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Morpho-anatomical, palynological studies on *Astragalus microcephalus*; Anatolian tragacanth

Ayla KAYA

Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskisehir, Türkiye.
Corresponding Author. E-mail: aykaya@anadolu.edu.tr (A.K.); Tel. +90-222-3350580

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ABSTRACT: *Astragalus microcephalus* Willd. (Fabaceae) is a member of the section Rhacophorus Bunge and is an element of the Irano-Turanian phytogeographic region. It grows mainly in the steppes of central Anatolia. Extraction of tragacanth and selenium is a valuable tool in pharmacy and is also important economically and ecologically. In this study, morphological, anatomical, pollen characteristics and seed surface properties of *A. microcephalus* were investigated in detail. The plant consists of thorny bushes that make up a pillow. Morphological characters such as the shape of paripinnate leaves, stipules and bracts, flowers whitish-yellow and purple colored in margins, the flower number in the inflorescence, the stenonychioid type of standard and reniform type of seed can be helpful to distinguish species of *Astragalus*. There is secondary growth in stem anatomy. The stem of this species has got tragacanth canals in pith. Sclerenchyma tissue is well developed in the petiole. *A. microcephalus* has equifasial leaves. The vascular bundles are surrounded by a bundle sheath. The pollen grains of *A. microcephalus* are tricolpate and prolate-spheroidal. The exine sculpturing is microreticulate with perforate while the seed surface is rugulate. Scanning electron microscopy (SEM) is used to determine pollen and seed morphology.

KEYWORDS: Anatomy; *Astragalus microcephalus; m*orphology; pollen

1. INTRODUCTION

Some *Astragalus* L. (Fabaceae) species such as *A. microcephalus* Willd., *A. aureus* Willd., *A. brachycalyx* Fischer, *A. gummifer* Lab. and *A. kurdicus* Boiss. are economically valuable because of produce selenium and tragacanth gum [1]. Tragacanth gum is recorded Tragacantha or Gummi Tragacantha in the name codex and pharmacopoeias. Top quality glue Fiyor is known by the name and it is used for the pharmaceutical emulsion, lozenges and tablets preparation in pharmacy. While it has numerous applications in pharmaceuticals as, a thickening agent in foods, it may also have applications in controlling cancer cells. In addition, the adhesive in the fabric and paper industry and finishing is also used as the transmitter in a pharmacy outside. Tragacanth gum is also the most important export product of Turkey. *Astragalus* species are also ecologically important because most of them provide pillows and they play a major role in preventing erosion. However, they are consumed in mountainous and rural areas as firewood and animal feed [2].

Astragalus is the richest genera of vascular plants with about 2530 taxa and 245 sections worldwide and 459 taxa and 63 sections in Turkey. It has high endemism rates (51%) in Turkey [3-5]. The sections of the genus are determined based on morphological characteristics such as stem length, stipule connection, leaf shape, inflorescence form and fruit state [6]. Although there are some systematical [7, 8], anatomical [5, 9-13], palynological [5, 13-20] and micromorphological [5, 20-22] studies, some systematic problems with *Astragalus* species are still unresolved [16].

A. microcephalus Willd. is a member of the section Rhacophorus Bunge, in the form of a spiny shrub, consisting of 140 species. Furthermore, Rhacophorus is one of the most important and difficult groups. *A. microcephalus* is one of the most problematic species in this section [3, 11]. Moreover, it is one of the most common and variable species in Turkey [3] and is known as Anadolu kitresi [23].

Here, it is aimed to contribute to the Flora of Turkey by investigating the morphological, anatomical (stem, leaflet and petiole), and palynological features of the plant in detail.

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2. RESULTS

2.1. Morphological

Astragalus microcephalus consists of cushion-forming shrubs. Leaves paripennate. Leaf rachis 1,3–3,4 cm, spiny, erect and below pubescent. Leaflets in 4-5 (-6)-pairs, 4-14 x 1-3,1 mm, elliptic, spine-tipped (0,3–1,6 mm) at the apex, cuneate at base, densely covered on both sides with long and white tomentose. Stipules 6–11 x 5-9 mm, narrowly lanceolate, densely covered white-haired in below, ciliate at above margins, united for half their length.

Flowers sessile, 2-3 per leaf axil. Inflorescence globose to cylindrical 0,5–1,3 cm diam, 10-30 flowered. Bracts 4–6 mm, linear-spathulate, carinate, dense and long-haired, acuminate at the apex (0,5–1 mm). Bracteoles absent. Calyx 5–7 mm, densely pilose but glabrous at the base; calyx five lobed and lobes divided almost to the base. Corolla whitish-yellow with purple veins. Standard stenonychioid type, 8,5-10,3 mm, oblong–oblanceolate, obtuse-rotundate at apex; large in the upper part, largest in the middle part, narrowed at base. Wings 8-10 mm, triangular, obtuse at the apex, clearly constricted above the auricle. Keel 8,5–9, 3 mm, blades falcate. Stamenler 8,5–9 mm, diadelphous. Ovary 4-5,2 x 0,8–1,5 mm, elliptic, dense and long-haired.

Legume 5-6,5 x 2,5–4 mm, broadly elliptic–ovate, dense and long-haired, one loculus and one seed. Seeds 2,5-3 x 1,3-2 mm, yellowish-brown, dark-brown, black spotted, reniform shaped and seed surface is rugulate (Figures 1-4).
Flowering: June
Fruiting: August
Habitat: Steps
Altitude: 850–2700 m
Distribution: Mainly Inner and East Anatolia

Figure 1. a. *Astragalus microcephalus* in natural **b.** Distribution of *Astragalus microcephalus* in Turkey

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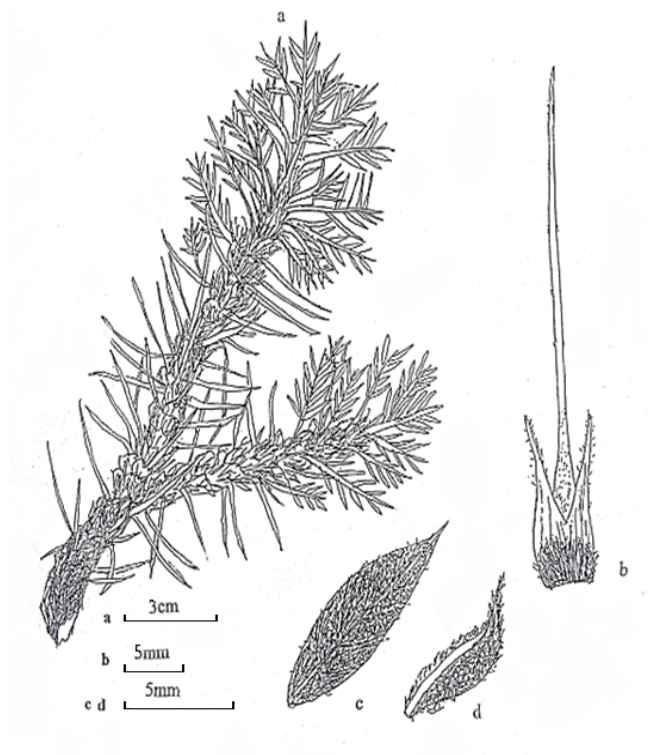

Figure 2. *A. microcephalus,* a. Habit, b. Stipule c. Leaflet, d. Bract

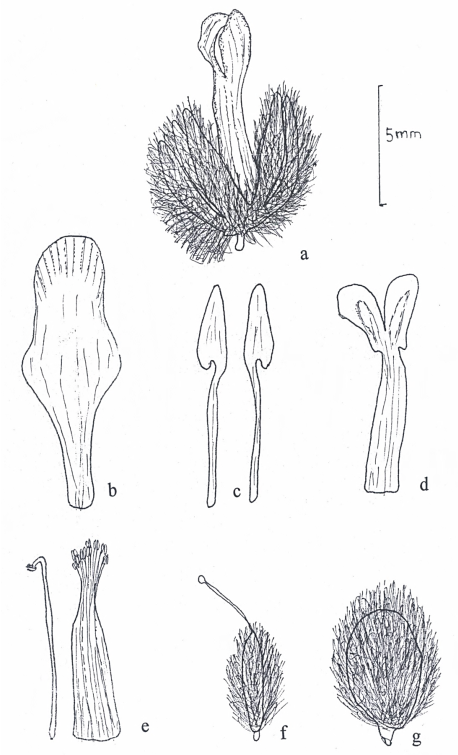

Figure 3. *A. microcephalus,* a. Flower, b. Standard c. Wing, d. Keel, e. Diadelphous stamens, f. Ovary g. Legume

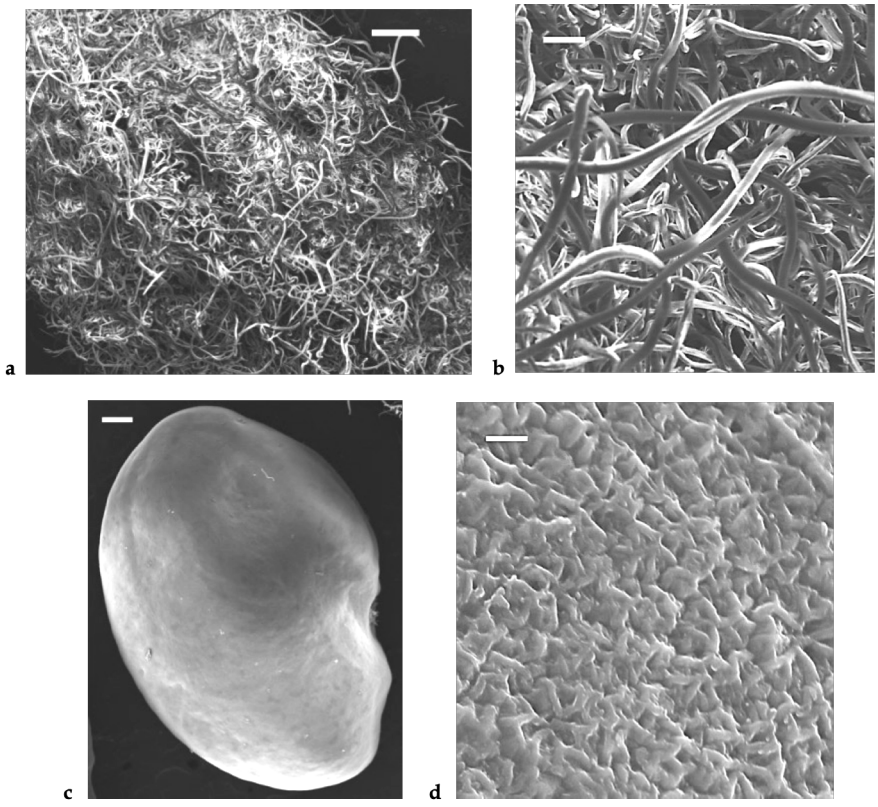

Figure 4. Leaflet upper surface view of *A. microcephalus* (SEM): **a**. 200 µm, **b**. 40 µm; **c**. Seed view of *Astragalus microcephalus*: 200 µm, **d**. Seed surface 4 µm

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2.2. Palynological

Pollen grains are radially symmetrical, isopolar, tricolporate. The polar axis is 17.4-20.8 µm and the equatorial axis is 15.8–19.4 µm. P/E 1.07-1.1 µm. Their shape of them is prolate-spheroidal. Amb is also rotundate. Colpi narrow and long, margin fairly clear. Clg 16.3–19.0 µm, Clt 1.6–2.4 µm. The shape of the pores is ellipsoid-circular, the length of the pores is 5.5–7.0 µm, and the width of the pores is 5.0–6.9 µm. Exine sculpturing is microreticulate with perforate (Figure 5).

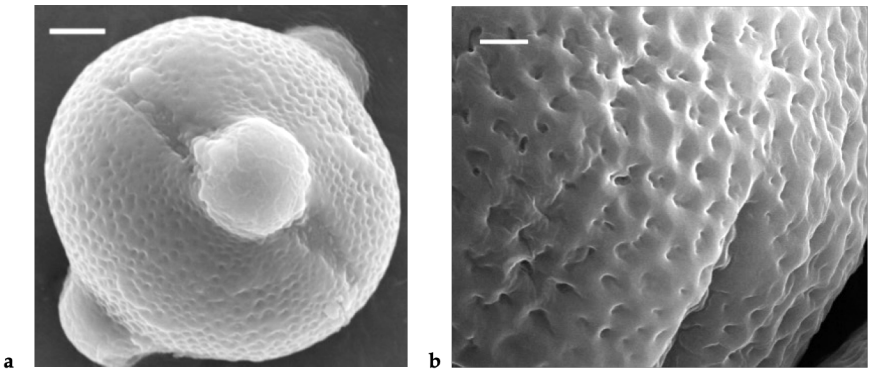

Figure 5. *A. microcephalus* (SEM); **a**. pollen grain (1 µm) **b**. ornemantasyon (3 µm)

2.3. Anatomical

2.3.1. Petiole

The shape of the transversal section of the petiole is almost orbicular. The uppermost of the petiole is a thick cuticle layer and there is an epidermis with one-layered cells under the cuticle. The collenchyma tissue under the epidermis consists of 4-5-layered and transversally oval-rectangular shaped cells. Vascular bundles are found in sclerenchyma tissue. While the larger one of these bundles is located in the center, 7-8 smaller ones are located partially around the larger one. The vascular bundles are arranged as phloem on the outside and xylem on the inside. The big vascular bundle in the center is elliptically shaped while the others are broadly ovate, ± triangular or almost rounded shaped. Pith is composed of scleranchymatic cells which are polygonal shaped (Figure 6).

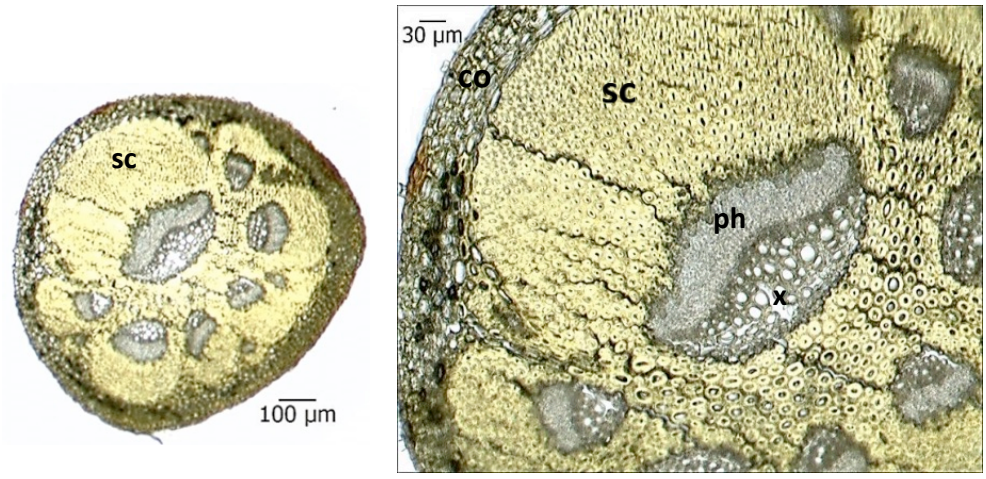

Figure 6. Transverse section of the petiole. **a**-general view **b**-magnification view, co. collenchyma, sc. sclerenchyma, ph. phloem, x. xylem

2.3.2. Leaflet

In the transverse section, the upper and lower epidermises consist of a single row of oval and rectangular cells and they are covered by a thick cuticle. Cells of the upper epidermis are larger than those of the lower. There are non-glandular trichomes in both epidermises and they are dense, long and curled. The stomata are of the anomocytic type and are found on both leaflet epidermises, more abundant on the lower surface. They are located on the epidermal cell level or at a slightly lower level than the epidermal cells. The leaflets are the equifacial type which consists of cylindrical palisade parenchyma cells, 2-layered, on both surfaces of the leaflets. Spongy parenchyma is one layered or absent. The midrib region is protruding and bigger than the others. There are also 5-6 small vascular bundles in lateral regions. The vascular bundles are; collateral type, and surrounded by a parenchymatic bundle sheath. A group of scleranchymatic cells is found below the phloem and they are thick walled and polygonal shaped. The xylem occurs in a narrow area and it faces toward the adaxial epidermis while the phloem is surrounded by the xylem and faces toward the abaxial epidermis (Figure 7).

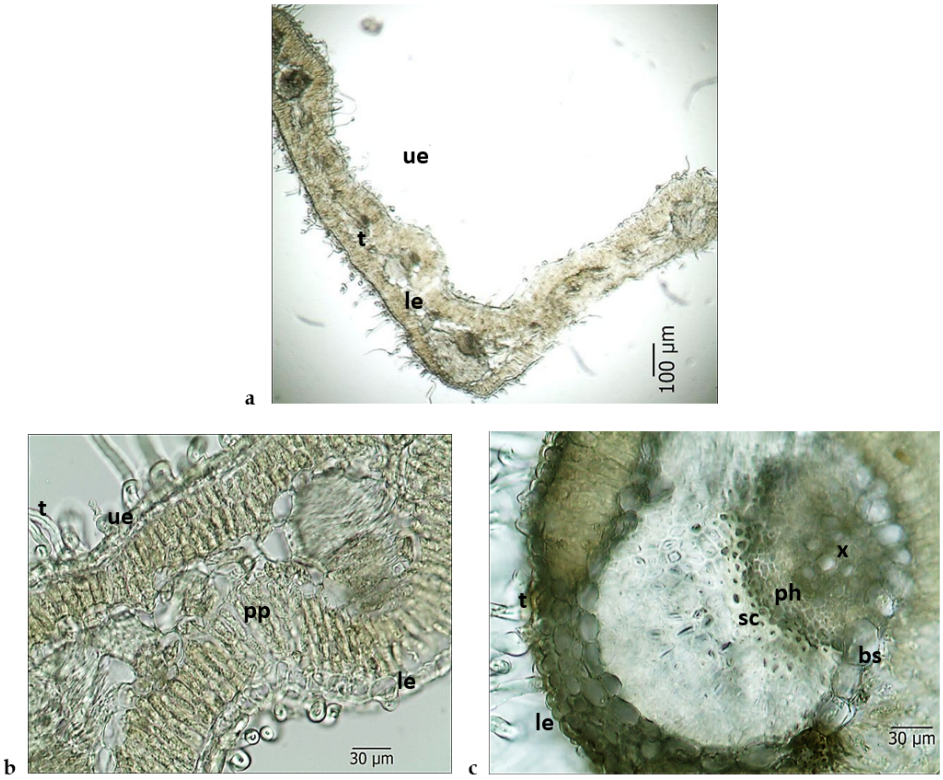

Figure 7. Transverse section of Leaflet. **a.** general view, **b.** mesophyll, **c.** midrib bs. bundle sheat t.trichome ue. upper epidermis, le. lower epidermis, pp. palisade parenchyma, ph. phloem, x. xylem

2.3.3. Stem

In the transverse section, the epidermis consists of a single layer of almost small, square cells and is covered with a thick cuticle. Covering trichomes consists of the non-glandular type which is simple, dense and long. The collenchyma tissue under the epidermis occurs from 5-10 layered. The primary cortex, which is composed of parenchyma cells, is 3-7 layered in between the collenchyma and sclerenchyma. The parenchyma cells are oval-rectangular shaped and include starch. The secondary cortex, which is composed of sclerenchyma cells, is located on the vascular bundles which are of the collateral type. The shape of phloem cells is irregular-polygonal. The endodermis could not be distinguished. The cambium is 2-3 layered with a flattened, rectangular shape. There are secondary xylem under the cambium and the primary xylem faces toward the pith. Rays are 3-9 layered and are included starch. In the upper sections of stems, the pith is parenchymatic and contains abundant starch. After several layered parenchyma cells, tears occur in the middle and lower stems and a wide tragacanth cavity is formed in the pith (Figure 8).

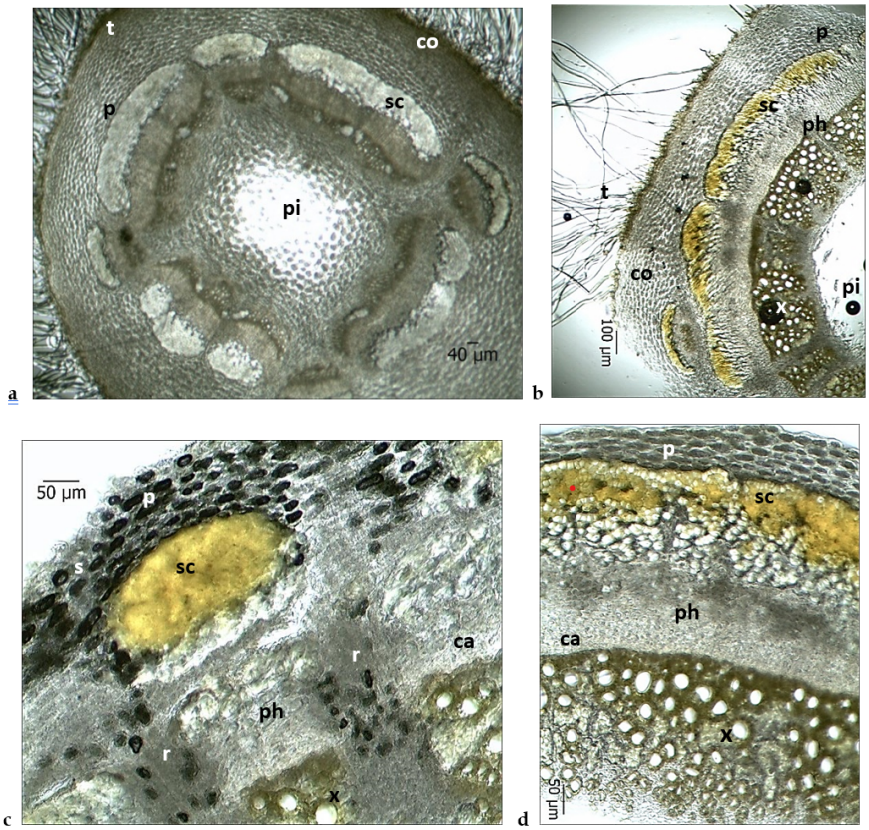

Figure 8. Transverse section of stem. **a**-general view **b**-general view partly **c**-middle part **d**-magnification view; ca-cambium co-collenchyma ph-phloem x-xylem p-parenchyma s-starch pi-pith r-ray sc-sclerenchyma t-trichome

3. DISCUSSION

This study investigated the detailed morphological, anatomical and palynological characteristics of *A. microcephalus*, which are extremely important because of their production of tragacanth gum and selenium. All morphological characters are described in morphological studies. Our results are compared with Flora of Turkey in Table 1. According to the table, our results are almost similar to Flora of Turkey [3]. However, the wing, keel, stamen, ovarium, legume and seed properties are described firstly in this present study.

Table 1. To compare with Flora of Turkey of *A. microcephalus* morphological properties

	Our findings	**Flora of Turkey**
Petiole	1,3-3,4 cm	1,5-2,5 cm
Leaflet	4–14 x 1,1 -3,1 mm	5 -14 mm
Stipul	6-11 x 5–9 mm	6 – 10 mm
Inflorasens diam	0, -1,3 cm	1-1,5 cm
Flower number	10-30	10-30
Bract	4-6 mm	4-6 mm
Calyx	5–7 mm	5–6 mm

Standard	8,5-10,3 mm	8–10 mm
Wing	8–10 mm	-
Keel	8,5–9,3 mm	-
Stamen	8,5–9 mm	-
Ovary	4-5,2 x 0,8 -1,5 mm	-
Legume	5-6,5 x 2,5–4 mm	-
Seed	2, -3 x 1,3–2 mm	-

A. microcephalus is a member of the Rhacopharus section and it is close to those species: *A. erinaceus, A. adustus* and *A. crassinerrus*. The calyx base is glabrous in *A. microcephalus* and it is easily separated from other species in the section [3]. The shape of *A. microcephalus's* stipula is the most important character that differentiates it from its close species. In *A. microcephalus* stipules are narrowly lanceolate while in other species they are broadly or triangular ovate. The smallest rachis is also found in the *A. microcephalus.* In addition, the figures showing the morphological features of the species, which contain systematic values, are given in detail, thus completing the missing information of *A. microcephalus* in the Flora [3]. *Astragalus* species' seeds are reniform shaped and usually have rugulate or rugulate-reticulate surfaces and they are almost the same as our seeds [8, 18, 20, 21].

A. microcephalus' pollen is radially symmetrical, isopolar, tricolporate and prolate-spheroidal shaped. Amb is also rotundate. Colpi narrow and long, margin fairly clear. The shape of the pores is ellipsoid-circular. Exine sculpturing is microreticulate with perforate. In Turkey, pollen grains of *Astragalus* species are generally stenolapynous and radial symmetry, isopolar, tricolpate (rarely tetracolpate) and microreticulate, microreticulate-perforate (rarely reticulate or rugulate) show ornamentation [5, 13-20]. Our pollen findings are similar to the previous evidence. However, some features were different. *A. microcephalus'* pollen is prolate-spheroidal shaped in the present study and Özbek et al.' study [5] while it was reported as prolate by Atasagun et al. [13], prolate, subprolate and prolate-spheroidal in sect. Onobrychoidei by Pınar et al. [17] and in sect. Hololeuce by Çeter et al. [18], oblate-spheroidal in sect. Hymenostegis by Bagheri et al. [19] and subprolate and prolate-spheroidal by Metin et al. [21]. There is also no information about pore characteristics in Özbek et al. [5] and Metin et al.' studies [20].

As a result of anatomical studies, the petiole, leaflet and stem structure of the species were determined in detail and It has been observed that the leaflet of *A. microcephalus* shows xeromorph plant characteristics such as the presence of a thick cuticle, dense trichomes, and the absence or very little sponge parenchyma [24]. The anatomic result of *A. microcephalus* has been compared to the study of different species of *Astragalus.* In Uysal's study [10], the stem and petiole anatomic characteristics of the *A. trojonus* show similarity to that of *A. microcephalus.* In addition, parenchymatic cells containing starch in the stem of *A. microcephalus* is similar to *A. trojanus* [10] and *A. argeus* [13]. In previous studies, cross sections of the petiole of some *Astragalus* species in Rhacophorus [11], and Incani sections [12] have investigated and reported similar findings as the outline of the petiole, a single layer epidermis, the number and size of vascular bundles and surrounded by sclerenchyma tissue of each vascular bundle. The equifacial leaflet type, absence or very little spongy parenchyma, anomocyctic stomata type, presence of stomata in both leaflet epidermises, presence of trichome and bundle sheath and the structure of the vascular bundle are similar to the studies by Metcalfe and Chalk [9], Uysal [10], Atasagun et al. [13] and Özbek et al. [5].

4. CONCLUSION

In this study, morphological, anatomical and pollen properties of *A. microcephalus* have been studied in detail. Morphological characteristics such as the shape of paripinnate leaves, stipules and bracts, the structure of the flower, fruit and seed can be helpful to distinguish *A. microcephalus.* In addition, the wing, keel, stamen, ovarium, legume and seed properties are described firstly in this present study and all parts of the plant are supported by drawing contributing to the Flora of Turkey. The stem, leaf and petiole characters of the plant were examined in anatomical studies. Secondary growth, dense-long trichomes and the presence of amylum

are remarkable in stem anatomy. The plant has equifacial leaves and anomocytic stomata. Sclerenchyma tissue surrounds the vascular bundles in the petiole. *A. microcephalus'* pollen has a tricolpate aperture, prolate-spheroidal shape and microreticulate-perforate surface.

5. MATERIAL AND METHODS

A. microcephalus was gathered during at flowering and fruiting periods (July and September; 2011) in Eskişehir (Eşenkara village) province of Turkey. Voucher specimens are stored in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskişehir (ESSE 14575), Turkey. Macromorphological observations were carried out under a binocular stereomicroscope, Olympus SZX12 with a drawing tube. Live material was stored in 70% ethanol for anatomical studies. All sections were manually taken from the middle regions of the plant and were in glycerin-gelatine and placed on microscope slides with Canadian Balsam. Photographs of anatomical sections were viewed with Olympus BX51 binocular light microscope and camera. For SEM study, pollen, seed and leaves were installed on SEM stubs using double-sided adhesive tape and covered with gold. Photos were taken with an electron microscope (Zeiss EVO 50).

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