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Morphological, Anatomical and Ecological Features of *Ajuga salicifolia* (L.) Schreber (Lamiaceae) with Natural Spreading

Sibel ULCAY *¹

Abstract

The aim of this study was to determine the morphological, anatomical and ecological characteristics of *Ajuga salicifolia* (L.) Schreber plant belonging to Lamiaceae family. The plant was collected between April and June 2019 from the open fields of Bağbaşı location in Kırşehir province. The collected samples were placed in 70% alcohol and fixed. Transverse sections were taken from the root, stem and leaf of the plant for anatomical research. In addition, superficial sections were taken from both the lower and upper parts of the leaf. According to the morphological examination, the stem of the plant is upright and occasionally accumbent. The stem is red from the basal to the middle and has hispid hairs on it. Leaves are usually oblong-lanceolate. The corolla is yellow and 1-2 very prominent red spots are seen at the far end. In the root cross section of the plant, the periderm is 1-2 layers, rectangular. Phloem is 7-8 rows and xylem are evident. Pith region is observed in the root. According to the stem cross section, the epidermis cells are circular and square shaped and covered with capitate glandular hairs and 1-3 cell glandular hairs. The vascular bundles are in a continuous ring. Sclerenchyma also continues around the vascular bundles. Collenchyma cells are seen in the corners of the stem. The leaf of the *A. salicifolia* plant is bifacial. Stomata are anomocytic. The soils in which it grows of *A. salicifolia* are clayey, slightly alkaline, salt-free, poor in phosphorus, and rich in potassium and organic matter. As a result, the examination of the species in terms of morphology and anatomical aspects provides important benefits in the systematic of plants. For this reason, it is expected that our study will benefit the studies to be carried out on the *Ajuga* genus.

Keywords: *Ajuga*, Lamiaceae, morphology, anatomy, ecology

1. INTRODUCTION

The Lamiaceae family is distributed in the world with 400 genera and 3200 species [1]. In Turkey, it is represented by 46 genera and 580 species. Of

these species, 260 species are endemic and the rate of endemism is about 44% [2, 3]. 23 taxa of *Ajuga*, a genus in Lamiaceae family, are spread in our country [4].

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Since secondary metabolites of family members are rich, most of them have medical and aromatic value [5]. Essential oil and phenolic compounds are also rich in flavonoids [6], and have antioxidant features [7, 8]. Species of the genus *Ajuga* include medicinal and pharmacological chemical compounds such as flavonoid, triglyceride, essential oil phytosteroid, diterpene [9, 10, 11, 12]. *Ajuga salicifolia* (L.) Schreber in Turkey "Sivrimayası" is known as [13]. Some species of the genus *Ajuga* are also valuable ethnobotanically. *Ajuga* types are used in [14], treatment of sore throat [15], jaundice, gout, joint pain, Fever, asthma, gut, hemorrhoids [16] and diabetes [17], as vasoconstrictor, blood thinner and blood purifier [18].

Morphological and anatomical features of some species of the genus *Ajuga* were examined [19, 20, 21, 22]. There are also studies on petiole anatomy and trichome morphology of some *Ajuga* taxa [23]. Pfeiffer revealed the morpho-ecological characteristics of the species *Ajuga reptans* L. and performed molecular analysis of this species [24].

In species with similar morphological features, significant differences may occur in the anatomical structures of vegetative organs such as roots, stems, leaves. Accordingly, anatomical characters have been used in taxonomy in recent years [25]. In our study, it is aimed to determine the morphological, anatomical and ecological features of *Ajuga salicifolia* (L.) Schreber plant, which was not examined morphologically, anatomically and ecologically, and to compare it with other species. We believe that the findings we obtain will also be a source for the studies to be conducted on other *Ajuga* species.

2. MATERIALS AND METHODS

The samples of *A. salicifolia* species, which is our research topic, were collected from Kırşehir province and its surroundings in June 2019 (Locality: Bağbaşı district, herbarium number: Sulcay361). The diagnoses of 15 samples collected were made according to Flora of Turkey [3]. Plant samples were taken in 70% alcohol and fixed. For anatomical examinations, the cross-

section from the root, stem, and leaf were by hand (Figure 2, figure 3A). In addition, superficial sections were taken from the leaf (Figure 3B, C). The sections taken were made into a permanent preparation by the glycerin-gelatin method [26]. In anatomical examinations, epidermis, collenchyma, parenchyma, in root, stem and leaf sections, and trachea and phloem elements were measured. Photographs were taken with the Nikon Eclipse Ni-U microscope and imaging system from the anatomical sections of the species. The number of stomata and epidermis cells in plants was calculated from the area of 1mm² on the lower and upper surfaces of the same age leaves of the plant [27].

For ecological studies, soil samples have been taken from the area where plant samples were collected. During the fieldwork, after removing the upper surface of the soil, a section of approximately 1 kg was taken with depth and diameter in the range of 0-20 cm. After these samples were dried in air and passed through a 2 mm sieve, they were made ready for analysis [28]. Soil analyzes have been conducted in two replications. Saturation percentage was obtained by saturating the colloid surface areas with water [29]. The pH and total salinity determinations were made in the saturation sludge [30,31]. Organic material has been classified by using modified Walkley-Black wet burning method [32]. Total lime was made with Scheibler calcimeter [33] and it was classified according to Ülgen and Yurtsever [34]. Useful phosphorus determination was made by extracting soils with sodium bicarbonate (pH: 8.5, 0.5 N NaHCO₃) [35]. Changeable potassium was determined ([36] by extracting it with ammonium acetate (pH: 7, 1 N NH₄OAc). Among the elements passing into the solution phase, concentration of phosphorus was determined by using the UV-VIS Spectrometer device, while the concentration of potassium was determined by using the Flame Spectrometer device.

3. RESULTS AND DISCUSSION

3.1. Morphological Results

The stem is upright, simple and occasionally accumbent. The stem is colored red from the basal

part to the middle and is covered with hispid hairs. The stem is cylindrical in shape and branches from the base part. The leaves are oblong-lanceolate, oblong-elliptical in shape, opposite order, short petiole.



Figure 1 General view of *A. salicifolia*, ca: calyx, fr: fruit, lf: leaf, p: petal, st: stem

The terminal of the leaf is acute. Base of floral leaves is truncate. There are pilose hairs on the leaf. The flowers are verticillate and two flowered. Corolla is yellow and forms a tube on the base of the corolla. The number of petals is 4. 3 of the petals are reduced. One of these petals are reduced more than the other two. The outside of the petals has velutinous hairs. The long petal has two gears and there are red spots on it (one or

two). The style is bifurcated, and has the same length or shorter than the pistil corolla. The number of stamens is four. The calyx is green in color and consists of four sepals. Calyx takes the form of a tube towards the base. The fruit is nutlet (Figure 1).

Table 1 Morphological measurement results of *A. salicifolia* plant

Parts of plant	Length Min. (mm)	Length Mak (mm)	Length Mean- SD. (mm)	Breadth Min.(mm)	Breadth Max. (mm)	Breadth Mean – SD. (mm)
Stem	135	170	150,83±5,23	0,4	3,0	1,51±0,23
Root	3,5	9,0	6,900±0,88	4	13	9,33±1,30
Petiole	2	7	5,50±,56			
Leaf	12,0	40,0	25.17±4.96	7	20	13.5±4.66
Sepal	6,0	13,0	8,518±0.78			
Petal	12	30	22,00±2,22			
Pistil	12	25	17,78±1,234			
Fruit	1,5	3,0	2,15±0,15	-	-	

Anatomical Results

Periderm consists of 1-2 rows of rectangular shaped cells in the root cross-section of *A. salicifolia*. The cortex consists of polygonal shaped large cells. Phloem cells are 7-8 rows. Xylem is evident. Cells irregularly arranged in the pith region are circular and parenchymatic (Figure 2A).

Epidermis cells are 1-2 rows on the outer part of the stem cross-section of the plant, they are circular, square shaped, and the stem is slightly angular. Capitate glandular (Figure 2D) and 1-3 cell eglandular hairs (Figure 2C) are seen on the epidermis. The vascular bundles are in a continuous ring. Phloem is 3-4 rows and circular shape. There is sclerenchyma with 1-2 row and continuous ring on the phloem. The cortex consists of 4-6 layer cells. The cortex cells are circular and polygonal in shape. There are collenchyma cells in the corners of the cortex towards the epidermis. There are 3-4 rows of cambium (Figure 2B).

Epidermis cells are circular and rectangular in *A. salicifolia* leaf cross-section and its walls are wavy. Papillae (Figure 3D) and simple eglandular (Figure 3F) with 1-4 cells are seen on the epidermis. In addition, short-stem glandular hair are present on the epidermis (Figure 3E). There are 3-4 rows of palisade parenchyma and 2-3 rows of spongy parenchyma in the cross-section of *A. salicifolia* leaf and is mesophyll bifacial.

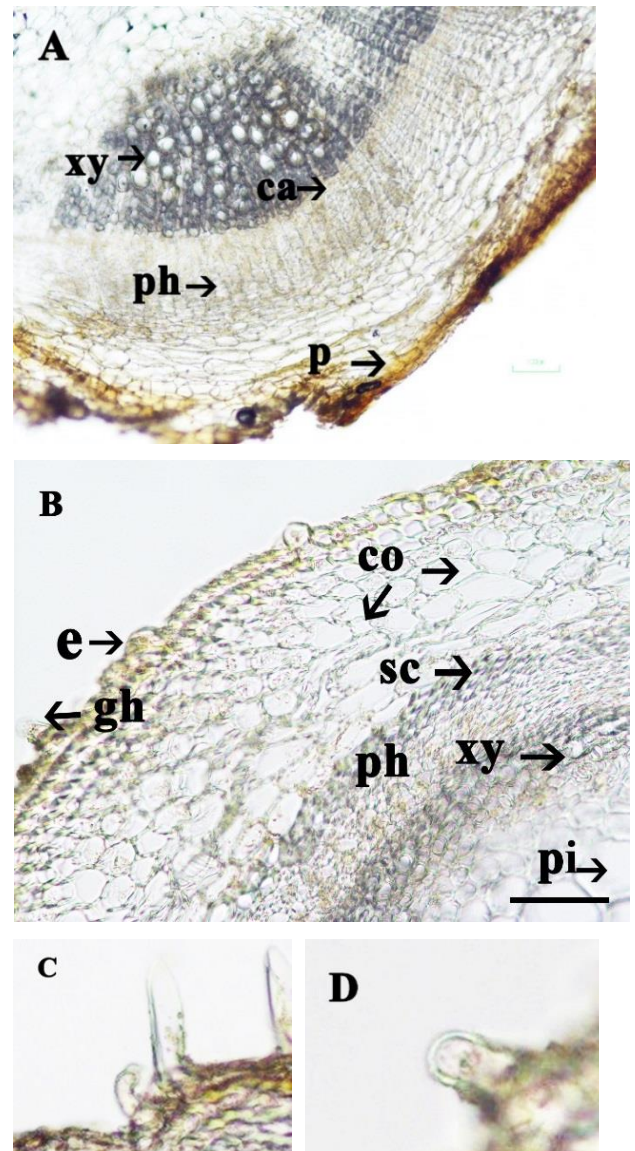


Figure 2 A Root cross section of *A. salicifolia*, B Stem cross section of *A. salicifolia*, C eglandular hair, D glandular hair, ca: cambium, co: cortex, e: epidermis, gh: glandular hair, p: periderm, ph: phloem, pi: pith, xy: xylem (Scale bar 100 μ m)

Table 2 Anatomical measurement results of *A. salicifolia*

	Width (μm) Mean \pm SD.	Length (μm)-Mean \pm SD.
Root	Periderm	17.30 \pm 0.64
	Diameter of cortex cells	72.77 \pm 5.54
	Diameter of phloem	12.95 \pm
	Diameter of xylem	24,69 \pm 1,69
	Cell of pith	45.12 \pm 2.34
Stem	Epidermis cells	17.31 \pm 1.28
	Diameter of collenchyma cells	15..49 \pm 1.62
	Diameter of cortex cells	30.91 \pm 2.49
	Diameter of pith cells	79.71 \pm 7.33
	Diameter sclerenchyma cells	7.3789 \pm 0.36
	Diameter of phloem	9.17 \pm 1.40
	Diameter of xylem	28.22 \pm 2.75
Leaf	Upper epidermis	39.70 \pm 0.68
	Lower epidermis	16.19 \pm 1.08
	Palisade parenchyma	21.78 \pm 0.42
	Diameter of spongy parenchyma	9.08 \pm 0.69
	Diameter of xylem	6.71 \pm 0.60

Table 3 The stoma features on the upper and lower epidermis of *A. salicifolia*

	Lower surface mean/SD.	Upper surface mean/SD.
Stomata width (μm)	22.09 \pm 0.65	21.36 \pm 0.77
Stomata length (μm)	29.69 \pm 1,11	28.71 \pm 1.02
Number of stomata (1 mm ²)	82	36
Number of epidermis cells (1 mm ²)	187	267
Stomata index	30.4	11.88
Type of stomata	Anomocytic	Anomocytic

There is one vascular bundle in the median vein. Stomata on the lower epidermis is more numerous. There are 82 stomata in 1 mm² area and the stomata index is 30.4. On the upper surface, the number of stomata is 36 and the stomata index is 11.88. There are anomocytic stomata on both surfaces.

3.3. Ecological Results

The habitat of the *A. salicifolia* open areas, field edges and slopes areas. Species has been recorded from 1078 m. Saturation percentage of soil where the species grow is 107.8% and the soil texture is

clayey. It has a pH value of 8.23 and it is slightly alkaline. Total amount of water-soluble salt of the soil is 0.02% and it is classified salt-free soil. It is 22.71% in terms of lime and it is considered as very calcareous soils. With respect to chemical features, available phosphorus amount of the soil is 0.8 kg/da and it is classified among very low phosphorus soils. Amount of potassium that can be obtained is 233.7 kg/da and it is classified as high. Amount of organic matter is 0.4% and it is classified in the group of high hummus soils (Table 4).

Table 4 Soil characteristics of *A. salicifolia*

Physical properties					
Saturation (%)			Texture		
107.8			Clayey		
Chemical properties					
Total Salt Soluble in Water (%)	pH	Lime (%)	Organic Matter (%)	Phosphorus (kg/da)	Potassium (kg/da)
0.02	8.23	22.71	0.4	0.8	233.7

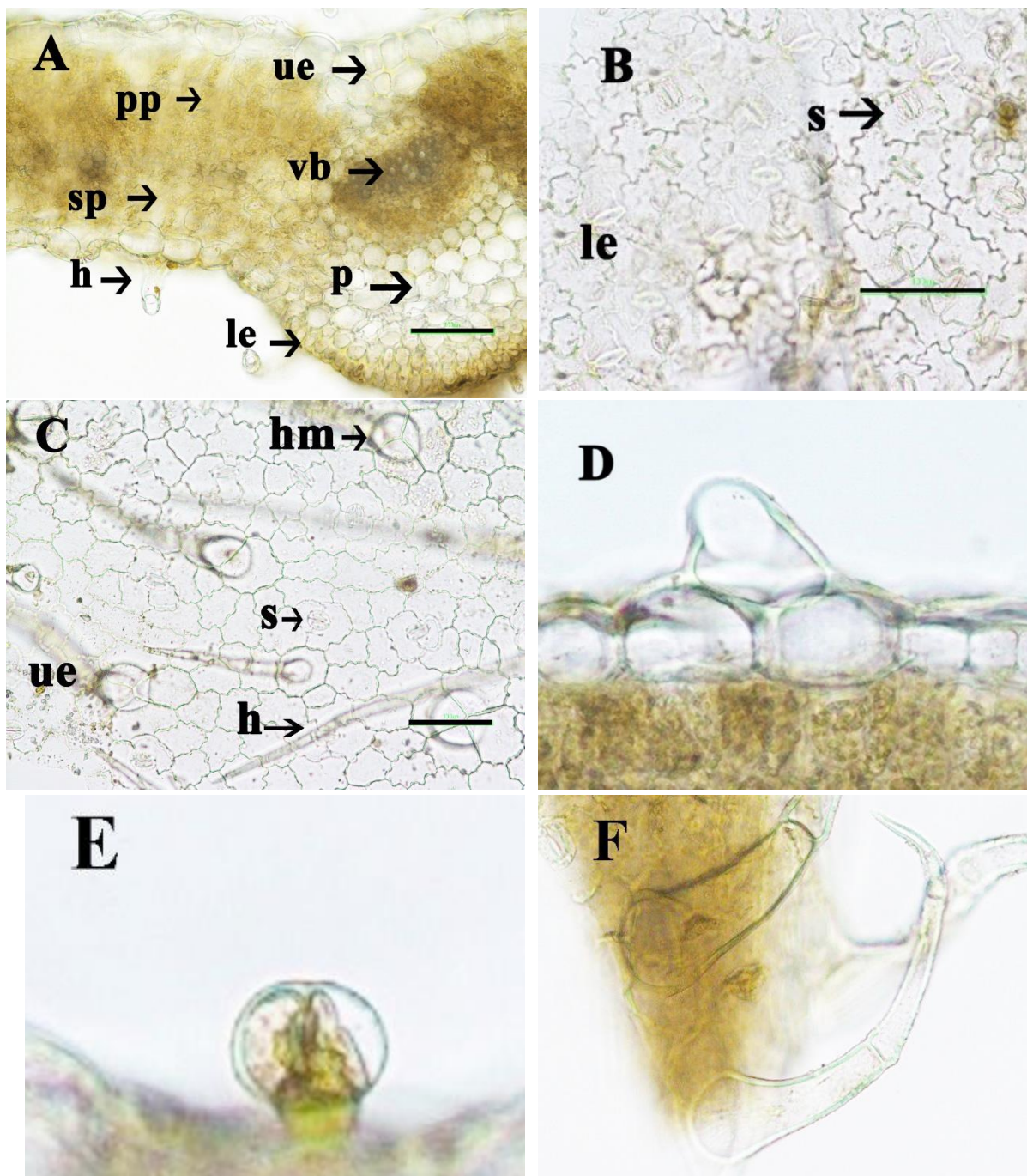


Figure 3. A Leaf cross section of *A. salicifolia*, B lower surface section of leaf, C upper surface section of leaf, D appearance of the papillae, E glandular hair, F eglandular hair, le: lower epidermis, h: hair, hm: main hair cell, p: papilla, pp: palisade parenchyma, s: stomata, sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle (Scale bar 100 μ m)

With this study, the morphological and anatomical features of the species of *A. salicifolia*, which belongs to the naturally distributed Lamiaceae family, are revealed.

Stem of *A. salicifolia* with pilose hair is about 15 cm in length. The stem length of *A. chamaepitys* (L.) Schreber subsp. *chia* (Schreber) Arcangeli var. *chia* has been reported to be 7-15 cm [19]. The shape of the leaves is usually oblong-lanceolate. The length of the petals is 12-30 mm. The petal length of *A. reptans* is 9-16 m [19]. One or two red spots are visible on the top of the petals. There are 2-3 rows of periderm outside the root of *A. salicifolia*. Epidermis is seen in the outmost part of the root of the species of *A. reptans* [19]. While parenchymatic cells are present in the *A. salicifolia* pith region, the root pith area is covered with secondary xylem elements in *Ajuga relictata* P.H. Davis taxon [22].

There are 1-2 rows of epidermis at the outermost part of the stem of *A. salicifolia*, and the stem is noticeably cylindrical. Metcalfe and Chalk state that the characteristic feature of the Lamiaceae family is the rectangle stem [37]. *A. salicifolia* stem shape complies with the general feature of the family. Collenchyma cells are seen just below the corner parts of the species that is our research topic. In the family's *Ajuga postii* Briq. and *A. relictata* species and *Lycopus europaeus* L. species, collenchyma cells were sequenced in the same way [22; 38]. Capitulate glandular and 1-3 cell eglandular hair is seen in the stem cross-section of the *A. salicifolia* plant. Similar hairs are seen in *A. reptans* L. and *A. chamaepitys* subsp. *chia* var. *chia* [19]. Metcalfe and Chalk state that glandular hairs are diagnostic for the Lamiaceae family [39]. In the cross section of the stem of *A. salicifolia*, a continuous ring-shaped sclerenchyma cells are seen just below the phloem. Erkara and Koyuncu mention about the presence of sclerenchyma in a small area above the xylem in the stem of the *A. reptans* species [20].

There are 3-4 rows of palisade parenchyma and 2-3 rows of spongy parenchyma in the cross section of *A. salicifolia* leaf, and is mesophyll bifacial. Sonmez and Kose state that *A. postii* leaf has a

bifacial type and diacytic stomata [22]. The number of stomata on the lower leaf surface of *A. chamaepitys* subsp. *chia* var. *chia* is higher than the number of stomata on the upper surface [19]. This feature is similar to *A. salicifolia* species.

As a result, in *A. salicifolia*, the redness on the stem and petal, the leaves being generally oblong-lanceolate, the hair on the petal and stem were observed morphologically. The presence of 7-8 layered phloem and pith on the root, the presence of glandular and eglandular hairs on the stem, the formation of a continuous ring of vascular bundles, the presence of a continuous ring-shaped sclerenchyma on the phloem, the waviness of the epidermis walls on the leaf, the number of palisade and spongy parenchyma layers, hair condition and types, are important features for *A. salicifolia*. The plant, which is our subject of study, spreads in open areas, field edges, and slopes. The soils in which it grows are clayey, slightly alkaline, salt-free, poor in phosphorus, and rich in potassium and organic matter.

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