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Comparative Anatomical Investigations on Some *Centaurea* (Asteraceae) Taxa from Türkiye

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ABSTRACT: One of the most abundant genera in the flora of Türkiye is *Centaurea* L. (Asteraceae). There are 787 species in the world and 174 species in Türkiye, 126 taxa are endemic to Türkiye. The *Centaurea antiochia* Boiss. var. *antiochia* Boiss., *Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bal.) Wagenitz, *Centaurea nerimaniae* Ş. Kültür are the subject of a comparative anatomical investigation. The aim of this study is to determine and compare the anatomical features of three different *Cantaurea* taxa, two of which are endemic. The morphological features of these three taxa are very similar to each other. For this reason, the determination of anatomical features has a special importance in terms of detecting the differences of species. *Centaurea* taxa were collected by authors. The *Centaurea* specimens were identified and placed in ISTE (The Herbarium of the Faculty of Pharmacy of Istanbul University). In order to conduct anatomical research, the sample was fixed in 70% alcohol. Sartur solution was used to stain cross sections of the stem, leaves, and leaf surface sections and the anatomical features have been investigated. The glandular and non glandular trichomes, mesophyll cells, stomatal index, and stomata structures were compared. This is the first anatomical study on these three *Centaurea* taxa. They have amphistomatic and isobilateral leaves. It has multicellular, long non-glandular hairs as well as short stalked, big-head glandular hairs on the epiderma. In sclerencymatic tissue, a vascular bundle is inserted. These anatomical characteristics match those of the *Centaurea* species that have already been studied.

KEYWORDS: Anatomy; Asteraceae; *Centaurea antiochia*; *Centaurea nerimaniae*.

1. INTRODUCTION

Centaurea L. is one of the most common genera in the Flora of Türkiye. There are 174 species in Türkiye, 787 species worldwide, and 126 taxa that are endemic to Türkiye. *Centaurea* L. is known by Turkish names such as Peygamber çiçeği, Zerdali dikeni, Çobankaldıran, Timurdikeni, Acımık, Çoban dikeni, Sarıbaş [1-5]. *Centaurea* species are rarely tiny shrubs with spiny branches or bigger subshrubs with evergreen leaves, annual, biennial, or perennial herbs (Figure 1). The leaves have multicellular hairs and sessile glands. According to Flora of Turkey and East Aegean Islands very challenging taxonomic genus that requires extensive research. It is yet to be determined how to divide this enormous genus in a logical and natural way. In many areas, it is difficult to distinguish between different species, and because there are so many intermediates, some morphologically fairly severe types have had to be combined into a single species. Yet, there are a lot of slender endemics that have no near relatives. The genus' Central and South European species are widely known for their inter-species hybridization. *Centaurea antiochia* Boiss. is perennial with a short and erect stem that is 30-100 cm in height, usually hirsute. Leaves with scattered short hairs; lower interruptedly pinnatifid or pinnatisect with numerous lanceolate lateral segments; terminal segments occasionally slightly bigger; median leaves pinnatifid to lyrate. Subglobose to ovoid capitula measuring 20–27 x 16–25 mm. Little brown appendages with 5-13 cilia (2-7 mm) on each side and a terminal spine increasingly narrow as they grow. If the appendages (incl. spine) are 10-25 mm it is *Centaurea antiochia* Boiss. var. *antiochia* Boiss. (CAA), if they are 5-10 mm, it is *Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bal.) Wagenitz (CAP). Flowers are blackish-purple, flowering in May and June. Achene is 5-6 mm; pappus is 5-12 mm. [6]. *Centaurea nerimaniae* (CN) is a perennial herb with a rosette of leaves at the base and a thicker rootstock, branched most frequently in the upper part. The erect stem is 150-165 cm striate and usually floccose tomentose. Leaves are tomentose and glandular-punctate. The capitula is 30-40x17-30 mm, globose or almost cylindrical. Appendage of phyllaries is straw coloured, with 10-12 cilia on each side, 1-

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4 mm long, ending in a slender 1-3 mm long spinule. Flowers are dark purple, flowering in June-July. Achenes is 4.0-5.5 mm. Pappus biseriate, with an outside series of 8 to 10 mm and an inner series of 2-3 mm. [7].

Centaurea species are used alone or together with other plants for antidiabetic, antidiarrheic, antirheumatic, anti-inflammatory, antipyretic, antimalarial, antimicrobial, antibacterial, antiviral, antiulcerogenic purposes in Türkiye [8,9]. Studies with *Centaurea* L. species showed that they contain chemical groups such as flavonoids, phenylpropanoid, triterpene, sesquiterpene lactones [10]. The flavonoid profile and biological activity of *C. nerimaniae* were originally described in a study. According to this study, the presence of the five flavonoids (apigenin, apigenin 7-O-glucoside, cirsimaritin, hispidulin and isokaempferide) may be responsible for the observed antioxidant, antibacterial, anti-inflammatory and anti-cholinesterase properties [11]. The aerial part of *C. antiochia* var. *praealta* contains phenols and flavonoids and it has antibacterial, anti-inflammatory, acetylcholinesterase (AChE) inhibitory, and antioxidant properties [12].

A comparative anatomical study is conducted on the *Centaurea antiochia* var. *antiochia*, *Centaurea antiochia* var. *praealta* and *Centaurea nerimaniae*. This study compares and contrasts the anatomical characteristics of three distinct *Centaurea* taxa, two of which are endemic. These three species are very similar in terms of morphological characteristics. Because of this, identifying anatomical characteristics is particularly crucial for identifying species distinctions.

Figure 1. A- *Centaurea antiochia* var. *antiochia;* B- *Centaurea antiochia* var. *praealta;* C- *Centaurea nerimaniae*

2. RESULTS

This is the first anatomical study on these three *Centaurea* taxa. They have amphistomatic and equifacial leaves. They have multicellular, long non-glandular hairs as well as short stalked, big-head glandular hairs on the epiderma. In sclerencymatic tissue, a vascular bundle is inserted.

2.1. Leaves anatomy

Centaurea antiochia* var. *antiochia: As a result of the presence of palisade parenchyma on both sides, the leaf type can be said as isolateral or equifacial. On the upper and lower epidermis, there is a wavy cuticle with mesophytic stomata. Epidermis cells are not wavy-walled, but flat and thin-walled. In superficial sections of the leaf, stomatal cells were observed in both the upper and lower epidermis (Figure 2-A). Leaf type is amphistomatic according to stoma distribution. On both surfaces, stomata cells can be found with three or four neighboring cells. Upper and lower epidermis both have anomocytic stomata. Palisade parenchyma is present in 2-3 layers on either side which contains chloroplast. The 1-2 layered isodiametric spongy parenchyma is irregularly located between the two palisade parenchyma. Phloem is near the lower surface of vascular bundles, whereas xylem is on the top surface. The bundle is enclosed by a single layer of parenchymatous cells; the midvein has the biggest bundle. Upper epidermis stomata index is 18.83, stomata length is 38.25±2.37 µm, stoma width is 23.89±1.82 µm, cuticle thickness is 3.73±0.59 µm, epidermis cell length is 9.06±1.57 µm, epidermis cell width is 12.59±2.25 µm, whereas lower epidermis stomata index is 23.58, stomata length is 41.25±2.70 µm, stoma width is 25.28±2.32 µm, cuticle thickness is 3.32±0.57 µm, epidermis cell length is 8.64±1.24 µm, epidermis cell width is 13.07±1.97 µm (Table 1). Lower stomatas are bigger than upper ones. On both the upper and lower side of the leaves, there are short stalked, big head glandular hairs as well as uniseriate, unicellular, and multicellular non-glandular hairs (Figure 3-A). The cell walls of multicelllular non-glandular hairs are thick, and they have 3 to 5 cells at the base of their section, as well as very long white hair (Figure 4-A).
The midrib, which appears nearly triangular in cross sections of the lamina, has single-layered, flattened, and ovate cells make up the epidermis, 1-2 layered collenchyma beneath the epidermal cells. A thin cuticle covers it all. Under the upper and lower epidermis, there is palisade and spongy parenchyma. Collateral vascular bundles are made up of xylem, phloem, and sclerenchyma cells (Figure 5-A).

Centaurea antiochia* var. *praealta: Palisade parenchyma is present on both sides, indicating that the leaf type is equifacial. There is a mesophytic stomata-covered wavy cuticle on the upper and lower epidermis. The cells of the epidermis have thin, flat walls. Stomatal cells were seen in the upper and lower epidermis of the leaf's superficial sections (Figure 2-B). According to the distribution of stomas, the leaf type is amphistomatic. Stomata cells can be present on both surfaces with three or four neighboring cells, because of that they are anomocytic stomata. Chloroplasts are found in the 3-4 layers of palisade parenchyma that are present on either side. The two palisade parenchymas are unevenly separated by the 1-2 layered isodiametric spongy parenchyma. Vascular bundles have xylem on the top surface and phloem close to the lower surface. The midvein possesses the largest bundle, which is surrounded by a single layer of parenchymatous cells. Upper epidermis stomata index is 21.28, stomata length is 32.14±2.25 µm, stoma width is 22.91±1.88 µm, cuticle thickness is 2.91±0.74 µm, epidermis cell length is 4.60±1.10 µm, epidermis cell width is 8.09±1.17 µm, whereas lower epidermis stomata index is 20.62, stomata length is 43.55±3.38 µm, stoma width is 26.67±3.75 µm, cuticle thickness is 5.04±1.12 µm, epidermis cell length is 7.00±1.82 µm, epidermis cell width is 7.73±0.95 µm (Table 1). Lower stomatas and epidermis cellls are bigger than upper ones. There are uniseriate, unicellular, and multicellular non-glandular hairs as well as short stalked, huge head glandular hairs on the upper and lower sides of the leaves (Figure 3-B). The glandular hairs are more on the upper surface (Figure 4-B).
The epidermis of the midrib is made up of single-layered, flattened, and ovate cells. It is entirely covered with a cuticle in both sides, lower cuticle is thicker than upper one. Collenchyma of 1–2 layers lies below the epidermal cells. There is palisade and spongy parenchyma beneath the upper and lower epidermis. Cells from the sclerenchyma, xylem, and phloem make up the collateral vascular bundles (Figure 5-B).

Centaurea nerimaniae: The presence of palisade parenchyma on both sides reveals that the leaf type is equifacial. In both the top and lower epidermis, there is a wavy cuticle covered in mesophytic stomata. The walls of epidermis cells are flat and thin rather than wavelike. It is an amphistomatic leaf because the stomata are located on the upper and lower surfaces of the leaf (Figure 2-C). There are anomocytic stomata because they can be seen on both surfaces with 3-5 neighboring cells. The three to four layers of palisade parenchyma on either side contain chloroplasts. The 1-2 layered isodiametric spongy parenchyma unevenly divides the 2-3 layered palisade parenchymas. Phloem is located close to the lower surface of vascular bundles and xylem is on the upper surface. Upper epidermis stomata index is 12.88, stomata length is 31±2.24 µm, stoma width is 16.5±2.23 µm, cuticle thickness is 4.7±1.3 µm, epidermis cell length is 15.5±5.9 µm, epidermis cell width is 26.5±3.3 µm whereas lower epidermis stomata index is 11.03, stomata length is 27.77±4.41 µm, stoma width is 21.11±3.33 µm, cuticle thickness is 6.88±1.53 µm, epidermis cell length is 10.91±1.45 µm, epidermis cell width is 17.35±1.37 µm (Table 1). Lower epidermis cellls are bigger than upper ones and lower epidermis is thicker

than upper epidermis. On the upper and lower sides of the leaves, there are short stalked, enormous head glandular hairs (Figure 3-C) and uniseriate, unicellular, and multicellular non-glandular hairs (Figure 4-C). The cells that make up the midrib's epidermis are ovoid, single-layered, and flattened. Under the epidermal cells is 2-4 layered of collenchyma. Collateral vascular bundles are composed of cells from the sclerenchyma, xylem, and phloem (Figure 5-C).

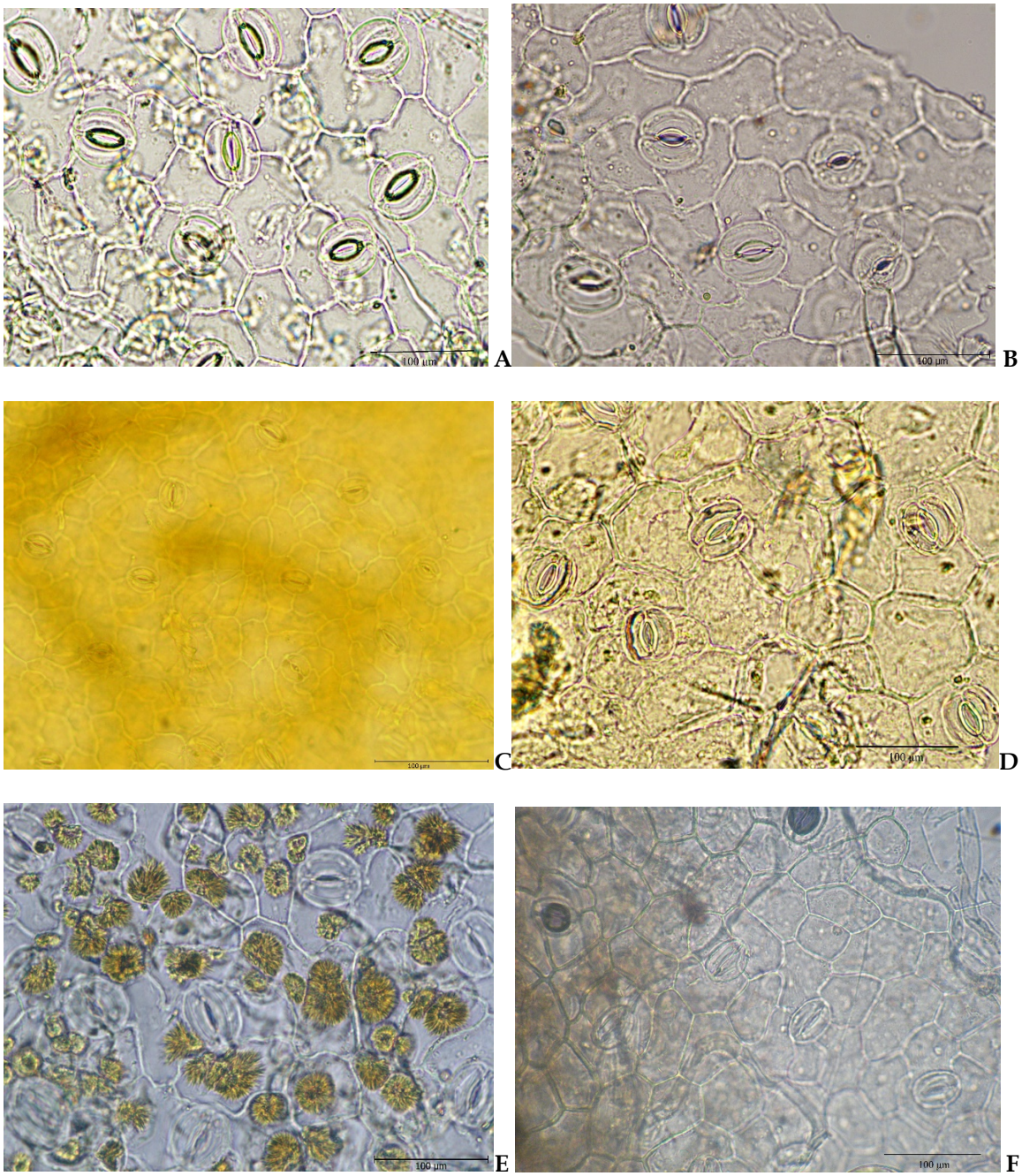

Figure 2. Lamina epidermal surface of *Centaurea* species.
A- CAA abaxial surface; B- CAP abaxial surface; C- CN abaxial surface; D- CAA adaxial surface; E- CAP adaxial surface; F- CN adaxial surface (Scale bars: 100 µm)

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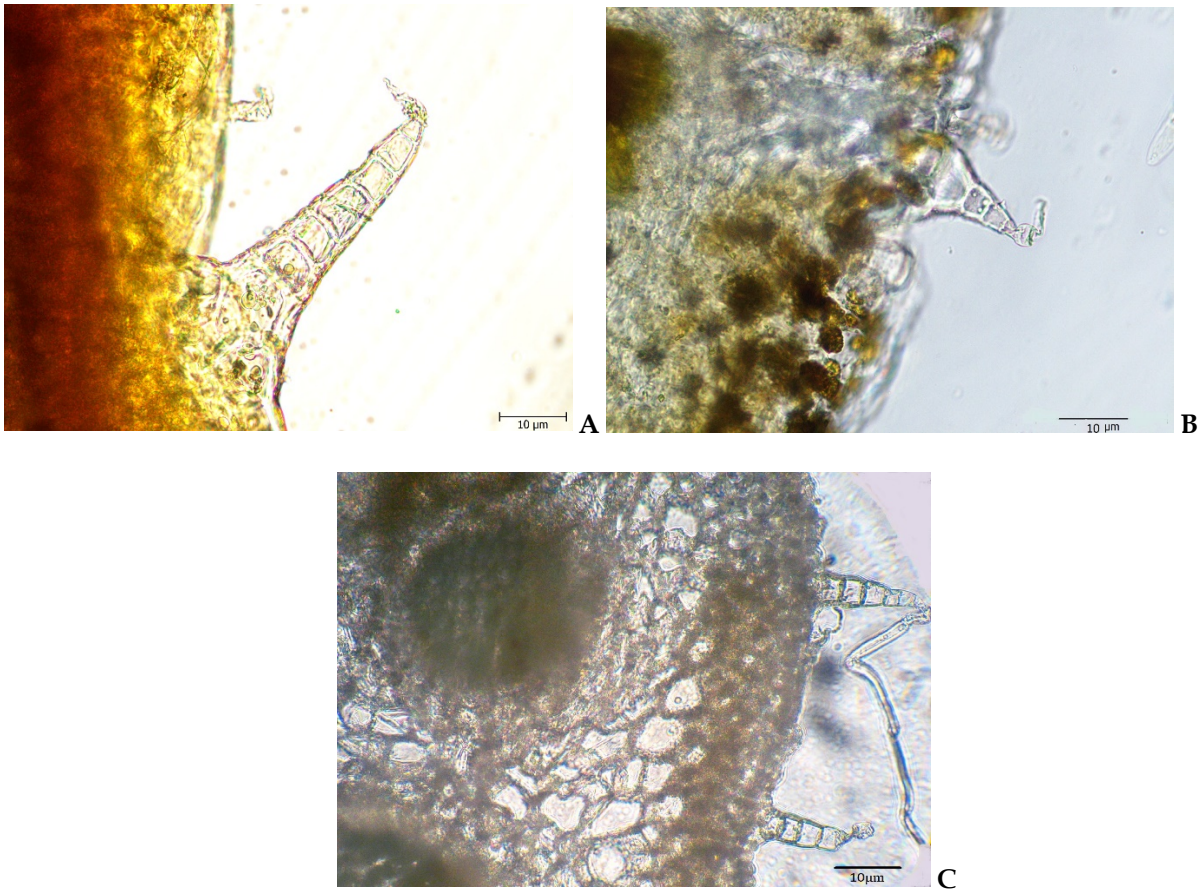

Figure 3. Non-glandular trichomes observed on studied *Cantaurea* species. A- CAA; B- CAP; C- CN (Scale bars: 10 µm)

Table 1. Anatomical measurements of leaf [Avr: average, Sd: standard deviation]

	CAA	CAP	CN
Upper epidermis			
Stomata type	anomocytic	anomocytic	anomocytic
Stomata index	18.83±2.49	21.28±3.26	12.88±2.58
Stomata length (µm) (Avr. ± Sd)	38.25±2.37	32.14±2.25	31±2.24
Stomata width (µm) (Avr. ± Sd)	23.89±1.82	22.91±1.88	16.5±2.23
Cuticle thickness (µm) (Avr. ± Sd)	3.73±0.59	2.91±0.74	4.7±1.3
Epidermis cell length (µm) (Avr. ± Sd)	9.06±1.57	4.60±1.10	15.5±5.9
Epidermis cell width (µm) (Avr. ± Sd)	12.59±2.25	8.09±1.17	26.5±3.3
Lower epidermis			
Stomata type	anomocytic	anomocytic	anomocytic
Stomata index	23.58±2.77	20.62±2.99	11.03±2.29
Stomata length (µm) (Avr. ± Sd)	41.25±2.70	43.55±3.38	27.77±4.41
Stomata width (µm) (Avr. ± Sd)	25.28±2.32	26.67±3.75	21.11±3.33
Cuticle thickness (µm) (Avr. ± Sd)	3.32±0.57	5.04±1.12	6.88±1.53
Epidermis cell length (µm) (Avr. ± Sd)	8.64±1.24	7.00±1.82	10.91±1.45
Epidermis cell width (µm) (Avr. ± Sd)	13.07±1.97	7.73±0.95	17.35±1.37

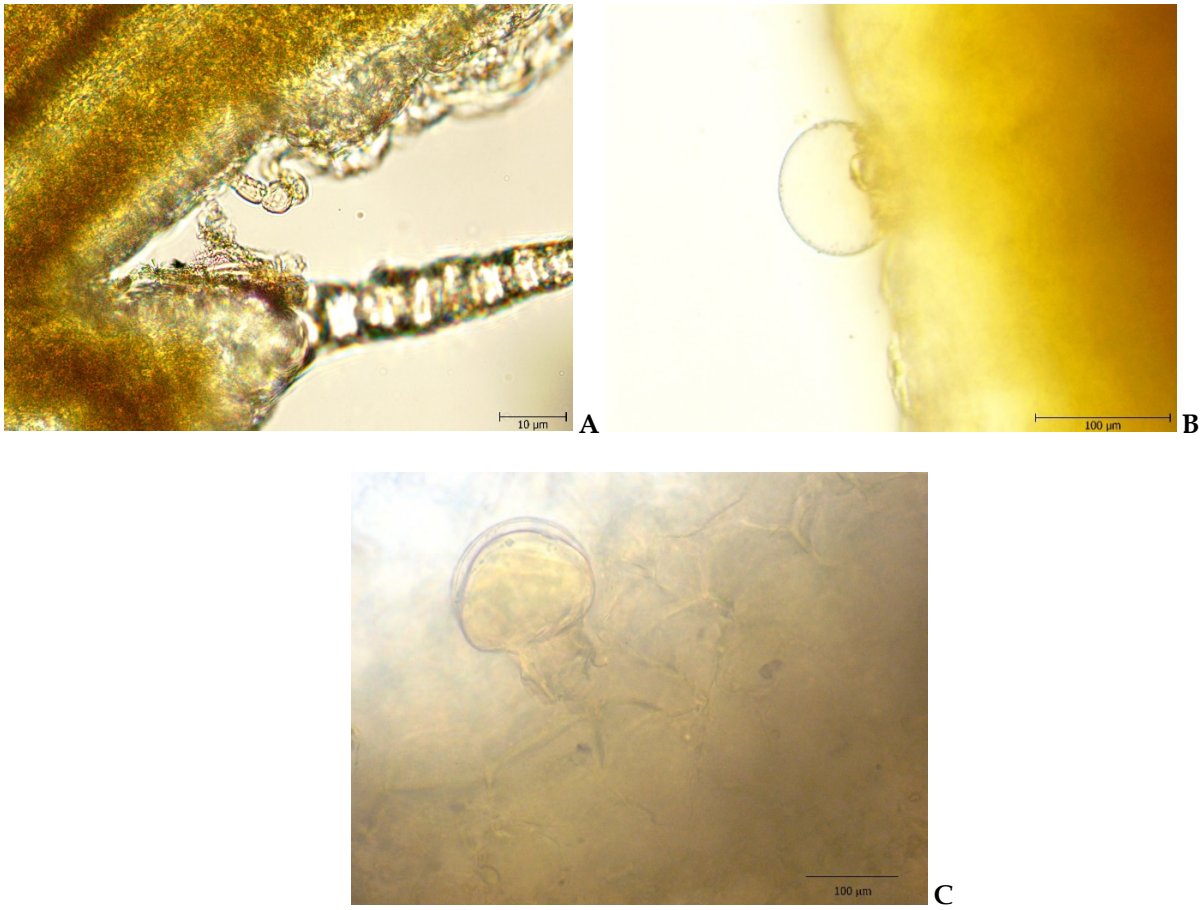

Figure 4. Glandular trichomes types observed on studied *Centaurea* species. A- CAA; B- CAP; C- CN

Stem anatomy

Centaurea antiochia* var. *antiochia: In cross section, the stem shows a slightly angular structure. Stem has non-glandular hairs that are numerous celled, uniseriate. The epidermis is made up of one layer of flattened, spherical cells. A single layer of epidermis follows a thin cuticle layer on the stem. The epidermis cell length is 7.5±1.8 µm, the epidermis cell width is 14.5±2 µm. Collenchyma tissue comes after the epidermis in the corners of the stem with 3-4 rows in CAA. Chlorenchyma tissue with abundant chloroplasts is seen under the epidermis. Between the sclerenchyma and the collenchyma is a single-layered endodermis. The endodermis cell length is 13.5±3.8 µm, the endodermis cell width is 19.5±4.8 µm. Between the chlorenchyma and the collenchyma, there exist sclerenchymatous bundles. After the chlorenchyma and collenchyma layer, 4-6 rows of cortical parenchyma cells come. Scleranchyma cells range in size from 4 to 5, and there are spaces between them. Bicollateral vascular bundles are scattered in a circular manner below the parenchymatous tissue. There are 19-20 various sized collaterall bundles of which 7 are large and 12 are small. Vascular bundles beneath the collenchymatic tissue have been found to be bigger. The number of small vascular bundles between large bundles varies between 2-3. Phloem only makes up a small portion of the bundles, which are mostly made of xylem and have sclerenchymatous tissue around them. Phloem sclerenchyma coming after the cortex is cap-shaped above the vascular bundles and has 7-10 cell rows. Phloem and xylem are both present beneath this layer. Between the phloem and xylem are 2-3 layer cambium. The mean diameter of the trachea in the conduction bundles is 10.5±3.3 µm (Table 2). The parenchymatous cells make up the pith. Polygonal and spherical parenchymatic pith cells are located in the middle of the stem. The average pith ray cell size is 80±23 µm. (Figure 6-A).

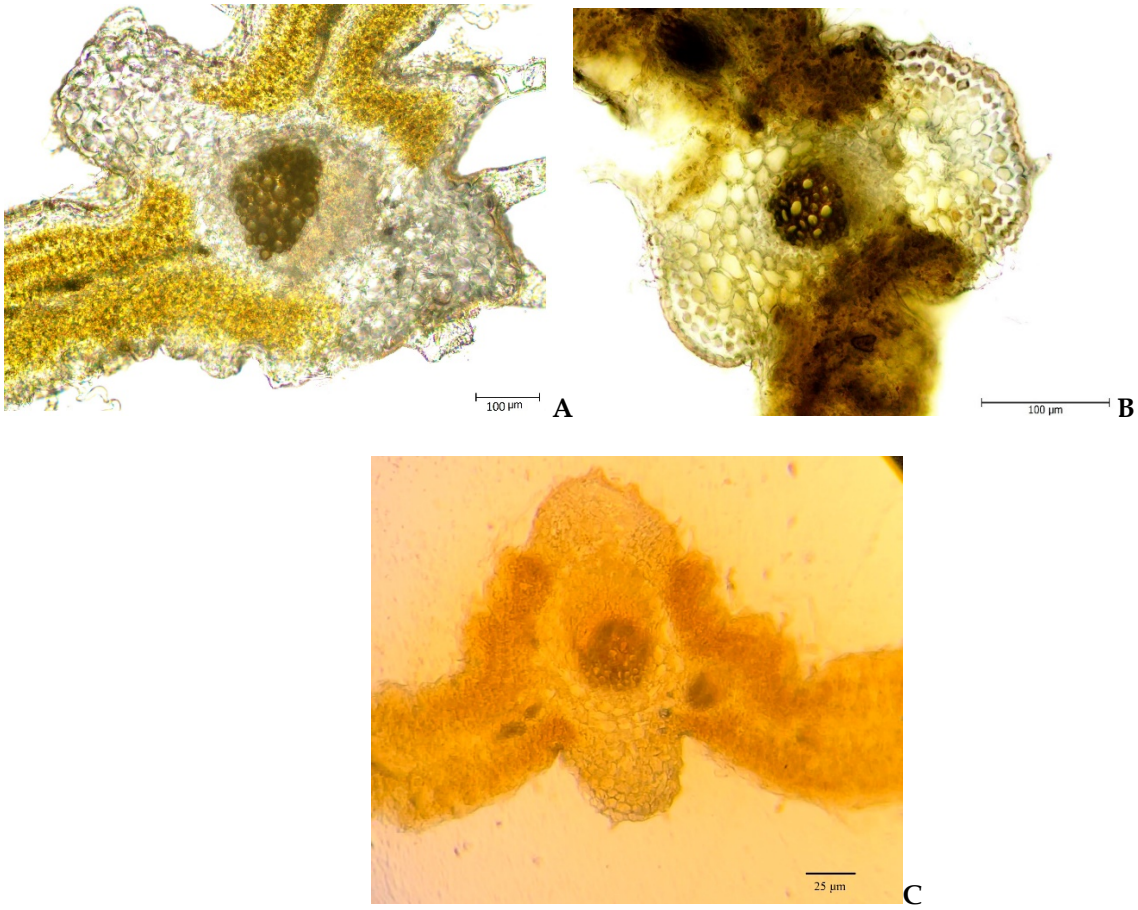

Figure 5. Cross section of leaves in *Centaurea* species.
A- CAA; B- CAP; C- CN

Centaurea antiochia* var. *praealta: The stem exhibits a slightly angular shape in cross section. In addition to glandular hairs with short stalks and big heads, the stem also has numerous celled, uniseriate, and recurved non-glandular hairs. One layer of flattened, ovoid-shaped, or spherical cells makes up the epidermis. The epidermis cell length is 8.5±3.8 µm, the epidermis cell width is 15.5±3.2 µm. On the stem, a thin layer of cuticle is followed by a single layer of epidermis. The corners of the stem have three to four layered of collenchyma tissue following the epidermis. Under the epidermis, chlorenchyma tissue containing lots of chloroplasts is visible. A single-layered endodermis lies between the collenchyma and the sclerenchyma. The endodermis cell length is 11.5±2.8 µm, the endodermis cell width is 19±3.3 µm. Sclerenchymatous bundles can be found between the collenchyma and the chlorenchyma. 4–5 rows of cortical parenchyma cells follow the layer of collenchyma and chlorenchyma. Underneath the parenchymatous tissue, bicollateral vascular bundles are dispersed in a circular pattern. There are between 19 and 20 collaterall bundles of different sizes, of which 6 are large and 13 are small. The two and three small vascular bundles are located between big bundles. After the cortex, the phloem sclerenchyma has 7–13 rows of cells and is cap-shaped above the vascular bundles. The average tracheal diameter in the conduction bundles is 15±3.9 µm. (Table 2). The centre of the stem is where the polygonal and spherical parenchymatic pith cells are found. Pith ray cell avarage is 60±13.6 µm (Figure 6-B).

***Centaurea nerimaniae*:** In cross section, the stem has a slightly angular form. The stem also features a large number of celled, uniseriate, and recurved non-glandular hairs in addition to glandular hairs with short stalks and large heads. The epidermis is composed of a single layer of ovoid cells. The epidermis cell length is 8±2 µm, the epidermis cell width is 16.5±2.8 µm. A single layer of epidermis is followed by a thin layer of cuticle on the stem. Following the epidermis, there are 4-5 layers of collenchyma tissue in the corners of the stem. There is chlorenchyma tissue containing chloroplasts. In between the collenchyma and the sclerenchyma is a single-layered endodermis. The endodermis cell length is 16.5±2.8 µm, the endodermis cell width is 32±6.4

µm.There are bicollateral vascular bundles in a circular pattern. There are 27 vascular bundles, among them 7 are large and 20 are small. The mean tracheal diameter in the conduction bundles is 10±2.5 µm. (Table 2). The pith ray calls are located in the cnetre of the stem and the mean of pith ray cell is 72±17 µm (Figure 6-C).

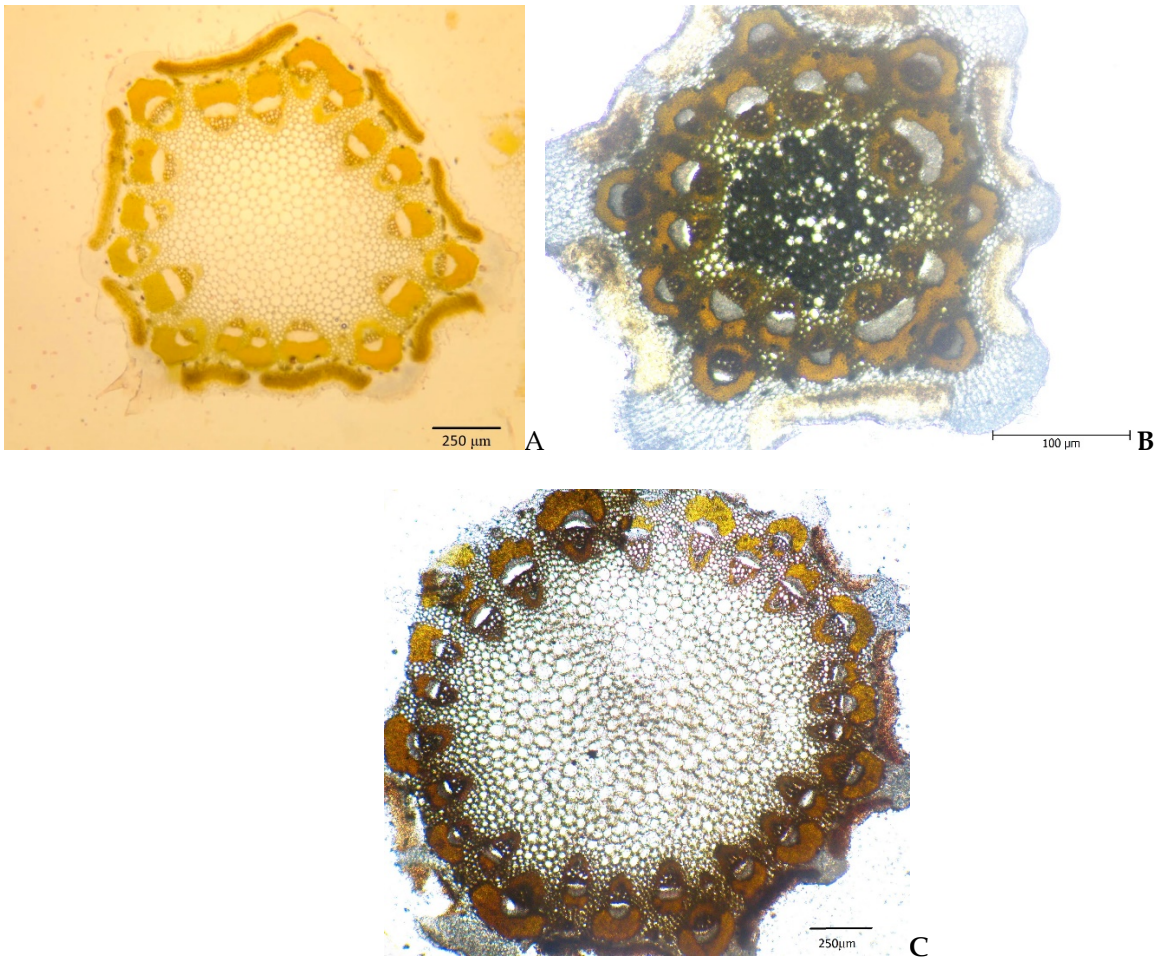

Figure 6. Stem of *Centaurea* species. A- CAA; B- CAP; C- CN

Table 2. Anatomical measurements of stem [Avr: average, Sd: standard deviation]

	CAA	CAP	CN
Epidermis cell length (µm) (Avr. ± Sd)	7.5±1.8	8.5±3.8	8±2
Epidermis cell width (µm) (Avr. ± Sd)	14.5±2	15.5±3.2	16.5±2.8
Pith ray cell	80±23	60±13.6	72±17
Trachea (µm) (Avr. ± Sd)	10.5±3.3	15±3.9	10±2.5
Endodermis cell length (µm) (Avr. ± Sd)	13.5±3.8	11.5±2.8	16.5±2.8
Endodermis cell width (µm) (Avr. ± Sd)	19.5±4.8	19±3.3	32±6.4

3. DISCUSSION

The anatomical characteristics of CAA, CAP and CN match those of the *Centaurea* species that have already been studied before [13-16].

As a result of this study, it was determined that the anatomy of 3 *Centaurea* taxa, which are very close to each other morphologically, have very similar anatomical features. The taxon with the most stomata on the lower surface of the leaf is CAA, while the one with the most stomata on the upper surface is CAP. The taxon with the longest and widest stomata on the lower leaf surface is CAP, while the taxon with the longest and widest stomata on the upper surface is CAA. The taxon with the thickest cuticle on the upper and lower leaf surfaces is CN. The taxon with the largest length and width of epidermis cells on the lower and upper surface of the leaf is CN. The dimensions of the stem epidermis cells are very close to each other. CAA has the largest pith ray cell and CAP has the largest trachea cells in stem. Among these three taxa, CN has the longest and widest

stem endodermis cells. The glandular and nonglandular trichomes are very similar. The findings were compared with the results of an anatomical study on 6 taxa of the genus *Centaurea*. This study was done with *Centaurea aggregata* subsp. *aggregata* Fisch & Mey, *Centaurea antitauri* Hayek, *Centaurea balsamita* Lam., *Centaurea cynarocephala* Wagenitz, *Centaurea urvillei* D. subsp. *armata* Wagenitz, *Centaurea urvillei* D. subsp. *hayekiana* Wagenitz [17]. Vascular bundles are bicollateral type in all 6 taxa. The number of vascular bundles is the highest in *C. antitauri* (73 vascular bundles), is the lowest in *C. aggregata* subsp. *aggregata (22* vascular bundles) but in our study the number of vascular bundles is the highest in CN (27 vascular bundles), and the CAA and CAP have 19-20 vascular bundles. Trachea diameter is the biggest in *C. cynarocephala (*46.62±10.26), lowest in C. *urvillei* subsp. *hayekiana* (18.36±3.82) but the trachea diameters of our species are even smaller, among three species the trachea diameter is the biggest in CAP (15±3.9). When we compared our results with these six taxa in terms of leaf anatomy, it was seen that leaves were isobilateral and amphystomatic for all taxa, and stoma types were anomocytic. In terms of stoma length and width, CN stoma sizes were similar to these six taxa, while CAA and CAP stomata had the greatest values in terms of length and width.

4. CONCLUSION

Centaurea species are important in terms of pharmacy due to the different active ingredients and important medicinal effects. 126 of 174 *Centaurea* species naturally found in Turkey are endemic and it is important for taxonomy to determine the anatomical structures of these species and to determine their similarities and differences with each other. In this study, the anatomical structures of 3 different *Centaurea* taxa, 2 of which are endemic, were studied for the first time and compared with each other. Common aspects in the anatomy of these three taxa were determined. An epidermal layer can be found on both the upper and lower surfaces of leaves. There is collateral vascular bundles. The xylem, which includes vessels and xylem parenchyma, is found near the upper epidermis and the phloem is located near the lower epidermis. They have amphistomatic leaf and the stomata type is anomocytic. The usual dicotyledonous stem anatomy is visible in their stem. There are epidermis with a cuticle, collenchymatous tissues in the cortex and multicellular stem trichomes. The innermost layer of the cortex, the endodermis, is distinctive. Vascular bundles are grouped in a ring around the pith and collateral. Parenchyma cells with thin walls and distinct intercellular spaces make up the pith. This study will also shed light on future studies on the anatomy of other *Centaurea* species.

5. MATERIALS AND METHODS

5.1. Plant Material

The *Centaurea* taxa used in this study were collected and identified by Prof. Dr. Şükran Kültür from different areas within the borders of Mersin province (Table 3). The *Centaurea* specimens were identified by using the "Flora of Turkey and the East Aegean Islands" and and recent research articles on these species [6,7]. The plant samples placed in ISTE (The Herbarium of the Faculty of Pharmacy of Istanbul University). In order to conduct anatomical research, the sample was fixed in 70% alcohol. Sartur solution was used to stain cross sections of the stem, leaves, and leaf surface sections. Using a Canon A 640 digital camera and an Olympus BH 2 light microscope, pictures of the well-stained slices were taken. The anatomical features of stems, leaves and leaf surfaces have been investigated. The glandular, non-glandular trichomes, mesophyll cells, stomatal index, stomata structures, epidermis cells of the leaves and trachea, pit ray cells, epidrmis cells and endodermis cells of the stem were compared.

Table 3. Collected species information

Species	Locality	Herbarium number
***Centaurea antiochia* Boiss. var. *antiochia* Boiss.**	Hacı Ahmetli köyü üstleri, Arsuz, Hatay	ISTE 102722
***Centaurea antiochia* Boiss. var. *praealta* (Boiss. & Bal.) Wagenitz**	Arslanköy, Mersin	ISTE 84004
***Centaurea nerimaniae* Ş.Kültür.**	Arslanköy, Mersin	ISTE 98163

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Author contributions: Concept – Ş.K.; Design – Ş.K., E.Ö.N.; Supervision – Ş.K.; Materials – Ş.K.; Anatomical experiments - Ş.K., E.Ö.N.; Data Collection – Ş.K., E.Ö.N; Analysis – Ş.K., E.Ö.N.; Literature Search – Ş.K., E.Ö.N.; Writing – Ş.K., E.Ö.N.; Critical Reviews – Ş.K., E.Ö.N.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

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