angles and sclerenchymatous tissue surrounds the phloem groups of vascular bundles. In the transverse section of *A. orientalis*, the stem of this species was quadrangular with well-defined collenchyma in the four angles. It was also determined that there were 2 to 3 layers of sclerenchyma in the outer side of phloem in this study. Quadrangular stem with well-defined collenchyma in the four angles and sclerenchymatous tissue surrounds the phloem groups of vascular bundles were observed in other members of Lamiaceae family (Metcalf and Chalk, 1972; Kandemir, 2003; Baran and Özdemir, 2006; Kahraman et al., 2010). The vascular cambium located between the phloem and the xylem was 1 to 2 rowed layer in the stem of *A. orientalis*. The vascular cambium was seen in the cross-sections of other members of Lamiaceae family (Dinç and Öztürk, 2008; Baran and Özdemir, 2009). There were big vascular bundles on the corners of *A. orientalis* stem, whereas a lot of small bundles between the corners were observed in the present study. There were also starch particles in the paranchymatous cortex cells of stem. The leaf of *A. orientalis* had 2 to 3 layered palisade parenchyma and 2 to 3 layered spongy parenchyma cells. Baran and Özdemir (2009) also stated that *Lamium lycium* which is the member of Lamiaceae family has 2 to 3 layered palisade parenchyma and 2 to 3 layered spongy parenchyma cells as well. These results were parallel to the results mentioned earlier. On the other hand, the stoma type of *A. orientalis* was diasitic and the leaf was bifacial. According to Metcalfe and Chalk (1972), diasitic stoma type was the most common in Lamiaceae family. Diasitic stoma and bifacial mesophyll type were also observed on the leaf of *Ajuga chamaepitys* and *Ajuga reptans* (Akçin et al., 2006). The stomata were

observed on both upper and lower surfaces of the leaf. Metcalfe and Chalk (1972) stated that the structure of the vascular bundles in the petiole of the species in the Lamiaceae could be used as a diagnostic character. The structure of petiole shows differences between genera and species. In addition to this, useful petiole anatomical characters are determined in designated taxonomical structures of some species (Shaheen, 2007; Eric et al., 2007). In the petiole of *A. orientalis*, there was one big vascular bundle in the center and 4 to 5 small vascular bundles at each corner of the petiole. Akçin et al. (2011) found that *A. reptans* has a total of nine vascular bundles: one big bundle in the middle and 4 vascular bundles at each corner. The vascular bundles of the leaf were surrounded by sclerenchymatic cells. Collenchyma in the petiole is 2 to 3 layered at each corners. In the cross-section of calyx and corolla, both adaxial and abaxial epidermis cells were covered with cuticle. In addition to this, there were a vascular bundle in the midrib of calyx and corolla. The adaxial epidermis cells of corolla were papillose type.

The most distinguishing characteristics of the species in the anatomical structure were the presence of glandular hairs on the surface layers on stem, leaves, petiole, calyx and corolla. Like other members of the Lamiaceae, *A. orientalis* had both glandular as well as non-glandular trichomes. Glandular trichomes were mainly observed on calyx and corolla, but non-glandular ones were found on the stem, leaf surface and petiole. According to Metcalfe and Chalk (1972), having glandular and non-glandular trichomes are important anatomical characters. Glandular trichomes significant taxonomic characters and act imported role for pollination in the Lamiaceae family (Navarro and El Oualidi, 2000). As a result, anatomical characters of medicinal species of *A. orientalis* were studied for the first time in this paper. According to the results, the results mentioned earlier, the anatomical features of root, stem, leaf, petiole, calyx and corolla provided useful characteristics for distinguishing species in *Ajuga* genus.

REFERENCES

- Akçin OE, Şenel G, Akçin Y (2006). The morphological and anatomical properties of *Ajuga reptans* L., and *Ajuga chamaepitys* (L.) Schreber subsp. *chia* (Schreber) Arcangel. var. *chia* (Lamiaceae) taxa. Pak. J. Biol. Sci. 9(2):289-293.
- Akçin OE, Özyurt MS, Şenel G (2011). Petiole anatomy of some Lamiaceae taxa. Pak. J. Bot. 43(3):1437-1443.
- Aktaş K, Özdemir C, Özkan M, Akyol Y, Baran P (2009). Morphological and anatomical characteristics of *Salvia tchihatceffii* endemic to Turkey. Afr. J. Biotechnol. 8(18):4519-4528.
- Baran P, Özdemir C (2006). The morphological and anatomical characters of *Salvia napifolia* Jacq., in Turkey. Bangladesh J. Bot. 35(1):77-84.
- Baran P, Özdemir C (2009). The morphological and anatomical properties of *Lamium lycium* (Lamiaceae), endemic to Turkey. Nord. J. Bot. 27:388-396.
- Başer KHC (1993). Essential oils of Anatolian Lamiaceae: A profile. Acta Hort. 333:217-238.
- Baytop T (1984). Türkiye'de bitkiler ile tedavi. İst. Üniv. Yay. No: 3255,

- Istanbul.
- Baytop T (1999). Therapy with medicinal plants in Turkey, Past and Present. (2nd ed.) Nobel Tip Press. İstanbul, Turkey.
- Ben Jannet H, Harzallah-Skhiri F, Mighri Z, Simmonds MSJ, Blaney WM (2000). Responses of Spo- doptera littoralis larvae to Tunisian plant extracts and to neo-clerodane diterpenoids isolated from *Ajuga pseudoiva* leaves. *Fitoterapia* 71:105-112.
- Chen H, Tan RX, Liu ZL, Zhang Y, Yang L (1996). Antibacterial neoclerodane diterpenoids from *Ajuga lupulina*. *J. Nat. Prod.* 59(7):668-670.
- Çelebioğlu S, Baytop T (1949). A new reagent for microscopical investigation of plant, Publication of the Institute of Pharmacognosy, No. 10, 19: 3001, İstanbul.
- Çobanoğlu D (1988). The morphological and cytological properties of *Salvia palaestina* Benth (Lamiaceae). *Turk. J. Bot.* 12:215-223.
- Davis PH (1982). Flora of Turkey and the east aegean islands, Vol 7, 10, (1982-1988). Edinburg University Press.
- Dinç M, Öztürk M (2008). Comparative morphological, anatomical and palynological studies on the genus *Stachys* L. sect. *Amblesia* Bentam (Lamiaceae) species in Turkey. *Turk. J. Bot.* 32:113–121.
- Eric TJ, Michael VA, Linda WE (2007). The importance of petiole structure on inhabitability by ants in *Piper* sect. *Macrostachys* (Piperaceae). *Bot. J. Linn. Soc.* 153(2):181-191.
- Heywood VH (1978). Flowering Plants of the World. Oxford, Oxford University Press.
- Kahraman A, Celep F, Doğan M (2010). Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *S. Afr. J. Bot.* 76:187–195.
- Kandemir N (2003). The morphological, anatomical and karyological properties of endemic *Salvia hypargeia* Fich. & meyer. (Lamiaceae) in Turkey. *Pak. J. Bot.* 35:219-236.
- Koyuncu O, Yaylacı ÖK, Öztürk D (2010). Risk categories and ethnobotanical features of the *Lamiaceae* taxa growing naturally in Osmaneli (Bilecik/Turkey) and environs. *Biodivers. Conserv.* 3(3):31-45.
- Metcalf CR, Chalk L (1972). Anatomy of the dicotyledons, vol. II. Oxford University Press, Oxford.
- Navarro T, El Oualidi J (2000). Trichome morphology in *Teucrium* L. (Labiatae), a taxonomic review. *Anales Jard. Bot. Madrid* 57:277–297.
- Özdemir C, Altan Y (2005). Morphological and anatomical investigations on endemic *Scutellaria orientalis* L. subsp. *bicolor* (Hochst) Edmund and subsp. *santolinoides* (Hauskn ex Bornm). *Pak. J. Bot.* 37(2):213-226.
- Özdemir C, Şenel G (2001). The morphological, anatomical and karyological properties of *Salvia forskahlei* L. (Lamiaceae) in Turkey. *J. Econ. Taxon. Bot.* 19:297-313.
- Özkan M, Soy E (2007). Morphology, anatomy, hair and karyotype structure of *Salvia blecharoclaena* Hedge and Hub.-Mor. (Lamiaceae), endemic to Turkey. *Pak. J. Biol. Sci.* 10:893-898.
- Sajjadi SE, Ghannadi A (2004). Volatile oil composition of the aerial parts of *Ajuga orientalis* L. from Iran. *Z. Naturforsch. C.* 59:166-168.
- Shaheen AM (2007). Characteristics of the stem-leaf transitional zone in some species of Caesalpinioidae (Leguminosae). *Turk. J. Bot.* 31:297-310.
- Thorne RF (1992). Classification and geography of the flowering plants. *Bot. Rev.* 58:225-348.
- Uysal İ, Öztürk M, Pirdal M (1991). Morphology, Anatomy and Ecology of Endemic Species of *Sideritis trojana* Bornm. *Turk. J. Bot.* 15:371-379.
- Werker E, Ravid U, Putievsky E (1985). Structure of glandular hairs and identification of the main components of their secreted material in some species of the Labiatae. *Israel J. Bot.* 34:31-45.