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COMPARATIVE ANATOMY OF SOME *SEDUM* SPECIES (CRASSULACEAE) IN TURKEY AND DISTINGUISHING CHARACTERISTICS OF THESE SPECIES

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ABSTRACT

Sedum is a large genus belonging to the Crassulaceae family. Some members of the genus are medically vital. This study investigated the anatomical characteristics of *Sedum acre* L., *Sedum album* L., and *Sedum pallidum* M. Bieb. distributed in Turkey. In the light of the findings obtained, the internal structure features distinguishing the species from each other are revealed. In the cross-sections taken by hand, it is determined that the species' roots are in the secondary structure. Large intercellular spaces are identified in the cortex of the *S. album* root. The cuticle layer is present on the epidermis of the stem cross-section and the alkaloids were found in the cortex of the stem. Both the upper and bottom surfaces of the leaves have anisocytic stomata, which may be seen on both sides of the leaf. In the cross section of the *S. acre* leaf, the vascular bundles are not scattered, unlike the *S. album* and *S. pallidum*, and are in regular rows in the center. The statistical results obtained reveal that some anatomical characters may be necessary for distinguishing these three species. Characteristics such as stem epidermis size, stem cuticle thickness, the diameter of the stem cortex parenchyma cell, stoma size on the lower and upper surface of the leaf can be considered to be of diagnostic importance.

Keywords: *Sedum*, Anatomy, Crassulaceae, Medicinal plants, Turkey
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INTRODUCTION

Crassulaceae is a cosmopolitan family, and it is distributed in regions such as Southern Africa, Mexico, Macaronesia, the Mediterranean and the Himalayas (Sponberg, 1978). Mort *et al*., (2001) The family spread from South Africa to the Mediterranean, Eastern Europe, and Asia, and that of the North African species spread to Macaronesia where they are later diversified. Members of the Crassulaceae are generally herb and shrubs, succulent plants, and are striking with their xeromorphic structures, especially the presence of water storage tissue in the leaves and the stem (Kean, 1924; Metcalfe, 1972).

The Crassulaceae family is generally monophyletic, and its members are closely related to members of the Saxifragaceae and Penthoraceae families (Albert *et al*., 1992). In addition, the family members, which have very high similarities in terms of floral and embryonic, also show similarity in vegetative and generative terms (Jensen, 1968). There are also some problems in distinguishing subfamilies (Ham and Hart, 1998). Ham and Hart (1998) carried out DNA analysis studies intending to eliminate the problems related to subfamily. As a result, they identified some sister subfamilies and reported that they were Cotyledonoideae, Sempervivoideae, Sedoideae, and Echeverioideae (Ham and Hart, 1998). *Sedum* is a large genus belonging to the Crassulaceae. According to Thiede and Eggli (2007), the genus comprises 430 species that are found in North America, Mexico, East Asia, and the Mediterranean basin (Hart and Bleij, 2003; Thiede and Eggli, 2007). There have been various sectional names and segregate genera published in the literature for this genus, which suggests that it is paraphyletic and taxonomically somewhat difficult (Hart and Bleij, 2003; Carrillo-Reyes *et al*., 2009). The genus *Sedum* comprises a large number of species that are employed in pharmaceutical production. Alkaloids, tannins, flavonoids, and cyanogenic chemicals have all been isolated from *Sedum* species as a result of chemical research carried out on them (Stevens *et al*., 1995). Some species belonging to the genus are known to have antiseptic and anti-inflammatory effects, and species such as *Sedum dendroideum* Moc & Sessé are known for their medicinal properties. (De Melo *et al*., 2009). *Sedum album* L. and *Sedum pallidum* Bieb. is used to treat hemorrhoids, pain in the ears and skin in Turkey (Tuzlacı, 2016). Till now, anatomical characteristics of some *Sedum* species were examined by Esau (1977), Metcalfe and Chalk (1950, 1979), Ardelean *et al*. (2009), Wu *et al*. (2013), Akhmetzhanova *et al*. (2016), Shahrestani *et al*. (2020) essential features in their anatomies were reported. Esau (1977) reported the stomatal configuration of *Sedum*. Metcalfe and Chalk (1950, 1979) studied the root, stem, petiole, and flower anatomies of dicotyledons, including *Sedum*. It has been reported that the root of *Sedum telephium* L. ssp. *maximum* (L.) Krock. has a thin periderm as well as many bundles of vascular tissue, according to Ardelean (2009). Zlatkovi *et al*. (2017) studied the epicuticular wax and phytochemical features of the *Sedum album* leaf and published their findings. According to Akhmetzhanova *et*

al. (2016), sclerenchymatical cells were found in the stem of a *Sedum acre* stem that was found in Central Kazakhstan. A study conducted by Shahrestani *et al*. (2020) investigated the comparative anatomy of certain *Sedum* species found in Iran.

In light of its close proximity to other families, issues in subfamilies, and difficulty differentiating genera, the Crassulaceae is a family that warrants further investigation. The genus *Sedum* presents extra difficulties since it contains species that exhibit homoplastic and transitional phenotypes to a greater or lesser extent, and because it contains species that exhibit similar family problems (Uhl, 1976). To account for these factors, the most taxonomically challenging genus in the family is *Sedum* (Messerschmid *et al*., 2020). When solving taxonomic problems, some anatomical characteristics are taken into consideration (Stace, 1984; Zheng and Gong, 1999; Zheng *et al*., 2001; Lu *et al*., 2008).

Anatomical characteristics of various medicinal species of *Sedum* genus are being investigated in this study, with the goal of reporting some discoveries that may aid in the identification of *Sedum* species. These three taxa are defined anatomically by transverse sections of root, stem and leaf as well as superficial sections of leaf, and this study adds new information to the taxonomy of the genera *Sedum* by discussing the characteristics investigated in relation to their potential value and previous work in the field. In addition, our study will be beneficial in terms of determining the distinctive anatomical characters of plants that spread in the same environmental conditions and in similar climates. The results of the research will contribute to future studies on these plant.

MATERIALS AND METHODS

Specimens of the species were collected between April and July (Table 1). Some of the specimens were put in 70% alcohol; others were turned into herbarium samples. In identifying the species, the work named Flora of Turkey was used (Davis, 1972). Sections were taken by hand, and glycerin was used as an analysis medium. The preparations were made permanent by the glycerin-gelatin method (Vardar, 1998). The cell types obtained from the root, stem and leaf sections of the species were determined using the 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 4), and the stoma index was calculated (Meidner and Mansfield, 1968). A total of 20 measurements were taken from tissues such as epidermis, periderm, parenchyma and vascular bundle elements (Table2, Table 3).

The IBM SPSS V23 statistical package was used for data analysis, calculating mean values and standard deviations (Table 2). The Shapiro Wilk test was used to determine whether numerical values of anatomical features were compatible with the normal distribution. Analysis of Variance (One-way ANOVA) was used to compare and contrast some characteristics of the species' root, stem, and leaf in order to identify similarities and differences, for this purpose post hoc (LSD) tests were used to determine the difference between the two species under consideration (Table 5).

Table 1. Information collected from the taxa researched.

Taxa	Locality and date of collection	Coordinates	Altitude (m)	Voucher	Alcohol stock number
S. acre	Kervansaray Dağı (Boztepe- Kırşehir)	39°19′258″N, 34°23′14″E	1684 m	SULCAY60, 2020.07.05	A69
S. album	Kervansaray Dağı (Boztepe- Kırşehir)	39°19′25″N, 34°15′100″E	1684 m	SULCAY60, 2020.07.05	A72
S. pallidum	Çamiçi (Niksar-Tokat)	40°38′16″N,36° 59′ 34″E	1165 m	SULCAY60, 2020.20.09	A123

Table 2. Quantitatively studied anatomical characters.

1	Length of stem epidermis	12	Width of upper surface stomata
2	Width of stem epidermis	13	Length of upper surface stomata
3	The thickness of the stem cuticle	14	Length of leaf epidermis
4	Diameter of cortex parenchyma cells	15	Width of leaf epidermis
5	Diameter of xylem	16	Diameter of leaf parenchyma cells
6	Diameter of pith parenchyma cells	17	The thickness of the leaf cuticle
7	Lower surface stomata number	18	Length of root periderm
8	Width of lower surface stomata	19	Width of root periderm

9	Length of lower surface stomata	20	Diameter of root xylem
10	Upper surface stomata number	21	Diameter of root cortex parenchyma cells

RESULTS

Anatomical Results

Root: *S. acre* root has a secondary structure. The periderm is 7-8 layers according to the cross-section of the root. The 7-8 layered cortex parenchyma cells have abundant starch, and their walls are thickened. The central cylinder is filled with xylem elements. Cambium is indistinguishable. The trachea is smooth circular-shaped 3-4 layered phloem consisting of crushed cells (Fig. 1-A).

Cross-sections of the *S. album* root show that the periderm has 5-6 layers, which is consistent with previous findings. As a result of the crushing of the cortical cells, huge intercellular gaps can be noticed in the cortex. Cambium is indistinguishable from other plants. The trachea is more pronounced in the direction of the cortex. Phloem is composed of 5-6 layers and is rectangular in shape (Fig. 1-B).

As with other species, the root of *S. pallidum* is a secondary structure. Periderm cells are found in two to three layers. In the cortex, the cells are irregularly polygonal in shape, and there are gaps between them. The xylem components are concentrated in the pith area. The phloem does not appear to be clear (Fig. 1-C).

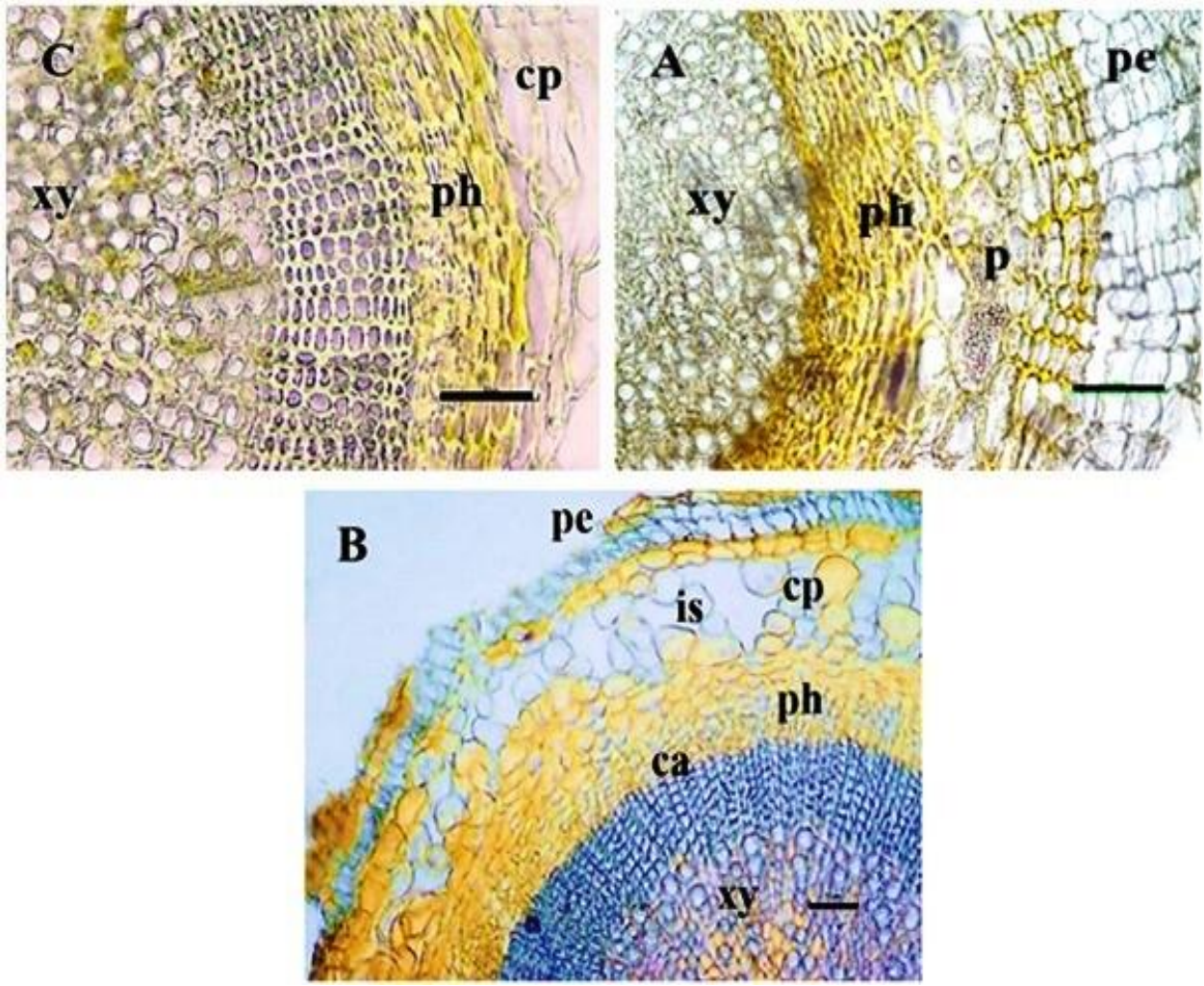

Fig. 1. The root cross-section of *Sedum*. *S. acre* (A), *S. album* (B), *S. pallium* (C), ca cambium, cp cortex parenchyma cell, is intercellular space, pe periderm, ph phloem, xy xylem (Scale bar 50 µm).

Table 3. The anatomical measurements of various tissues (Se: Standart error)

Characters	*S. acre*		*S. album*		*S. pallidum*	
	Width (µm) ±Se	Length (µm) ± Se	Width (µm) ± Se	Length (µm) ± Se	Width (µm) ± Se	Length (µm) ± Se
Root Periderm	26.76±4.52	42.56±15.07	29.065±5.39	32.68±3.94	31.11±14.01	32.68±14.85
Diameter of root cortex cells	27.18±6.76	52.30±11.21	26.01±11.64	52.60±14.09	8.45±1.85	27.57±9.28
Root diameter of trachea	15.88±2.81		14.77±2.94		11.88±1.68	
Stem diameter of pith cells	42.29±9.12	42.06±14.38	30.34±9.21	32.5±14.38	40.30±14.05	39.92±13.03
Stem diameter of trachea	10.49±2.02		11.21±2.09		10.13±1.68	
Leaf parenchyma	41.22±13.05		38.72±12.09		104.77±40.65	

Stem: According to the cross-section of a *S. acre* stem, epidermal cells are single-layered and round in shape, whereas dermal cells are bilayers. A cuticle protects the epidermis from the environment. The cortical region is quite vast and is made up of parenchyma cells that have a circular shape. The vascular parts come together to create a cylinder in the middle. While the xylem elements can be distinguished, the phloem elements cannot be distinguished. Alkaloids are found in little amounts throughout the stem (Fig. 2-D).

Epidermis cells are single-layered, round and rectangular in shape, according to the cross-section of the *S. album* stem. A cuticle protects the epidermis from the environment. The cortex is made up of 13-14 layers of parenchyma cells arranged in a layered pattern. In the heart of the plant, the xylem forms a continuous ring. Phloem is indistinguishable from other flowers. Alkaloids are found in little amounts throughout the stem (Fig. 2-E).

The epidermis cell of *S. pallidum* is similar to that of *S. album* in that it is single-layered, rectangular, and circular in shape. A cuticle protects the epidermis from the environment. The cortex is made up of 10-11 layers of parenchyma cells, which are interconnected. It is possible to tell the difference between xylem, phloem, and pith. The stem does not contain any alkaloids (Figure 2-F-G).

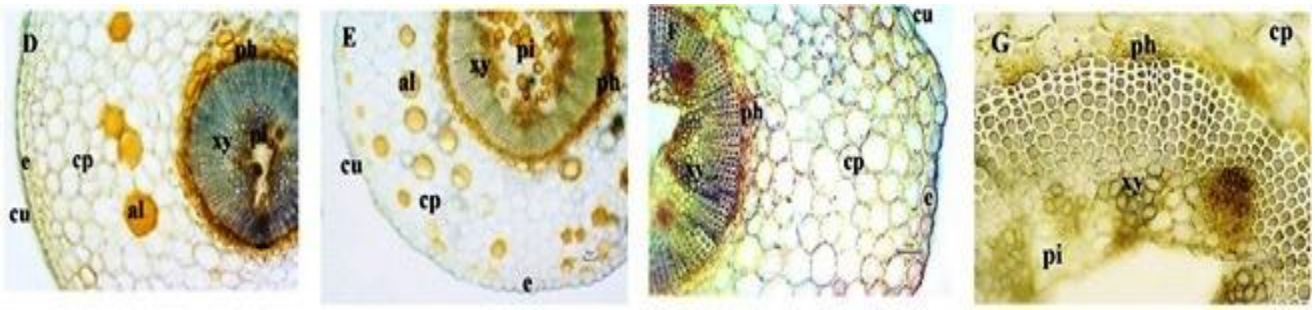

Fig. 2. Stem cross-section of *Sedum*. *S. acre* (D), *S. album* (E), *S. pallium* (F-G), al alkaloid, cu cuticle, cp cortex parenchyma cell, e epidermis, ph phloem, pi pith, xy xylem (Scale bar 50 µm).

Leaf: Leaves of the species are succulent. Cross-sections of the epidermis reveal that the cells are one layered, round, and rectangular in shape. The walls of the cells are wavy. The walls of the upper epidermis cells of *S. pallidum* are more depressed and noticeably wavy than those of the lower epidermis cells (Fig. 3-J). The mesophyll is not distinguished from the rest of the plant. Mesophyll is made up of parenchymal cells that are round in shape. In the central region of the *S. acre* leaf, the vascular bundles are arranged straight, not dispersed, and the number of vascular bundles is 5-6 (Figure 3-H). The number of vascular bundles in *S. album* is 10-13, while the number of vascular bundles in *S. pallidum* is 9-10 and dispersed. Alkaloids are found only in the leaves of *S. album* (Fig. 3-I). Anisocyticis the stomata type found in all of the species under investigation (Fig. 4). The *S. album* lower leaf surface had the fewest stomata of any of the species we evaluated, with only 20 stomata total (Table 2). The stomatal index of the species' bottom and upper leaf surfaces is almost the same on both sides. Stomata indices are reported in table 4.

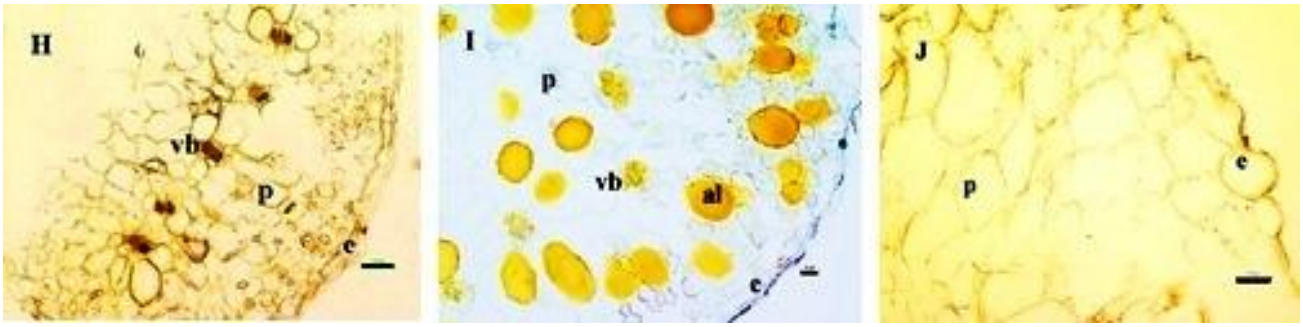

Fig. 3. Leaf cross-section of *Sedum*. *S. acre* (H), *S. album* (I), *S. pallium* (J), al alkaloid, p parenchyma cell, e epidermis, vb vascular bundle (Scale bar 50 µm).

Table 4. The stomata feature on the upper and lower epidermis of the *Sedum.*

Characters	*S. acre* Lower/Upper	*S. album* Lower/Upper	*S. pallidum* Lower/Upper
Number of stomata (1 mm²)	300/256	112/180	288/251
Number of epidermal cells	28/32	20/40	36/32
Stomatal index	91/88	84/81	88/89
Stomata type	Anisocytic	Anisocytic	Anisocytic

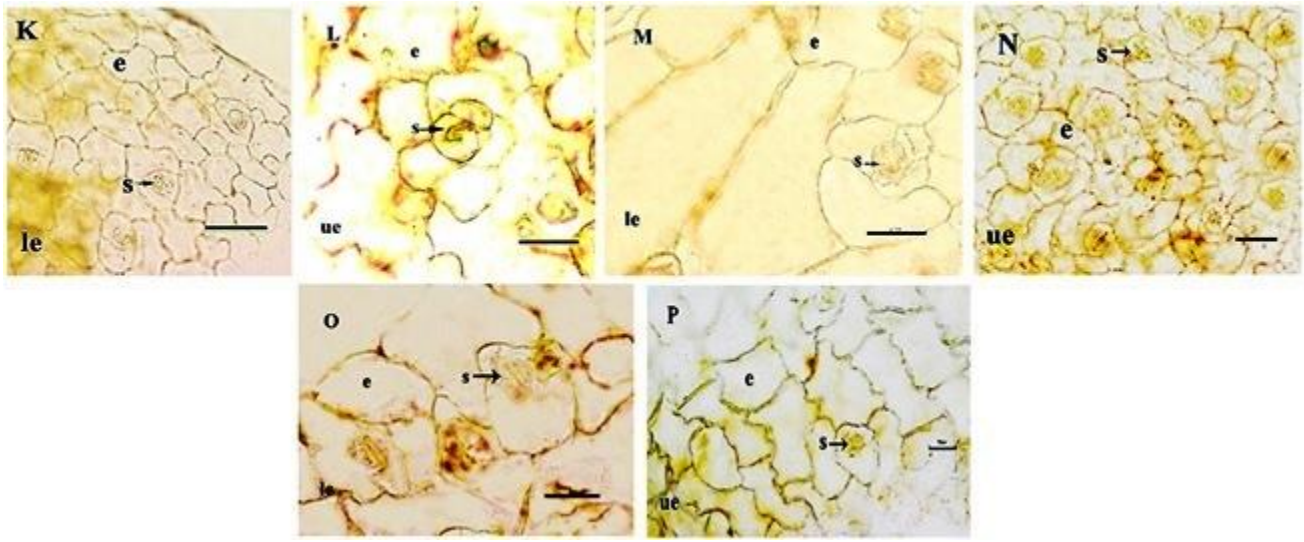

Fig. 4. The surface section of *Sedum*. *S.* acre (K-L), *S. album* (M-N), *S. pallium* (O-P), e epidermis, le lower epidermis surface, s stoma, ue upper epidermis surface. (Scale bar 50 µm).

Analysis Results: In the course of the variance analysis, it was discovered that the breadth and length of the stem epidermis, as well as the diameters of the cortex parenchyma and the thickness of the cuticle, were significantly different from one another. A considerable difference in the size of stomata was detected on both the upper and lower surfaces of the species' leaves, which was confirmed once again. While the stem epidermis width of the *S. acre* differed greatly from the epidermis width of the *S. album*, there was no difference between the stem epidermis width of the *S. pallidum* and the *S. album*. It was discovered that the stem epidermis length of *S. acre* differed statistically from that of *S. album* and *S. pallidum* species, although no statistically significant difference was identified between *S. album* and *S. pallidum*. Significant differences were discovered between *S. acre* and the *S. album* and *S. pallidum* species in terms of stem cuticle thickness, but no significant differences were found between *S. album* and *S. pallidum* in terms of stem cuticle thickness. The diameter of the cortex parenchyma in the stem, the width of the lower surface stomata on the leaf, and the length of the lower surface stomata on the leaf of *S. acre* were found to be significantly different from those of *S. album* and *S. pallidum* species, while no significant difference was found between *S. album* and *S. pallidum* species.

Significant differences were detected between *S. pallidum* and *S. album* species in terms of upper surface stomata width, but no significant differences were discovered between *S. album* and *S. acre* species in terms of lower surface stomata width. A statistically significant difference was identified between *S. acre* and *S. album* in terms of top surface stomata length, however there was no statistically significant difference between the two species in terms of stomatal length (Table5).

Table 5. Anatomical characters with significant differences as a result of statistical analysis (Se: Standart error).

Taxa	Stem epidermis width Mean± Se	Stem epidermis length Mean±Se	Stem Cuticle thickness Mean±Se	Diameter of cortex parenchyma Mean±Se	Lower stomata width Mean±Se	Lower stomata length Mean±Se	Upper stomata width Mean±Se	Upper stomata length Mean±Se
S. acre	20.44∓6.06[a]	51.06∓7.86[a]	14.03∓2.2[a]	83.82∓17.69[a]	30.48∓5.39[a]	40.95∓9.1[a]	26.53∓0.94[a]	33,176∓2.7[a]
S. album	14,57±4,51[b]	37.62∓10.06[b]	10.56∓2.1[b]	56.07∓9.79[b]	21.141∓2.5[b]	28.51∓2.37[b]	22.91∓3.44[a]	28.36∓2.56[b]
S.pallidum	23.15∓3.12[a]	40.02∓8.30[b]	7.79∓2.73[b]	48.02∓8.48[b]	23.10∓4.57[b]	25.80∓4.12[b]	17.80∓1.52[b]	32.32∓2.23[a]
P	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000

DISCUSSION

The anatomical features of some species belonging to the *Sedum* genus, which are valuable for medical and folk medicine, were studied in detail. The roots of all three species are secondary. The number of periderm and cortex parenchyma layers of taxa is different from each other. Again, the number of phloem layers in the root cross-sections of the species were determined to be different from each other. Unlike other species, wide gaps in the root cortex have been detected in the *S. album*. *S. telephium* ssp. *maximum* (L.) Krock. the root is also secondary, and the periderm consists of flattened cells (Ardelean, 2009).

In all three species, vascular bundles form a continuous cylindrical ring in the center according to stem cross-sections. A similar feature has been determined in the stem *S. telephium* ssp. *maximum* (Ardelean, 2009). Shahrestani *et al*. (2020) talked about the presence of cortical bundles in the stem of *S. pallidum* in a study they conducted in Iran. In our study, cortical bundles were not detected. In the same study, *S. pallidum* and *S. album* stalk did not find any cuticle layer (Shahrestani *et al*., 2020). In our study, we defined cuticles in three species.

The leaves of three species belonging to the genus *Sedum*, which constitute our study, are succulent, and the mesophyll is not differentiated. In the middle part of the *S. acre* leaf, while the vascular bundles are arranged stripe-shaped, they are scattered in other species. Stomata are anisocytic. Esau (1965) reported the presence of anisocytic stomata in the genus *Sedum*. Alkaloid is observed only on the lower leaf surface of *S. album*.

Messerschmid *et al*. (2020*)* asserted that the *Sedum* genus has classification challenges, and they also explored the evolutionary relationships of the *Sedum* genus in detail. Many anatomical characteristics are useful in categorizing species, despite the fact that they are influenced by environmental variables (Metcalfe and Chalk, 1950; 1979). As a result, taxonomists have been searching for anatomical characteristics that can aid in the identification of the species in question. There may be anatomical characteristics in the stem, and particularly in the leaves, that will help to distinguish plants from other species (Stace, 1984; Lu *et al*., 2008). It has been suggested that the epidermis of the leaf (Zheng and Gong, 1999) and the morphological aspects of the stem (Zheng *et al*., 2001) are useful in the identification of *Sedum* species (Zheng *et al*., 2001). The results of the Anova tests also demonstrate that anatomical characteristics might be regarded differentiating characteristics. The thickness of the stem cuticle, the diameters of the stem cortical parenchyma cells, and the width of the stem epidermis differ across the species with which we operate. Additional distinguishing characteristics include the widths and lengths of the epidermis on the lower and upper sides of a species's leaf, as well as the width and length of the stoma on the lower and upper surfaces. Particularly in light of the fact that the *S. acre* and *S. album* species that are found in the same environment were both gathered from the same geographic location, the significance of the characteristics that we deem to be different increases.

As a result, the anatomies of three species belonging to the *Sedum* genus, which have a problem in the diagnosis, were examined in detail, and comparisons were made. Again, comparisons have been made with other anatomical studies on species. The findings of the root anatomy of the species were presented for the first time by us. In addition, the statistical findings obtained revealed features such as stem epidermis size, stem cuticle thickness, the diameter of stem cortex parenchyma, stomata size on the lower and upper surface of the leaf, distribution patterns of leaf vascular bundles, alkaloid carriage can be important distinguishing characters.

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Authors' contribution: S. U. conducted the experiment, analyzed the results of the experiment, interpreted the data, revised the manuscript, and approved the final version.

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