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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Comparative Anatomical Features Study of the Some Medicinal *Rumex* Species Distributed in Turkey

Türkiye'de Yayılış Gösteren Bazı Tıbbi Rumex Türlerinin Anatomik Özelliklerinin Karşılaştırmalı Olarak İncelenmesi

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Abstract
Distributed in Turkey *Rumex crispus* L., *Rumex patientia* L., anatomical features of *Rumex obtusifolius* species were comparatively investigated in this study. For anatomical studies, cross-sections were taken from root, stem and leaf. In addition, superficial sections were taken from the leaf and stomata index and stomata features were determined. Druse crystals were found at the root of the *R. obtusifolius*. The radial-shaped vascular bundles in the root of *R. crispus* had 4-5 arms. Glandular hair was found only in the stem of *R. obtusifolius*. It is also characteristic that vascular bundles were scattered in the stem of this species. There was cuticle in the stem of *R. crispus*. Stem corner numbers of taxa also differed in terms of species. *R. crispus* was 10, *R. obtusifolius* was 8, *R. patientia* was 14 cornered. The number and type of collenchyma layer in the stem of the species were also different. Outward bulge was detected in the mid vein region in leaf cross-sections of taxa. This bulge was less pronounced in *R. crispus* and the most prominent in *R. obtusifolius*. At least the number of stomata was observed on the *R. obtusfolius* leaf. Leaf epidermis cell walls were wavy in *R. patientia*. A large number of druse crystals had been identified in the leaves of all species.
Keywords: *Rumex crispus*, *Rumex patientia*, *Rumex obtusifolius*, Anatomy, Medicinal plants.

Öz
Bu çalışmada, Türkiye'de yayılış gösteren *Rumex* cinsine ait. *R. crispus* L., *R. patientia* L., *R. obtusifolius* L. türlerinin anatomik özellikleri karşılaştırmalı olarak incelenmiştir. Anatomik çalışmalar için kök, gövde ve yapraktan enine kesitler alınmıştır. Ayrıca yapraktan yüzeysel kesitler alınarak stoma indeksi ve stoma özellikleri belirlenmiştir. *R. obtusifolius* kökünde druz kristallare rastlanmıştır. *R. crispus* kökünde 4-5 kollu demetler radyal şekilde dizilmiştir. Sadece *R. obtusifolius* gövdesinde glandular örtü tüyüne rastlanmıştır. *R. crispus* gövdesinde kutikula mevcuttur. Taksonların gövde köşe sayıları da türler açısından farklılık göstermektedir. *R. crispus* 10, *R. obtusifolius* 8, *R. patientia* 14 köşelidir. Türlerin gövdelerindeki kollenkima tabaka sayıları ve tipleri de birbirinden farklıdır. Taksonların yaprak enine kesitlerinde orta damar bölgesinde dışarıya doğru çıkıntı tespit edilmiştir. Bu çıkıntı *R. crispus*'ta az belirgin, *R. obtusifolius*'ta ise çok belirgindir. Yaprak yüzeylerindeki stoma sayısı en az *R. obtusifolius* türünde gözlenmiştir. *R. patientia* türünde yaprak epidermis hücre çeperleri dalgalıdır. Tüm türlerin yapraklarında çok sayıda druz kristali belirlenmiştir.
Anahtar Kelimeler: *Rumex crispus, Rumex patientia, Rumex obtusifolius*, Anatomi, Tıbbi Bitkiler.

I. INTRODUCTION

Polygonaceae, which has 32 genera and around 800 species in the world, was generally distributed in the Northern Hemisphere [1]. *Rumex* (Polygonaceae) genus, which is the only or perennial herbaceous plants that bloom in May-September, had about 200 species on earth, and 25 species and 7 hybrids were common in Turkey, 6 of which were endemic [2,3]. *Rumex* species contain anthropoids, tannins, flavonoids, naphthalene derivatives, leucoanthocyanidol, steroidal compounds, fixed oils and essential oils, saponosides and polysaccharides [3]. Some species had been found to be effective against fungi and bacteria. In addition, some species were found to show anti-inflammatory effect. Antitumoral, cardiovascular, antihistaminic, hematological and laxative activities were mentioned according to the substance groups they contain [4, 5]. It was also stated that some species of the genus show antioxidant and antimicrobial activity [6, 7]. Members of the family were used in folk medicine. *R. crispus* L. was useful in diseases such as skin, respiratory, digestive hemorrhoids, edema, excretion, sugar, rheumatism, diarrhea, pile, lung bleeding [8]. *R. patientia* L. was used as immunosuppressant and in the treatment of tonsillitis and *R. obtusifolius* in the treatment of hemorrhoids. *R. obtusifolius* L. seeds had been shown to be effective in the treatment of hyperglycemia by Aghajanyan et al. [9]. *Rumex* species were also important ethnobotanically. usually, leaves of *Rumex* species were consumed raw in salads, while stems, branches, and leaves were consumed by cooking [8,10,11].

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Despite being affected by ecological conditions, many anatomical characters helped to solve systematic problems. [12,13]. Therefore, taxonomists had explored anatomical characters that could support the diagnosis of species. Stems, and especially leaves, were some of the few anatomical characters that had proven important in classification [14, 15]. Carlquist studied the wood anatomy of the Polygonaceae family. Carlquist reported that some species belonging to the family possess features such as contain nonbordered perforation plates, the existence of vested pith on the vessel, the existence of silica bodies [16]. Micco and Aronne, in a study related to *Rumex scutatus*, determined that the plants on steep slopes roots develop asymmetrically, the lateral roots act like connecting rods. They also found that in roots growing on flat ground, lignified xylem was surrounded by non-lignified parenchyma cells filled with starch [17]. Ghazalah et al. investigated foliar anatomy of some *Rumex* species. They reported that epidermal cells were generally polygonal in shape, and glandular and non-glandular trichomes showed variation [18]. Sahney and Vibhasa examined the body anatomy of *Rumex hastatus*. They reported that the stem epidermis of *R. hastatus* had sessile mucilage glands and druse crystals [19]. Soleimani et al. they revealed the anatomical and palynological characteristics of *Rumex* species [20]. Goremykina and Dinekina reported that the maximum vessel diameter in the stem of some species belonging to the Polygonaceae family supported kinship relations between the species.

The aim of this study is to investigate the anatomical features of some medicinal species belonging to the genus *Rumex* and to provide a source for the research to be done with *Rumex* species. In addition, the study is thought to will be beneficial in the diagnosis of *Rumex* species.

II. MATERIALS AND METHODS

The samples of *Rumex crispus*, *R. patientia* and *R. obtusifolius*, which were selected as research subjects, were collected from various parts of Tokat in 2015-2016 (Table 1). Species were identified according to Flora of Turkey [22]. The sections from 15 plant samples were made into a permanent preparation according to the glycerin gelatin method [23]. The cell types obtained from the root, stem and leaf sections of the species were determined by using Upright Microscope Eclipse Ni-U imaging system and photographed. Cell measurements were made from transverse and superficial sections of taxa. Stoma and epidermis cell numbers per 1 mm^2 were found on the lower and upper surfaces of the leaves of the same age (Table 5) and the stoma index was calculated [24] .25 measurements were taken from tissues such as epiderma, periderma, parenchyma, collenchyma, and sclerenchyma (Table2, Table 3, Table 4).

Table 1. General information about taxa.

Taxa	Locality and date of collection	Coordinates	Altitude (m)	Voucher
R. crispus	Pazar (Districts), Tatar Village	40°16′46″N,36°17′21″E; 40°16′20″N,36°14′38″E	572m, 555m	SULCAY344. 04.06.2015
R. patientia	Tokat-Ulaş Village	40°18′48″N,36°26′15″E;	645m	SULCAY337. 04.08.2016.
R. obtusifolius	Tokat-Kızılköy Village, Pazar (Districts)	40°16′54″N,36°17′33″E; 40°22′52″N,36°40′37″E	567,656m	SULCAY338. 04.08.2016

III. RESULTS AND DISCUSSIONS

3.1. Root Anatomical Features

Rumex crispus had thickened rhizome. Root epidermis cells were 1-2 layered, wavy-walled and polygonal. Radial bundles with 5-6 arms were found in the transverse sections of the roots taken from the rhizome. Endodermis cells were circular in shape. The cortex consisted of plenty of aerenchyma cells. These cells were polygonal shaped. The cortex was 9-10 layered (Figure 1-A).

R. obtisufolius had a secondary root. The root peridermis of *R. obtisufolius* had 3-4 layers and were circular or quadratic shaped (Table 2). The cortex was narrow and there were many gaps between the parenchyma cells. Cambium was unclear, phloems were crushed (Figure 1-B).

R. patientia had rhizome. Cross-sections were taken from the lateral roots on the rhizome and were showed that the central cylinder was radially shaped. The peridermis cells had 3-4 layered and were square, rectangular or polygonal shaped. Cortex cells were polygonal or circular shaped (Figure 1-C). Druse crystals were observed in the cortex (Figure 1-D, Table 2) and cortex consisted of aerenchyma cells (Figure 1-C).

Table 2. Comparative root features in *Rumex* species studied

Properties of species	*R. crispus*	*R. obtusifolius*	*R. patientia*
Layer of epidermis	1-2 layered	-	-
Druse Crystal	Absent	Absent	Present
Layer of peridermis	-	3-4 layered	3-4 layered
Xylem arm count	5-6	-	-
Endodermis	Present	Absent	Absent
Phloem	Crushed	Clear	Crushed
Vascular Bundle Type	Primer -Radial	Secondary	Secondary

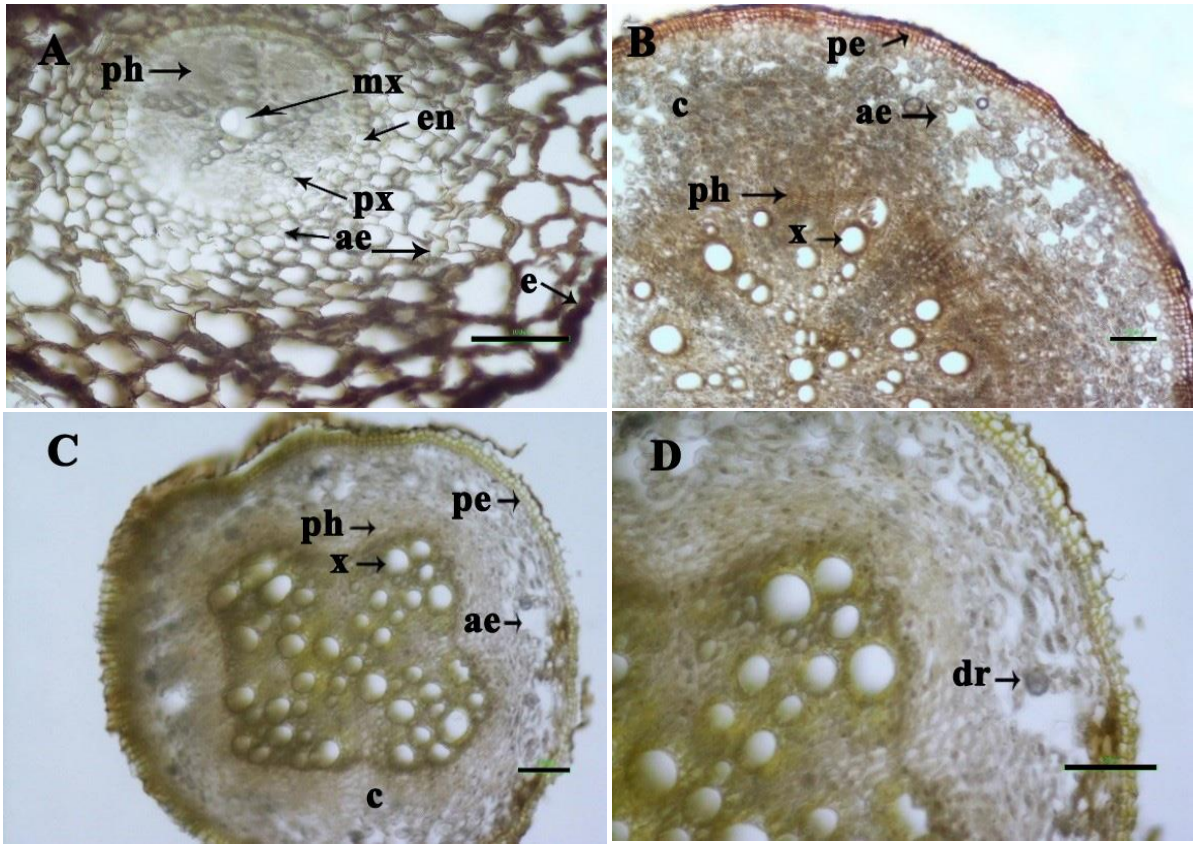

Figure 1. Transverse sections of root. A) *R. crispus*, B) *R. obtusifolius*, C-D) *R. patientia*. e: Epidermis, pe: Peridermis, mx: Metaxylem, px: Protoxylem, ae: Aerenchima, x: Xylem, ph: Phloem, dr: Druse crystal (scale bar 100 µm)

3.2. Stem Anatomical Features

The stem of the *R. crispus* plant had 10 cornered and contained collenchyma cells. The epidermis cells were circular shaped and had a thin cuticle. The vascular bundles were arranged in two rings. Sclerenchyma cells were around the bundles. The cortex was 3-4-layered and was narrowed. Druse crystals were observed in the pith region. Druse crystal number was 16-17. The parenchyma cells in pith were of different sizes and were circular in shape. The parenchyma cells of the pith were of different dimensions and were circular in shape. Vascular bundles were in form of collateral and bicollateral bundles. In addition, some vascular bundles were internal bundles. Collenchyma was a lacunar type. Sclerenchyma on the vascular bundles was dome-shaped (Figure 2-E, Table 3).

The stem of *R. obtusifolius* was 8 cornered and irregular wave. The epidermis cells of *R. obtusifolius* were circular shaped. There were sparse trichomes on these cells. Angular collenchyma cells were present in the waves. Scattered vascular bundles appeared in the stem transverse sections of the taxon. Vascular bundles had bundle sheath. Druse crystals were scattered throughout the stem. Druse crystal number was 45-50. There is no specific pith region. Parenchyma cells were very different in size and had gaps between them. Vascular bundles were in form of collateral. Internal

bundle was not detected. Some vascular bundles were obcollateral. There is no sclerenchyma on vascular bundles (Figure 2-F, Table 3).

The stem of *R. patientia* was 14 cornered and regular wave. The stem was hairless. The stem epidermis cells of the *R. patientia* taxa were circular shaped. Vascular bundles continued in intervascular areas. Vascular bundles were in form of concentric and bicollateral bundles. In addition to these bundles, types of internal bundles were seen. The cortex parenchyma cells were circular. Druse crystals were numerous in the cortex. The pith was very large and carried abundant druse crystals. Druse crystal number was 95-100. In the pith region, there were very prominent and numerous intercellular spaces. Collenchyma was a lamellar type (Figure 2-H). Sclerenchyma on the vascular bundles was flat or polygonal shaped (Figure 2-G, Table 3).

Table 3. Comparative stem features in *Rumex* species studied

Properties of species	*R. crispus*	*R. obtusifolius*	*R. patientia*
Rhizome	Present	Absent	Present
Sclerenchyma	Upper and lower of bundle	Absent	Upper and lower of bundle
Number of internal vascular bundles	Medium	Absent	Many
Number of corner of stem	10	8	14
Vascular Bundle Type	Collateral, Bicollateral	Collateral	Concentric, Bicollateral
Shape of stem	Regular	Irregular	Regular
Trichome on outer stem surface	Absent	Present	Absent
Cortex Parenchyma layers	3-4	No distinction	5-6
Druse crystals in pith	Few (16-17)	Medium (45-50)	Many (95-100)
Vascular bundle	Regular	Scattered	Irregular
Shape of sclerenchyma cap on phloem	Dome	Absent	Flat or polygonal
Collenchyma type	Lacunar	Angular	Lamellar
Cuticle	Present	Absent	Absent

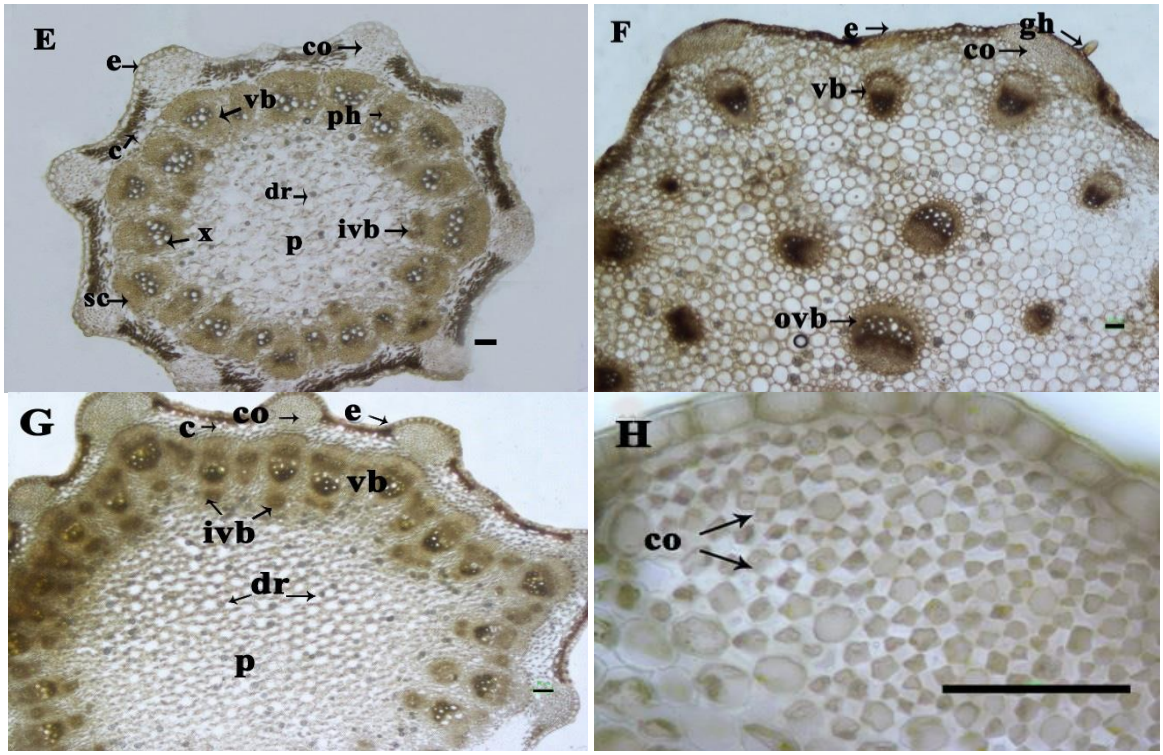

Figure 2. Transverse sections of stem. E) *R. crispus*, F) *R. obtusifolius*, G-H) *R. patientia*. e: Epidermis, p: Pith, x: Xylem, ph: Phloem, dr: Druse crystal, co: Collenchyma, c: Cortex, vb: Vascular bundle, ivb: Internal vascular bundle, ovb: Obcollateral vascular bundle, sc: Sclerenchyma, gh: Glandular hair (scale bar 100 µm)

3.3. Leaf Anatomical Features

In the leaf transverse sections of *R. crispus*, upper and lower epidermis cells were polygonal shaped. Epidermis cell walls were flat. Cuticle layer was present on the epidermis. There was a slightly prominent bulge on the upper epidermis side of the middle vessel region. The number of vascular bundles in the middle vessel region was 3 and these vascular bundles were collateral. Collenchyma cells were 1-2 layered. Mesophyll consisted of 2-3 rows of palisade parenchyma and 2-3 rows of spongy parenchyma cells. Anisocytic stomata was observed on the lower and upper surfaces. The number of stomata per mm^2 was 126 in abaxial surface of *R. crispus* and the number of stomata per mm^2 was 102 in adaxial surface.

The leaf epidermis cells of *R. obtusifolius* had wavy walls. Epidermis cells were circular shaped. Also a thin cuticle was available on the epidermis. There was a very prominent bulge on the upper epidermis side of the middle vessel region. The number of vascular bundles in the middle vessel region was 4 and these bundles were collateral type. Collenchyma cells were 4-5 layered. Mesophyll consisted of 2-3 layered of palisade parenchyma and 5-6 layered of spongy parenchyma cells. Anomocytic stomata were seen on both the adaxial and abaxial surfaces. Leaf surface was not seen hair. As in other species, druse crystals were observed in the leaf cross-section. The number of stomata per mm^2 was 78 in abaxial surface of *R. obtusifolius* and the number of stomata per mm^2 was 64 in adaxial surface.

In the *R. patientia* leaf cross-section, the epidermis cells were circular or rectangular shaped. The cell walls of both the upper and lower epidermis were wavy. Furthermore, cuticle was seen in the lower and upper epidermis. There was a bulge in the upper epidermis of the middle vessel region. This bulge was prominent. Collenchyma cells were located in this bulge and were 4-5 layered. Two small and two large vascular bundles were observed in the middle vessel region and vascular bundles were collateral and concentric type. One of these bundles was internal and another was obcollateral. Gaps occurred between parenchymal cells in this region. Druse crystals spread throughout the leaf. No hair on the leaf surface. Mesophyll consisted of 2 layered of palisade parenchyma and 2 layered of spongy parenchyma cells. The stomata on the abaxial surface (lower) were anomocytic. The stomata on the adaxial surface (upper) were anisocytic. The number of stomata per mm^2 was 136 in abaxial surface of *R. patienta* and the number of stomata per mm^2 was 87 in adaxial surface.

Table 4. Comparative leaf features in *Rumex* species studied

	R. crispus	***R. obtusifolius***	***R. patientia***
Bulge of the upper epidermis	Less prominent.	Very prominent.	Prominent
Cell wall of the epidermis	Flat	Flat	Wavy
Mesophyll type	Bifacial	Bifacial	Bifacial
Layer of palisade parenchyma	2-3	2-3	2
Layer of spongy parenchyma	2-3	5-6	2
Bundle sheath	Absent	Absent	Present
Layer number of collenchyma	1-2	4	4-5
Number of vascular bundles	3	3	4
Vascular bundle type	Collateral	Collateral	Collateral, Concentric
Druse crystals	Many	Medium	Many
Cuticle	Present	Present	Present

Table 5. The stoma features in the upper and lower epidermis

	R. crispus **Abaxial / Adaxial**	***R. obtusifolius*** **Abaxial / Adaxial**	***R. patientia*** **Abaxial / Adaxial**
Number of stomata (1 mm^2)	126/102	78/64	136/87
Width of stomata (μm)	28,06/ 34,22	25,19/ 24,89	23,19/ 25,89
Length of the stomata (μm)	40,78/ 43,30	38,25/ 33,77	31,267/ 34,22
Number of epidermal cell	158/185	120/122	187/125
Stomatal index	44,3/35,5	39,3/20,7	42,1/41,0
Stomata type	Anisocytic/Anisocytic	Anomocytic/Anomocytic	Anomocytic/Anisocytic

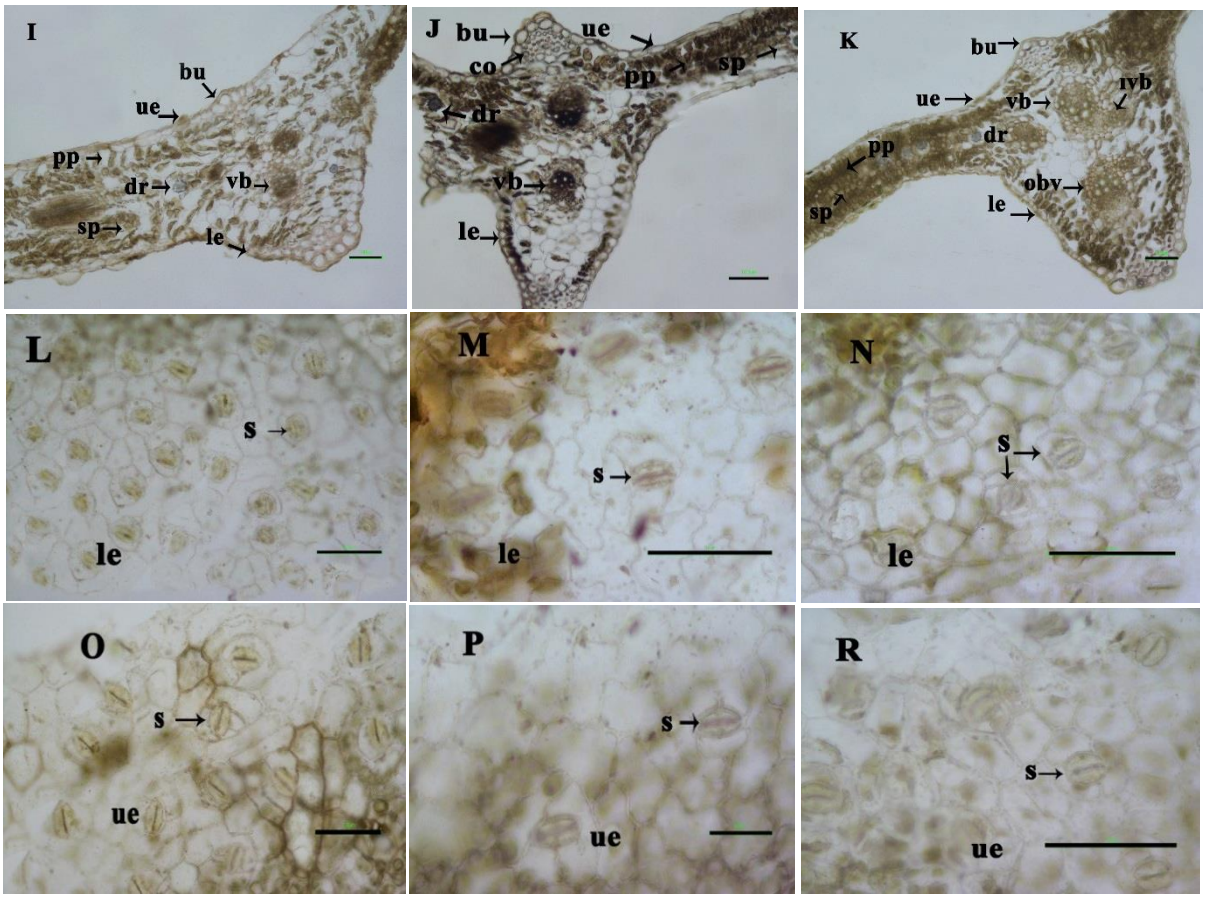

Figure 3. Transverse and superficial sections of leaf. I-L-O) *R. crispus*, J-M-P) *R. obtusifolius*, K-N-R) *R. patientia*. ue: Upper epidermis, le: Lower epidermis, pp: Palisade parenchyma, sp: Spongy parenchyma, s: Stoma, dr: Druse crystal, co: Collenchyma, vb: Vascular bundle, bu: Bulge, obv: Obcollateral bundle, ıvb: Internal vascular bundle (scale bar 100 µm)

In this study, the anatomical features of the vegetative organs of three species belonging to the genus *Rumex* were examined comparatively. *R. crispus* and *R. patientia* had rhizomes. The root anatomy of the taxa was examined from the lateral roots taken from the rhizome. *R. crispus* roots were in primary structure and there were 1-2 layered of epidermis. The numbers of xylem arms were 5-6. The cortex was 9-10 layered. De Micco and Aronne stated that the cortex of *R. scatatus* had a few layered of aerenchyma was observed [17]. Laan et al. in a study about *R. crispus* species found the presence of aerenchyma cells and the amount of aerenchyma was reported to increase with the exposure of the root flood [25]. Peridermis cells of *R. patientia* were in 2-3 layered, peridermis cells of *R. obtusifolius* were in 3-4 layered. Phloem cells were most distinct in *R. obtusifolius*. Druse crystal was observed only at the root of *R. obtusifolius*.

In the cross-sections of stem that constitute our research subject, the number of corners of their stem was different from each other. The corner number of *R. crispus* was 10, *R. patientia* was 14 and *R. obtusifolius* was 8. The stem of *R. obtusifolius* was irregular-wavy shaped and hair only on this species. *R. crispus* had cuticle on the stem epidermis. In the stem of *R. crispus* and *R. patienta* species, while the vascular bundles were in regular order, in *R. obtusifolius* observed scattered bundles unique to monocotyledons [26]. Internal bundles were detected in stem of *R. crispus* and *R. patienta*, while no internal bundles were seen in *R. obtusifolius*. In *R. hastatus* there were no internal bundles, as was *R. obtusifolius* [19]. Soleimani et al. stated that there were internal bundles in the stem of *R. crispus*. There were also studies that mention the presence of internal and obcollateral vascular bundles in the stem of the *R. patientia* [27, 28]. Sclerenchyma cells on the vascular bundles were arranged in different ways. Sclerenchyma cells of *R. crispus* were dome-shaped and, they were flat or polygonal shaped in *R. patienta*. No sclerenchyma was found on the vascular bundles of *R. obtusifolius*. Soleimani et al. stated the sclerenchyma shapes on phloem as broad crescent-shaped in *R. conglomeratus*, circular cap-shaped in *R. crispus*, and narrow crescent-shaped in *R. dentatus* [20]. Collenchyma of varieties of taxa was different from each other. The lacunar collenchyma was observed in *R. crispus*, lamellar collenchyma was in *R. patienta*, and the angular collenchyma was in *R. obtusifolius*. *R. obtusifolius*, *R. crispus* and *R. patienta*

had 45-50, 16-17 and 95-100 druse crystals, respectively. In addition, Sahney and Vibhasa showed that druse crystals were found in *R. hastatus* species [19].

In *R. obtusifolius* leaf cross-section, unlike other species, the edges of the cell walls of the epidermis were wavy. Leaf cross-sections of the species showed an outward bulge in the upper epidermis of the middle vessel region. This bulge was most prominent in *R. obtusifolius*. The presence of this bulge was first detected by us. There were 4 vascular bundles in the middle vascular region of *R. patienta* taxa, while in *R. crispus* and *R. obtusifolius* were 3. *R. vesicarius* L. species had 2 vascular bundles in the middle vessel region [29]. Anomocytic stomata was detected on the abaxial surface of *R. patienta* and anisocytic stomata on the adaxial surface. The leaf mesophyll of *R. patientia* consisted of 2-layer palisade parenchyma and 2-layer spongy parenchyma. Soleimani et al. showed that the cells of the palisade parenchyma were 2-layered in a way they do in *R. patientia* [20]. The presence of of anisocytic and paracytic stomatas in *R. patienta* was mentioned by Khan and Hayat [30].

IV. CONCLUSION

With this study, the anatomical features of the vegetative organs of three species in *Rumex* genus were examined in detail. The presence of vascular bundle type and druse crystals in the roots of taxa may be distinctive. The presence of cuticles and feathers on the epidermis of the stem, the number of corners and shapes of stems, the arrangement of the vascular bundles, the amount of internal bundles, the number of druse crystals in the pith, the collenchyma types can be considered taxonomically important. In leaf cross-sections, outward bulge was detected of upper epidermis. This bulge was less pronounced in *R. crispus* and *R. obtusifolius* was very pronounced. The bulge in the epidermis, the number of collenchyma layers, the number of layers of palisade and spongy parenchyma, and the stoma index may be important characters in distinguishing the species belonging to the *Rumex* genus.

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