



Morpho-anatomical investigations on *Ajuga postii* Briq and *Ajuga relictta* P.H.Davis

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Abstract

In the present study morphological and anatomical features of *Ajuga postii* and *Ajuga relictta* (Lamiaceae) which are local endemic for Turkey have been carried out. Corolla of *A. postii* is dark purple-pink (drying purplish) and upper lip obsolescent or lost; corolla of *A. relictta* is cream colored when dried yellowish-beige and upper lip 1 mm, bidentate to the base. Anatomical verities have been represented in cross sections of roots, stems, and leaves of the species. *A. relictta* has been observed without trichomes and *A. postii* with sparsely non-glandular trichomes on the stems. In root cross sections of the species, sclerenchyma cells have been observed in the cortexes. In the leaf cross-sections, glandular trichomes have not been observed, only non-glandular trichomes have been determined.

Key words: *Ajuga*, morphology, anatomy, local-endemic, Turkey

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Ajuga postii Briq ve *Ajuga relictta* P.H.Davis üzerine morfolojik-anatomik bir araştırma

Özet

Yapılan çalışma ile Türkiye’de lokal endemik türler olan *Ajuga postii* ve *Ajuga relictta* (Lamiaceae)’nın morfolojik ve anatomik özellikleri belirlenmiştir. Buna göre *A. postii*’nin korollası koyu pembe-mor (kuruyunca morumsu) renkli, üst dudak eksilmiş veya kaybolmuş; *A. relictta*’nın korollası krem rengi kurduğunda ise sarımsı-bej rengi, üst dudak 1 mm, tabanda iki dişlidir. Anatomik özellikler çalışılan örneklerin kök, gövde ve yaprak anatomik kesitleri üzerinden verilmiştir. *A. relictta*’nın gövdesi üzerinde herhangi bir tüy oluşumu görülmezken, *A. postii*’nin gövdesi üzerinde seyrek olarak örtü tüyleri gözlenmiştir. Çalışılan taksonların kök anatomik kesitlerinde sklerenkima hücreleri korteks tabakasında belirgindir. Yaprak enine kesitlerde salgı tüyleri gözlenmezken, örtü tüyleri gözlenmiştir.

Anahtar kelimeler: *Ajuga*, morfoloji, anatomi, lokal-endemik, Turkey

1. Introduction

Lamiaceae is represented by 236 genera and about 7,200 species in the worldwide (Heywood et al., 2007). In Turkey, Lamiaceae is the third largest plant family, is represented by 46 genera and 580 species, 260 species are endemic, and endemism rate of the family is about 44% (Davis, 1982; Davis et al., 1988; Güner et al., 2000; Dirmenci, 2012).

Ajuga L. is located in the subfamily Ajugoideae of Lamiaceae. Ajugoideae has four-lobed drupa and nonpermanent stylus (Judd, 2008). Members of the *Ajuga* are grown in Europe, Asia, Africa, Australia and North America. *Ajuga* species are used in traditional medicine; in Uganda tea of *A. alba* (Gürke) Robyns is used for cold and tiredness (Hamill et al., 2003); in Morocco *A. iva* (L.) Schreber is used as anthelmintic and for gastrointestinal disorders and breast cancer (Ziyyat et al., 1997; Ouhaddou et al., 2014); in Kenya *A. remota* Benth. is used against malaria and diabetes (Keter and Mutiso, 2012). In Turkey, *A. orientalis* L. is used for hemorrhoids (Güneş and Özhatay, 2011) and skin diseases (Koyuncu et al., 2010); *A. chamaepitys* (L.) Schreber is used for wound healing (Tümen et al., 2006), hemorrhoids, as diuretic and antivenom (Sarac and Ugur, 2007). *A. chamaepitys* (L.) Schreber subsp. *chia*

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(Schreber) Arcangeli var. *chia* (Schreber) Arcangeli is used for hemorrhoids and wound healing (Kılıç and Bağcı, 2013).

Ajuga is represented with 13 species and 10 subspecies in Turkey (Güner et al. 2012). *Ajuga postii* Briq and *Ajuga relictata* P.H. Davis are endemic species of Turkey. The studied species distribute between 1350-1500 m.

In the Flora of Turkey (Davis, 1982), localities of *A. postii* are shown as Hatay and Mersin provinces. However, the species was not found in Hatay. On the other hand, type locality of *A. relictata* is demonstrated on Kahramanmaraş - Ahir Mountain, but like *A. postii* the species was not found determined locality, however, *A. relictata* was rediscovered close to the stated locality (Kahramanmaraş - Çimen Mountain) (Varol et al., 1998). Restricted localities and endemic features of the species make the present study crucial for the future perspective. The aim of the study is to determine morphological and anatomical features of *A. postii* and *A. relictata* which are local endemics for Turkey flora.

2. Materials and methods

2.1. Plant materials

A. postii was collected from İçel: Çamlıyayla, Namrun Castle, 37° 16.8' 54.5'' N 34° 60.1' 16.5'' E, 1350 m, 10.07.2015 and *A. relictata* was collected from Kahramanmaraş: Çimen Mountain, Yavşan Hill, Pekmezpınarı, , 37° 28' 42'' N 36° 42' 21'' E, 1500 m, 03.06.2015. Localities of the species have been shown in Figure 1. Collected samples are stored at Anadolu University Faculty of Pharmacy Herbarium (ESSE no 15083-15084).



Figure 1. Localities of collected *Ajuga* species.

2.2. Morpho-Anatomical studies

Morphological features of the herbs were determined from different plant parts. At least 10 plant samples were used for average measurements. Anatomical structures were studied on the roots, stems, and leaves. Plant materials were maintained in the 70% alcohol, were stained with Sartur reagent (60 ml lactic acid, 45 ml Sudan III, 2 gr aniline, 0.2 gr I, 1 gr KI, 10 ml alcohol (95 %) and 80 ml distilled water), and chloral hydrate. The permanent preparates were prepared with glycerine-gelatine. Photographs were captured with Zeiss AXIO Lab. A1 binocular light microscope and Axiocam ERc 5s camera.

3. Results

Strongly perennial herb, 77-84 cm with a short woody rhizome. Stems virgate, simple or branched above, pilose on angles. Cauline and floral leaves shortly petiolate, leaves ovate-lanceolate, 3.5-10 × 1-4 cm, entire or irregularly serrate or dentate with sparsely villose, base attenuate, apex acute. Flower 2 per verticillaster, large, c. ½ as long as floral leaves. Calyx 8-12 mm, divided to ½ into lanceolate 5 teeth, glandular-villose teeth or glabrous. Corolla dark purple-pink (drying purplish), 24-50 mm, upper lip obsolescent or lost, emarginate; lower lip 3 x tube, resupinate, densely pubescent (Figure 2 and 3). Stamen 4, 13-18 mm, filament simple, trichomes lanate-villose. Stylus 22-24 mm, glabrous, not gynobasic, inserted well above the base of ovary lobes, bifid stigma. Fruit baccate, obscurely 4-lobed, c. 3-6 mm with a fleshy purplish-black pericarp (Table 1).

Figure 2. Habit of *A. postii*Figure 3. Flowers of *A. postii*Table 1. Morphological results comparison of *A. postii* in present study and the study of Flora of Turkey (Davis, 1982).

Characteristic	Flora of Turkey (Davis, 1982)	Present study
Habit		Perennial
Plant length	77-84 cm	c. 1 m
Stem	Branching simple or branched above	simple or branched above
	Indumentum with long trichomes on the corners	with long trichomes on the corners
Cauline leaves	Size (cm) 1.5-4 x 4-10	3.5-10 x 1-4
	Base -	attenuate
	Apex -	acute
	Margin entire or irregularly serrate or dentate	entire or irregularly serrate or dentate
	Indumentum with rare long trichomes	with rare long trichomes
Verticillasters	Flowers 2	2
Bracts	Size (cm) 1.5-4 x 4-10	3.5-10 x 1-4
	Shape ovate- lanceolate	ovate- lanceolate
	Margin entire or irregularly serrate or dentate	entire or irregularly serrate or dentate
	Apex -	acute
Calyx	Length (mm) 8-11	8-12
	The shape of teeth -	lanceolate
	The apex of teeth -	acute
	Indumentum teeth with glandular hairs	with glandular hairs or glabrous
Corolla	Color pink, when dried purple	dark purple, pink
	Length (mm) 30-50	24-50
	The shape of lobe reduced upper lip	reduced or lost upper, resupinate lower lip
	Indumentum -	inner side with lanate-villous trichomes
Stamen	Length (mm) -	13-18
Filament	Indumentum -	lanate-villous
Style	Length (mm) -	22-24
	Indumentum -	glabrous
Stigma	Type -	bifid
Nutlet	Width (mm) c.6	3-4

Distribution and ecology: *A. postii* is endemic to Turkey and grows only in *Fagus* L. forest, *Quercus* L. macchie and rocky mountain slopes elevations from 60-1600 m, Eastern Mediterranean element (Davis, 1982).

Phenology: Flowering from June to July.

3.2. Anatomy of *Ajuga postii*

Root: In the cross sections of the root, in the outer layer of the root, periderm tissue has been observed with 2-3 layers of flattened cells. Beneath the periderm, the cortex is multi-layered with parenchymatous and polygon-shaped cells. Sclerenchymatous cells take a large place in the cortex. The cambium which is generally located between phloem

and xylem cells is indistinguishable. Regular trachea and tracheid cells are located in xylem tissue. The pith of the root is located with 1 to 2 layers of primary pith rays between the secondary xylem cells (Figure 4).

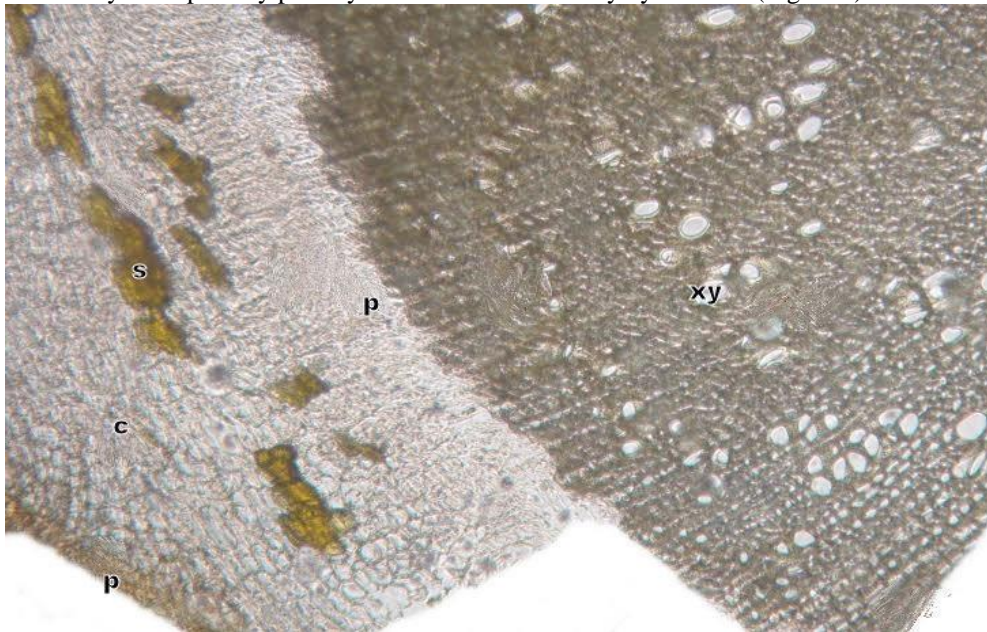


Figure 4. The cross section of the root of *A. postii* p: Periderm, c: Cortex, s: Sclerenchyma, p: Phloem, xy: Xylem

Stem: *A. postii* has the typical square stem of Lamiaceae. The stem epidermis is located monolayered, and the outer layer is covered with the cuticle. Beneath the epidermis, 5-6 layered of collenchyma is observed on the corners, and with 1-2 layers between the corners. Cortex has been observed with oval and parenchymatous shaped cells. In the cross section of the stem, endodermis and cambium layers were not observed. Although phloem is located with 2 to 3 layered cells, and xylem is taken larger place than phloem. The pith region is placed with large polygon shaped and parenchymatous cells (Figure 5).



Figure 5. The cross section of the stem of *A. postii* e: Epidermis, c: Cortex, p: Phloem, xy: Xylem

Leaf: In the leaf cross section, the epidermis is located uniseriate on the adaxial and abaxial sides. Epidermis is covered with the thin layered cuticle. The trichomes are non-glandular and are located with 1 to 5 cells (Figure 7). Palisade parenchyma and spongy parenchyma cells are not distinguished to each other. The collateral vascular system is located in the midrib region, and the outer of the phloem cells sclerenchyma is observed (Figure 6). The leaf is bifacial and the stoma is diasitic (Figure 8). The stoma is only observed on the lower surface of the leaf.

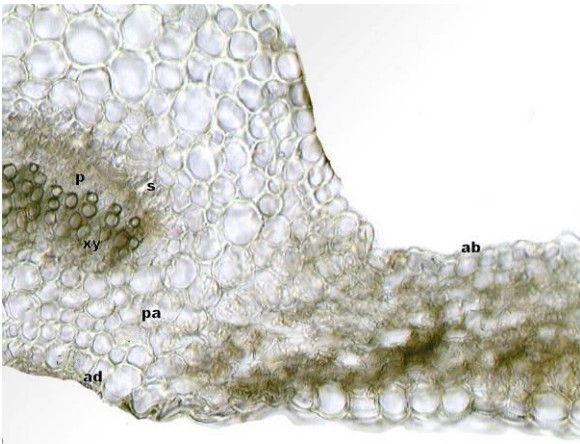


Figure 6. The cross section of the leaf of *A. postii* ad: Adaxial epidermis, ab: Abaxial epidermis, pa: Parenchyma, s: Sclerenchyma, p: Phloem, xy: Xylem

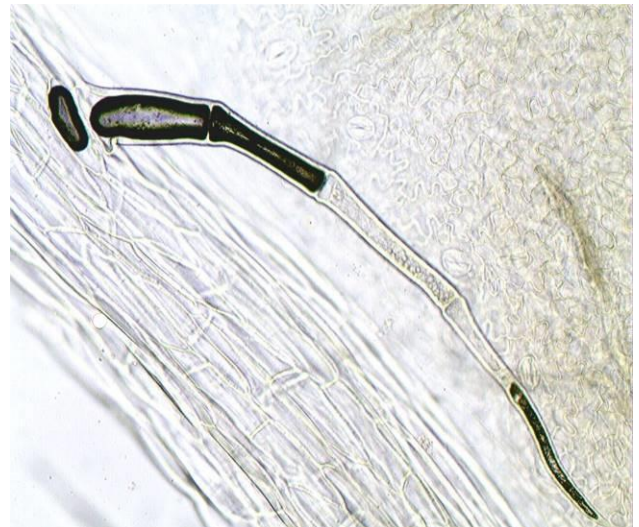


Figure 7. Non-glandular hair of the leaf of *A. postii*

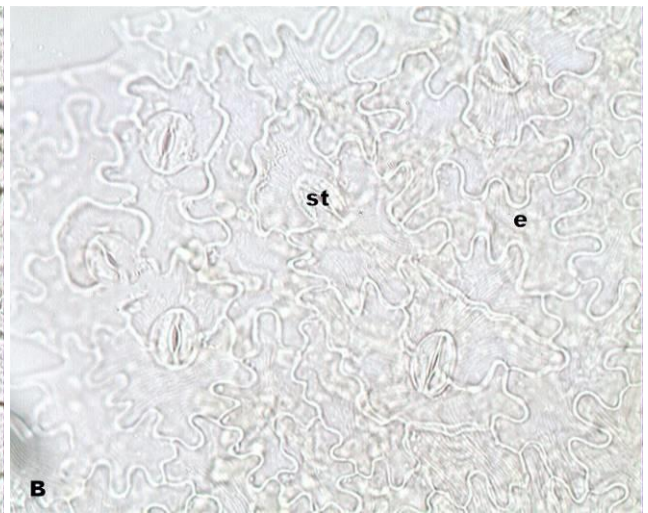


Figure 8. The surface sections of the leaf of *A. postii* (A) Adaxial surface (B) Abaxial surface e: Epidermis, st: stoma

3.3. Morphology of *Ajuga relictia*

Perennial herb, 23-49 cm. Stem strong, erect, shortly branched in the upper part. Leaves lanceolate (at least on axillary

shoots), glabrous, base leaves lanceolate, attenuate, edges entire or rarely toothed, apex acute, glabrous, 3-4.6 × 0.9-1.2 cm. Those on the main stem with petioles triangular and amplexicaul at the base (lamina withered); leaves on axillary shoots narrower, entire or rarely toothed, apex acute, glabrous 4-8.5 × 0.8-3.3 cm, attenuate at the base and sessile. Inflorescence terminal, spike-like 3-14 cm, axis glandular-villous, verticillasters densely 6-14 (16) flowered, at least lower ones subtended by leaf-like bracts, upper shorter. Bracts lanceolate, base semi-amplexicaul, edges entire, apex acute, glabrous, 1.7-5.0 × 1.0-1.2 cm. Calyx lanceolate, 7-11 mm, divided to ½ into glandular-villous narrowly lanceolate 5 teeth; apex acute-cuminate, gray lanate-villose. Corolla 11-19 mm, cream colored, yellowish-beige when dried, tube slender and twice as long as 3-lobed lower lip; upper lip 1 mm, bidentate to base, gray lanate-villose (Figure 9 and 10). Stamen 4, filament lanate-villose, 4-7 mm. Stylus 10-14 mm, not gynobasic, stigma bifid. Fruit nutlet, reticulate-rugulose, 1-1.5 mm, dark yellow (Table 2).



Figure 9. *A. relicta*



Figure 10. *A. relicta* flowers

Table 2. Compared data of *A. relicta* with description of Flora of Turkey

Characteristic	Flora of Turkey (Davis, 1982)	Present study
Habit		Perennial
Plant length (cm)	more 30	23-49
Stem	Branching	shortly branched above
	Indumentum	glabrous
Base leaves	Size (cm)	3-4.6 x 0.9-1.2
	Shape	lanceolate
	Base	attenuate
	Margin	entire or rarely dentate
	Apex	acute
	Indumentum	glabrous
Cauline leaves	Size (cm)	4-8.5 x 0.8-3.3
	Base	amplexicaul
	Apex	acute
	Margin	entire or rarely dentate
	Indumentum	glabrous
Verticillasters	Flowers	6-12
	Length (cm)	3-14
Bracts	Size (cm)	1.7-5 x 1-1.2
	Shape	lanceolate
	Margin	entire
	Apex	acute
	Base	semi-amplexicaul
Calyx	Length (mm)	8-11
	The shape of teeth	lanceolate
	The apex of teeth	acute-cuminate
	Indumentum	grey lanate-villous
Corolla	Color	cream, when dried yellowish-beige
	Length (mm)	11-19
	Number of lobe	3-lobed lower lip, reduced upper lip (2 dentate)
	Indumentum	grey lanate-villous
Filament	Length (mm)	4-7
	Indumentum	lanate-villous
Style	Length (mm)	10-14
	Indumentum	glabrous
Stigma	Type	bifid
Nutlet	Shape	reticulate-rugulose
	Size (mm)	1-1.5

Distribution and ecology: *A. relicta* is endemic to Turkey and grows only in open areas of *Cedrus libani* A. Rich. and *Abies cilicica* Ant. & Kotschy Carriere an elevation 1500 m. It is Eastern Mediterranean element (Davis, 1982).

Phenology: Flowering from June to July.

3.4. Anatomy of *Ajuga relictata*

Root: The outermost layer of the root is located with disrupted cells of the phellogen. Under the phellogen, several layered phelloderm is observed. Beneath the periderm, the multi-layered cortex stays with oval and parenchymatous cells. Sclerenchyma cells are observed in the cortex layer. Similarly *A. postii* between phloem and xylem cells, cambium has not been observed. Beneath the phloem, xylem is composed of the ordinate trachea and tracheid elements and xylem cells are extended to the pith. Pith region is located with 1 to 2 layers of primary pith rays between the secondary xylem cells (Figure 11).

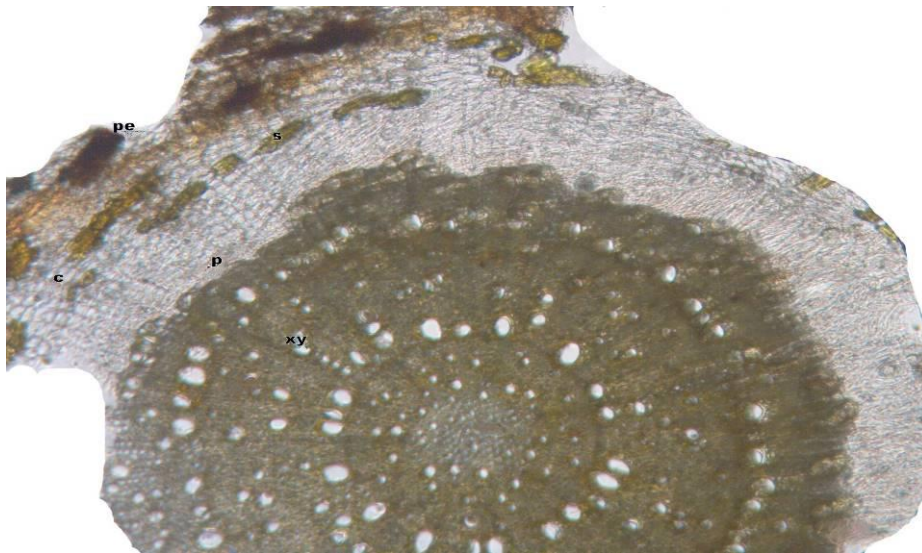


Figure 11. The cross section of the root of *A. relictata* pe: Periderm, c: Cortex, s: Sclerenchyma, p: Phloem, xy: Xylem

Stem: *A. relictata* has been observed with the quadrangular stem of Lamiaceae. Transverse section of the stem, epiderma is located with 1 to 2 layered cells with a thin layered cuticle. Beneath the epiderma, collenchyma cells are multilayered on the corners and are monolayered between the corners. The cortex is stated with parenchymatous cells, and sclerenchyma cells are observed on the phloem. In stem cross section of *A. relictata*, the vascular system is well developed, phloem consists of amorphous and oval-shaped cells, and xylem is located with round, oval and polygonal shaped cells. Cambium layer is not definite between phloem and xylem cells. Pith region occurs large, polygon shaped, rounded parenchymatous cells. (Figure 12).



Figure 12. The cross sections of the stem of *A. relictata* e: Epidermis, c: Cortex, s: Sclerenchyma, p: Phloem, xy: Xylem

Leaf: In the crosscut section of the leaf, adaxial and abaxial sides of the epidermis are located with 1-2 layered of flattened epidermal cells. Non-glandular hair has been observed on the edge of the leaf with 4-5 cells (Figure 14). The vascular system is the collateral type and is located in parenchymatous cells. Xylem is located in the upper epidermis side and phloem stays in the lower epidermis side. Sclerenchyma cells are located above the phloem (Figure 13). The stoma is diasitic and the leaf is bifacial. The stoma is presented on upper and lower surfaces of the leaf (Figure 15).



Figure 13. The cross section of leaf of *A. relictta* ad: Adaxial epidermis , ab: Abaxial epidermis , pa: Parenchyma, s: Sclerenchyma, p: Phloem, xy: Xylem



Figure 14. Non-glandular hair of *A. relictta* on the edge of the leaf

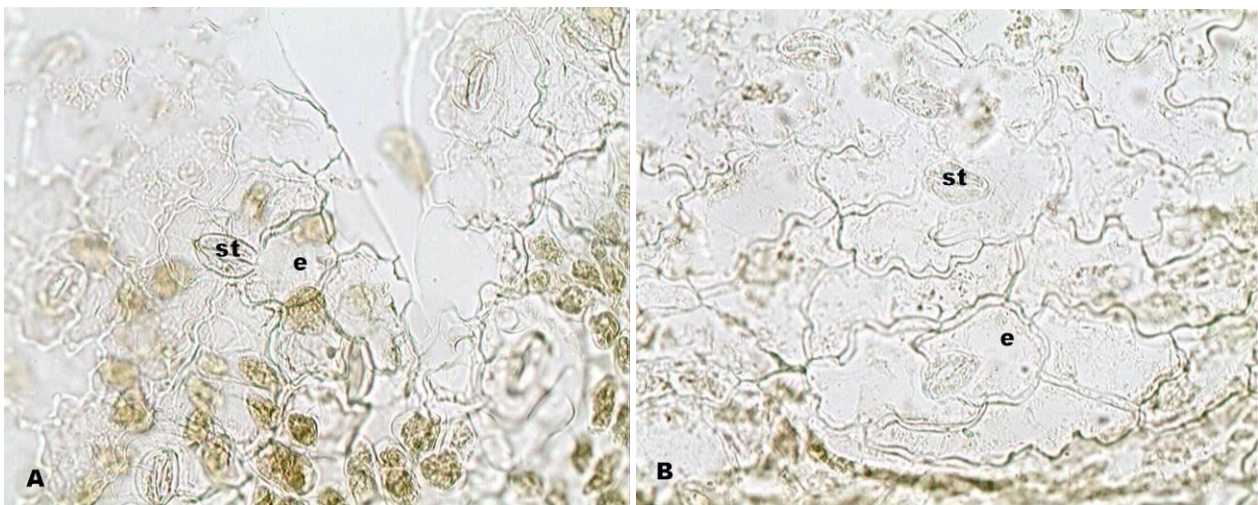


Figure 15. The surface sections of the leaf of *A. relictta* (A) Adaxial surface (B) Abaxial surface. e: Epidermis cell, st: stoma

4. Conclusions and discussion

In the present study morphologies of *A. postii* and *A. relictta* have been compared with Flora of Turkey (Davis, 1982), and anatomical structures of the two species have been studied for the first time.

In the present study, *A. postii* plant length has been found between 77-84 cm, however, in the Flora of Turkey (Davis, 1982), the length of the plant was shown about 1 m. In the present study, lengths and widths of the cauline leaves have been determined as 3.5-10 x 1-4 cm, on the other hand in the description of Flora of Turkey (Davis, 1982) measures were given as 4-10 x 1.5-4 cm. The base of the leaves has been determined to attenuate, and the apex of the leaves is acute in the present study, the specified features were not determined in the Flora of Turkey (Davis, 1982). Bract sizes are as similar as cauline leaves in the both present study and Flora of Turkey (Davis, 1982). The apex of bracts has been determined as acute in the present study, the feature was not determined in the Flora of Turkey (Davis, 1982). Calyx teeth shape and apex have been determined respectively as lanceolate and acute in the present study for

the first time. Corolla length has been found between 24-50 mm in the present study, however, in the Flora of Turkey (Davis, 1982) the length was determined between 30-50 mm. In the present study, the indumentum of corolla has been determined as the lanate-villous inner side of the corolla, in the Flora of Turkey (Davis, 1982) the feature was not found. In the present study, stamen length, indumentum of the filaments, the length of style, and indumentum feature have been determined for the first time. Stamen length is between 13-18 mm, indumentum of the filaments is the lanate-villous, the length of style is between 22-24 mm, and indumentum of the style is glabrous.

Morphological features of *A. relict*a have been compared with Flora of Turkey (Davis, 1982). In the present study, the plant length has been determined between 23-49 cm, however, the length was given more than 30 cm in Flora of Turkey (Davis, 1982). The size of the base leaves is found 3-4.6 x 0.9-1.2 cm, the margin is entire or rarely dentate, and the apex is acute in the present study, however, the measures, margin and the apex of the base leaves were not determined in Flora of Turkey (Davis, 1982). The size of the cauline leaves has been determined to 4-8.5 x 0.8-3.3 cm, on the other hand in the Flora of Turkey (Davis, 1982) only the length of the leaves was given and was determined as 2.5-5 cm. The margin and the apex of the cauline leaves have been determined respectively entire or rarely dentate, and acute in the present study. The number of the verticillaster was shown as 6-12 in the Flora of Turkey (Davis, 1982), however, in the present study, the number is changed from 6 to 16, the length of the verticillaster has been found 3-14 cm. Features of the bracts were not determined in the Flora of Turkey (Davis, 1982), however in the present study size, shape, margin, apex and base of the bracts have been determined. The other missing features of *A. relict*a have been determined in the present study, the apex of calyx teeth is acute-cuminate, indumentum of calyx is grey lanate-villous, corolla color is cream, indumentum of corolla is grey lanate-villous, length of filament is 4-7 mm, indumentum is lanate-villous, length of style is 10-14 mm, and indumentum is glabrous. In the Flora of Turkey (Davis, 1982), the length of corolla was determined as 16 mm, however, in the present study, the length has been found between 11-19 mm.

The investigated plant species are thought to be relict endemic, with regards to climatic diversity and sheltered sides of Turkey during the glacial periods “Şekercioglu et al., 2011”. Therefore, the study is important to identify the species. Studied two species are located in Lamiaceae, and the family is specific with trichomes (Navarro and El Oualie, 2000), and glandular hairs (Metcalf and Chalk, 1950), however in the present study *A. relict*a has been observed without trichomes on the stem, base-cauline leaves, and bracts. *A. postii* has sparsely non-glandular trichomes on the stem, cauline leaves, and bracts. *A. relict*a has been investigated with trichomes on the calyx, corolla, and filaments, and *A. postii* has been observed with trichomes on the calyx teeth, and the inner side of the filaments and corolla (Table 1 and 2). Anatomical features of *A. postii* and *A. relict*a have been studied for the first time in the present study. *A. postii* and *A. relict*a are perennial herbs and both species have been observed with periderm layers on the outer side of the cross sections of the roots, the same result was determined on the root cross section of *A. reptans* L. (Erkara and Koyuncu, 2009). In the present paper, the both roots of the species have been observed with sclerenchymatous cells in the cortex, and indefinite cambium layers between phloem and xylem.

Anatomical features of Lamiaceae were determined by Metcalfe and Chalk (1950), according to the study, Lamiaceae members are specific with square shape of the stem, and collenchyma cells on the corners. The leaf of Lamiaceae was detected as dorsiventral (Metcalf and Chalk, 1950), in the present study, leaves of the studied species have been determined dorsiventral like the previous study, however palisade and spongy parenchyma cells of the mesophyll of *A. postii* are not differentiated, the same result was observed on *A. reptans* (Erkara and Koyuncu, 2009). Stomata of Lamiaceae members were determined on one or both two sides of the leaves (Metcalf and Chalk, 1950), in the present study, the similar results have been observed. Stoma structure of *A. postii* has been only observed on the abaxial side of the leaf; on the other hand, *A. relict*a are located with the stomata on both two leaf sides like *A. orientalis* (Çalı, 2014), *A. chamaepitys* subsp. *chia* var. *chia* “Akçın et al., 2006”, and *A. reptans* (Erkara and Koyuncu, 2009). Stomata of Lamiaceae are specific with diasitic types (Metcalf and Chalk, 1950), the same results have been determined in the present study, and *A. orientalis* (Çalı, 2014), *A. chamaepitys* subsp. *chia* var. *chia*, and *A. reptans* “Akçın et al., 2006”. Xylem and phloem were specified as collateral bundles of the stems in some Lamiaceae members (Metcalf and Chalk, 1950), in the present study, studied species have shown the same result, however, in the stem cross section of *A. orientalis*, between phloem and xylem vascular cambium was observed and the property was collateral open (Çalı, 2014). Sclerenchyma was observed on the phloem in the stem cross sections (Metcalf and Chalk, 1950; Çalı, 2014), for the present paper the same result has been observed on *A. relict*a, however, in the stem crosscut section of *A. postii*, the structure has not been determined. Pith region of the stem was determined as homogeneous (Metcalf and Chalk, 1950), the same result has been observed in the present study for both two species. Hair of *Ajuga* genus was determined with the long handle (Metcalf and Chalk, 1950), in the present study, two species have the same features. In the present paper, studied plant species have been observed with non-glandular leaf hairs. Trichomes of *A. postii* are located on the leaf surface (Fig. 7), and non-glandular hair structure of *A. relict*a has been observed on the edge of the leaf (Fig. 14), however, in the morphological findings of the leaf of *A. relict*a has been determined as glabrous on the leaf surfaces. In the literature studies, glandular hairs were observed on the stem, petiole, leaf, calyx and corolla of *A. orientalis* (Çalı, 2014); glandular hairs and trichomes on the stem of *A. reptans*, and on the stem and leaves of *A. chamaepitys* subsp. *chia* var. *chia* “Akçın et al., 2006”. Vascular bundles of the leaves of the xerophytic plants

were observed with sclerenchymatous cells (Metcalf and Chalk, 1950), in the present study, the same result has been determined for the two species.

As a conclusion, in the present paper, morphological and anatomical features of the local endemic species *A. postii* and *A. relicta* have been determined. Anatomical characters of the taxa have been studied for the first time. Thus, the present study is a resource to identify the species, and to use for medicinal purposes.

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