

Investigation of the Stem and Leaf Anatomy of Five *Artemisia* L. Species Growing in Türkiye

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Received: 27.01.2023 Received in revised: 31.05.2023 Accepted: 10.08.2023

ABSTRACT

In this study, it is aimed to make anatomical analysis of different *Artemisia* L. species (*A. absinthium* L., *A. annua* L., *A. abrotanum* L., *A. incana* (L.) Druce, *A. tournefortiana* Rchb.) grown in Turkey and contribute to the definition of this genus. The anatomical study materials were preserved in alcohol (70%). The sections were cut manually with a sharp razor blade and stained with Chloralhydrate solution (50%) and Sartur Reagent. Anatomical structures were examined under the light microscope, and detailed structures were shown with photographs. The results showed that although the leaf and stem anatomical characteristics were compatible with the Asteraceae family, some characters could be specific for species. The stems of all studied species have more or less irregular rounded shapes, but ribs containing collenchyma tissue were much more prominent in the stem of *A. annua*. The leaves of *A. incana* and *A. absinthium* were covered on both sides with glandular and dense T-shaped non-glandular trichomes, but *A. tournefortiana* had almost no hair. In both, the leaf (near to xylem) and the stem (next to the endodermal cells) of *A. tournefortiana*, there were particularly large secretory canals located. The anatomical structure of stems and leaves of *A. incana* and *A. tournefortiana* growing in Turkey was investigated for the first time. As a result of the study, it was shown that the studied species were anatomically distinguishable from each other. The study also contributes to the definition of the genus *Artemisia* L., which has taxonomic problems.

Key words: *Artemisia*, Leaf, Secretory canals, Stem, Trichomes.

Türkiye’de Yetişen Beş *Artemisia* L. Türünün Gövde Ve Yaprak Anatomisinin İncelenmesi

ÖZ

Bu çalışmada Türkiye’de yetişen farklı *Artemisia* L. türlerinin (*A. absinthium* L., *A. annua* L., *A. abrotanum* L., *A. incana* (L.) Druce, *A. tournefortiana* Rchb.) anatomik olarak incelenmesi ve bu cinsin tanımlanmasına katkı sağlamak amaçlanmıştır. Anatomik çalışma materyalleri alkolde (%70) muhafaza edilmiştir. Keskin jiletler kullanılarak manuel olarak kesitler alınıp, Sartur Reaktif ve Kloralhidrat (%50) solüsyonu ile boyanmıştır. Anatomik yapılar ışık mikroskopunda incelenmiş, detaylı yapıları fotoğraflarla gösterilmiştir. Sonuçlar, gövde ve yaprak anatomik özelliklerinin Asteraceae familyası ile uyumlu olmasına rağmen bazı karakterlerin türe özgü olabileceğini göstermiştir. Enine kesitlerde, incelenen tüm türlerin gövdeleri aşağı yukarı düzensiz yuvarlak şekle sahiptir, ancak kollenkima dokusu içeren belirgin çıkıntılar sadece *A. annua* gövdesinde fark edilebilir. *A. absinthium* ve *A. incana*’nın yapraklarının her iki yüzü yoğun şekilde glandüler ve T şeklinde glandüler olmayan

tüylerle kaplıydı, *A. tournefortiana*'da ise neredeyse tüy yoktu. *A. tournefortiana*'nın hem gövdesinde (endodermal hücrelerin yanında) hem de yaprağında (ksilem yakınında) bulunan oldukça belirgin salgı kanalları vardı. Bu çalışma ile, Türkiye'de yetişen *A. tournefortiana* ve *A. incana*'nın yaprak ve gövdelerinin anatomik yapısı ilk kez incelenmiştir. Çalışma sonucunda incelenen türlerin anatomik olarak birbirinden ayırt edilebilir olduğu gösterilmiştir. Çalışma aynı zamanda taksonomik sorunlar yaşayan *Artemisia* L. cinsinin tanımlanmasına da katkı sağlamaktadır.

Anahtar kelimeler: *Artemisia*, gövde, Salgı kanalları, Trikomlar, Yaprak.

INTRODUCTION

The *Artemisia* L. genus (Asteraceae) having medicinal and economic significance in worldwide. It contains almost 500 species of both herbs and shrubs and is a diverse genus of the *Anthemideae* tribe (Hussain, 2020). *Artemisia* genus is represented by 27 taxa, including 21 species, 3 subspecies and 3 varieties in Turkey (Sancar et. al., 2021). *Artemisia* species, known as “pelin otu, Kâbe süpürgesi, yavşan otu, Kâbe kekiği, peygamber süpürgesi” are used in traditional Anatolian medicine as tonic, antimalarial, anthelmintic, antidiabetic, wound healer as much as for bronchitis, ulcer and tuberculosis (Kirbağ et. al., 2019; Güner et. al., 2012).

Different species of *Artemisia* - contain mainly terpenoids, flavonoids, sterols, and coumarins. *Artemisia* species have several biological activities including antibacterial, antifungal, antioxidant, antihepatotoxic, antimalarial, and cytotoxic activities (Bora and Sharma, 2011).

Many studies considering morphological, anatomical, cytogenetic, molecular and evolutionary features of *Artemisia* species have been done in worldwide. The anatomical studies have been carried out in various countries such as Pakistan, Iran, Serbia, (Nourbakhsh, 2008; Konowalik ve Kreitschitz, 2012; Hussain et al., 2019) but there are few data about anatomical aspects and their value in the classification of the species of *Artemisia* in Turkey. The aim of this study is to make comparative anatomical analyzes of *Artemisia* genus, which has taxonomic problems and difficult to distinguish between species due to similar morphological features, on five different *Artemisia* species grown in Turkey (*A. annua* L., *A. absinthium* L., *A. incana* (L.) Druce, *A. abrotanum* L. and *A. tournefortiana* Rchb.) (Fig 1).

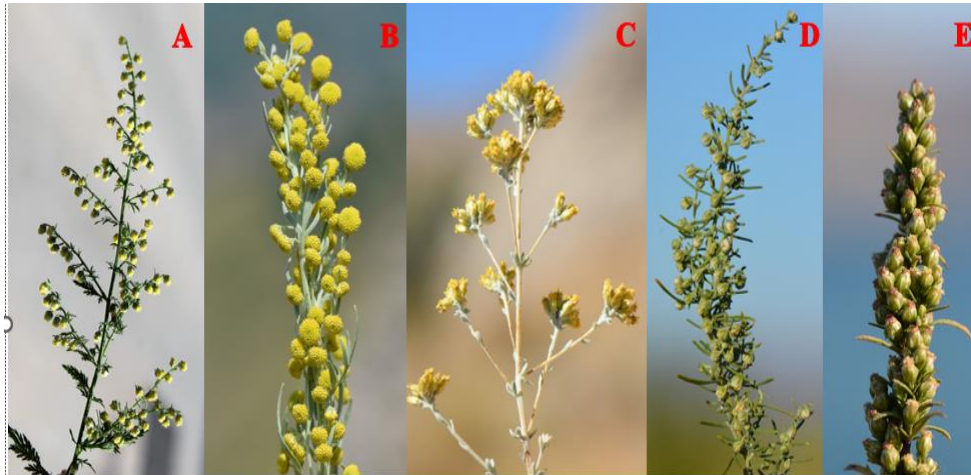


Figure 1. A: *A. annua*, B: *A. absinthium*, C: *A. incana*, D: *A. abrotanum*, E: *A. tournefortiana*.

MATERIAL AND METHOD

Plant Material

We have chosen five *Artemisia* species belonging to subgenus *Artemisia* Less.: *A. absinthium*, *A. annua*, *A. abrotanum*, *A. incana*, *A. tournefortiana*. Fresh plant materials were collected during field trips conducted in Turkey in 2019. The species were identified according to the morphological descriptions in Flora of Turkey and the East Aegean Islands (Cullen, 1975) and a doctoral thesis in which *Artemisia* species were revised (Kursat, 2010). The voucher specimens were prepared and deposited in the Herbarium of Ankara University, Faculty of Pharmacy (AEF).

Names of collected species, the dates and localities of their collection, and the (AEF) herbarium numbers of voucher specimens are given in Table 1.

Table 1. Names, collection dates/localities, and the herbarium numbers of the species.

Species	Locality and Date of Collection	AEF No.
<i>A. abrotanum</i> L.	C5, Bitlis: Güroymak, between Muş and Güroymak, 500 m away from Güroymak district, roadside, streamside, 1290 m, 05.09.2019	28823
<i>A. absinthium</i> L.	B5, Kars: 1 km from the city center, on the Tuzluca-Kars road, among the rubble piles on the roadside, 1765 m, 10.08.2019	28821
<i>A. annua</i> L.	B1, Sakarya, Akyazı, on the Kuzuluk road, roadside, 13.10.2019	28820
<i>A. incana</i> (L.) Druce	C5, Muş: Malazgirt, Between Aktuzla village and Karıcalı village, roadside, slopes, 1560 m, 09.08.2019	28822
<i>A. tournefortiana</i> Rchb.	B5, Kars: 1 km from the city center, on the Tuzluca-Kars road, among the rubble piles on the roadside, 1765 m, 10.08.2019	28824

Anatomical analysis

The anatomical materials were preserved in alcohol (70%). The sections were cut manually with a sharp razor blade and stained with Chloralhydrate solution (50%) and Sartur Reagent. Observations of the prepared slides were performed under a light microscope (Leica DM 1000) with a digital camera (Leica DFC290) and a computer with the imaging software Leica IM 1000.

RESULTS AND DISCUSSION

In this study, the stem and leaf anatomies of *A. absinthium*, *A. abrotanum*, *A. annua*, *A. incana*, and *A. tournefortiana* were examined in detail with cross-sections, and important characters in diagnosis were revealed. Findings of anatomical characters are presented comparatively in Table 2 and Table 3.

Stem

In cross-sections, the stems of all studied species have more or less irregular rounded shapes with ribs (Fig 2). These ribs contained collenchyma tissue are more prominent in *A. annua* stem and two or four stomata are located on its ribs. The one-layered epidermis, composed of oval and isodiametric cells, is present on the surface of all investigated species. The non-glandular and glandular trichomes occur on the stems. *A. incana* and *A. absinthium* species were densely covered with non-glandular T-type trichomes (Fig 3) on their stem while Asteraceae-type glandular trichomes (Capitate with unicellular stalk, multicellular head) were fewer as in *A. annua* and *A. abrotanum*. On the other hand, the *A. tournefortiana* stem had no hair at all.

The cortex is composed of collenchyma and parenchyma in all investigated species. The ribs contained collenchyma cells with a different number of layers (9-13 celled layers in *A. annua* and *A. absinthium*; 4-6 in *A. abrotanum* and *A. tournefortiana*; 1-3 in *A. incana*), whereas parenchyma is present between the ribs. The remainder of the cortex is composed of 2-4 layers of parenchyma cells. The vascular bundles are all collateral and arranged as forming a circle. In *A. absinthium* and *A. tournefortiana* stems, vascular bundles are divided by parenchyma cells. 2-12 layers of well-lignified sclerenchyma were above the phloem. The phloem was composed of crushed, small, irregular cells. The phloem layer of *A. annua* was relatively thinner. The xylem consists of 3-8 parallel rows of xylem elements; each row comprised 2–7 vessels. Sclerification of the central cylinder could be noticed clearly because well-developed xylem and sclerenchyma tissue make a ring shape. The thickness of this ring is greater in *A. incana* and *A. abrotanum* compared to the other 3 species.

Prismatic crystals were dispersed on the pith of *A. annua*, while *A. abrotanum* had druses on its pith. Very large secretory canals are present in the cortex of *A. tournefortiana* (Fig 4B), while small secretory canals could be seen in the cortex of *A. annua*, *A. absinthium*, *A. incana* and *A. abrotanum*. Small secretory canals in the pith are found only in the *A. absinthium* stem (Fig 4A).

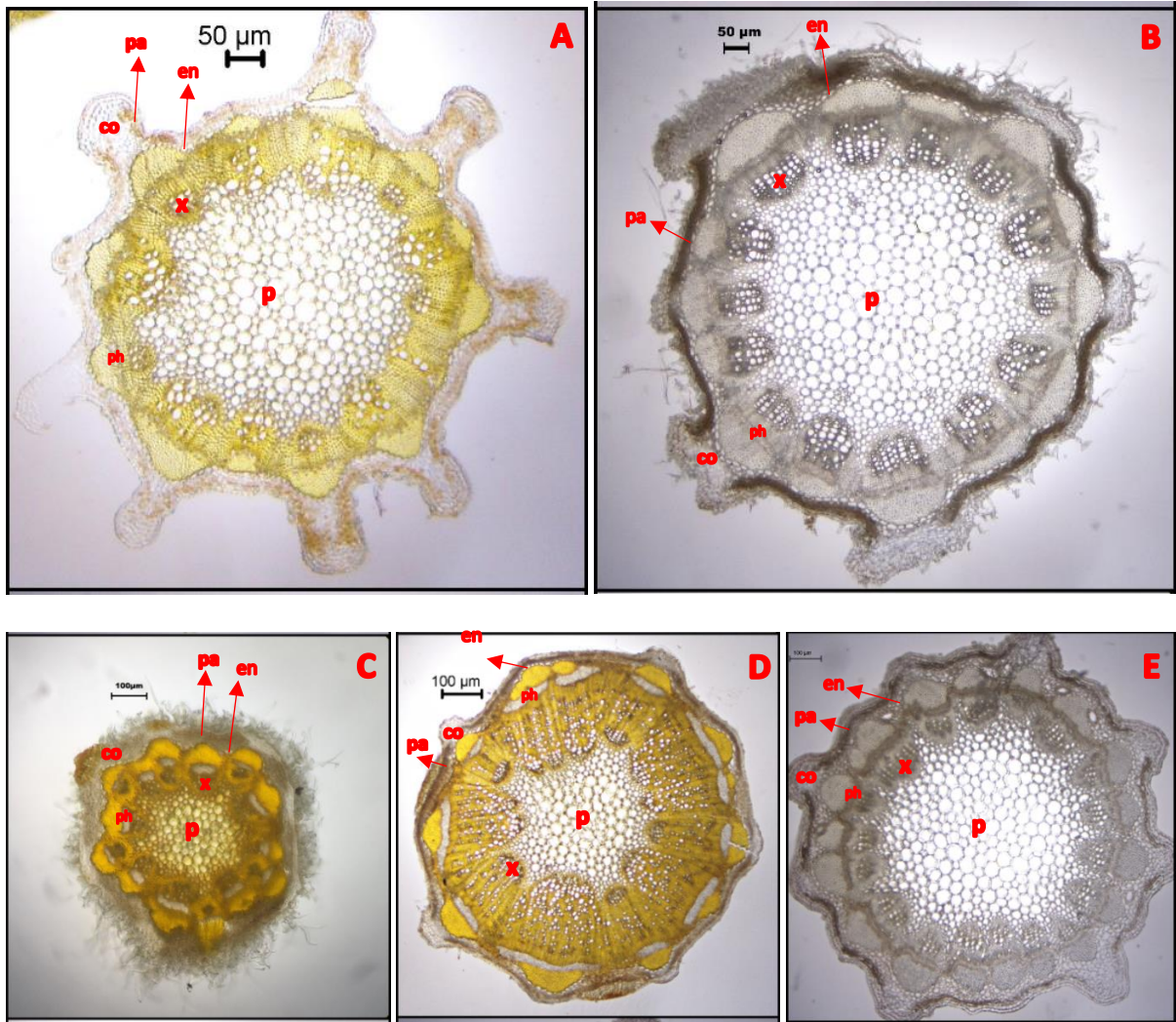


Figure 2. Cross section of stem. **A:** *A. annua*, **B:** *A. absinthium*, **C:** *A. incana*, **D:** *A. abrotanum*, **E:** *A. tournefortiana*. **co:** collenchyma, **en:** endoderm, **p:** pith, **pa:** parenchyma, **ph:** phloem, **x:** xylem.



Figure 3. Cross section of *A. absinthium* stem. T-shape trichome.

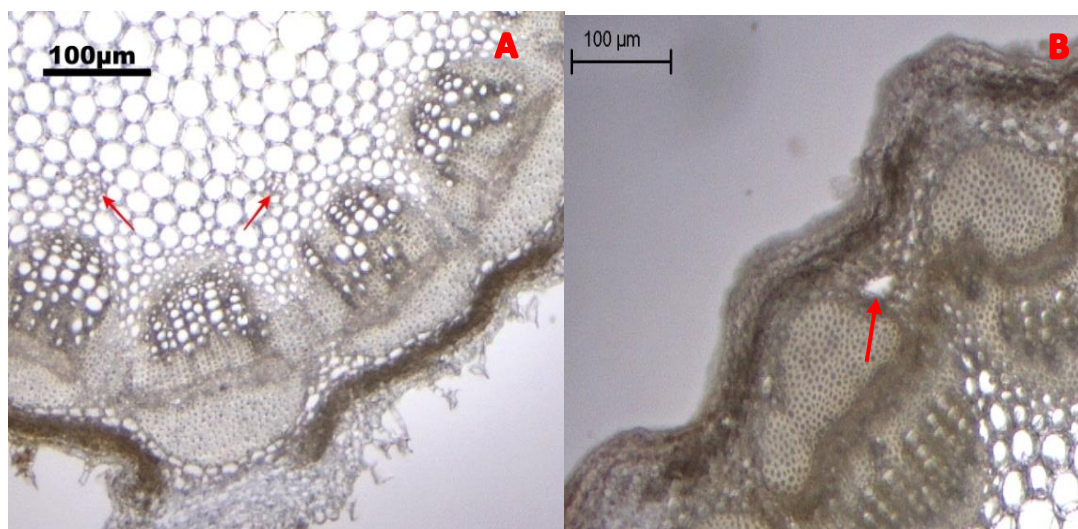


Figure 4. A: Cross section of *A. absinthium* stem, secretory canal on the pith, B: Cross section of *A. tournefortiana* stem, large secretory canal.

Leaf

Leaf cross-section is almost triangular in *A. incana* (Fig. 5C) and *A. abrotanum* (Fig. 5D), while in other species, *A. absinthium* (Fig. 5B), *A. annua* (Fig. 5A) and *A. tournefortiana* (Fig. 5E), has long and linear appearance. The leaf is monofacial; the epidermis is covered by a thin layer of cuticle in all five species. On the upper and lower leaf sides, a one-layered epidermis could be noticed (Fig 6A). The palisade tissues adjacent to both adaxial and abaxial sides are consisted of short columnar cells with chloroplasts arranged in one or two layers. In the midrib region collateral vascular bundles could be found. On the leaf cross sections, the main vein is slightly protruding with two lateral ribs in *A. absinthium* (Fig 6B). The ribs are more prominent in *A. annua* and *A. tournefortiana*. The leaves of *A. incana* and *A. absinthium* were covered by Asteraceae type glandular trichomes and densely T-shaped non-glandular trichomes on both sides. However, *A.abrotanum* has sparse; *A.annua*, and *A. tournefortiana* have very sparse glandular hairs. There are secretory canals located near to xylem or sclerenchyma tissues. However, these canals are more prominent in the leaf of *A. tournefortiana* (Fig 6C).

In plant systematics, various macro characters and leaf anatomy are used to solve the taxonomic problems of plants. The leaf epidermis has important structures (stomata, epidermal cells, subsidiary cells, guard cells, and trichomes, etc.) in the identification of species (Hussain, 2020; Odewo et. al., 2020).

The trichomes are found on the leaf surface of plants and act as a physical barrier to protect plants from pathogens and herbivorous attacks. The glandular trichomes in *Artemisia* species are the main sources from which the production of secondary metabolites occurs (Hussain, 2020; Janačković et. al., 2019). It was investigated whether there was an increase in the number and size of glandular trichomes of *A. annua* under stress conditions, but positive results could not be obtained (Kjær, 2012). In many anatomical studies with *Artemisia* species, trichomes were emphasized and the most common Asteraceae type (capitate) glandular trichomes were reported (Janačković et. al., 2019).

Findings for *A. absinthium*, *A. annua* and *A. incana* in an anatomical study conducted with 28 *Artemisia* taxa in 2008 are consistent with our findings (Nourbakhsh, 2008). The results obtained when various studies including *A. absinthium*, *A. annua* and *A. tournefortiana* species were examined are also consistent with our findings (Hayat et. al., 2009; Hussain et. al., 2019). A study conducted with the taxa *Artemisia absinthium* var. *absinthium* and *A. absinthium* var. *calcigena* analyzed the anatomical and morphological characters comparatively. However, it was observed that the leaves were bifacial. In our findings, there are palisade parenchyma cells on both sides of the leaf and it is monofacial (Konowalik and Kreitschitz, 2012). Our findings from the *A. abrotanum* plant are also compatible with the literature (Ivashchenko and Ivanenko, 2012).

Table 2. Comparison of stem anatomies of examined *Artemisia* species.

Anatomical character	<i>A. abrotanum</i>	<i>A. absinthium</i>	<i>A. annua</i>	<i>A. incana</i>	<i>A. tournefortiana</i>
Shape of the stem	Usually irregularly shaped, cylindrical, slightly protruding	Usually irregularly shaped, cylindrical, more or less protruding	Cylindrical with prominent protrusions	Usually irregularly shaped, cylindrical, slightly protruding	Usually irregularly cylindrical, prominently protruding
Non-glandular trichomes	Sparse, single-celled	Dense, T-shaped	Sparse, T-shaped	Dense, T-shaped	-
Asteraceae type glandular trichomes	Sparse	Dense	Sparse	Dense	Sparse
Epidermis cells	A single row, usually elliptic	A single row, usually elliptic	A single row, usually elliptic	A single row, usually elliptic	A single row, usually elliptic
Parenchyma cells	3-6 rows, flat or round	3-6 rows, flat or round	3-6 rows, flat or round	2-9 rows, flat or round	2-9 rows, flat
Collenchyma cells	6-7 rows, irregularly thickened	6-13 rows, irregularly thickened	4-12 rows, irregularly thickened	6-8 rows, irregularly thickened	6-8 rows, irregularly thickened
Endoderm cells	Single row, large, flat, or elliptic shaped	Single row, large, elliptic shaped and prominent throughout the stem	Single row, large, flat, or elliptic shaped and prominent throughout the stem	Single row, large, elliptic shaped	Single row, large, flat, or elliptic shaped and prominent throughout the stem
Sclerenchyma cells	3-12 rows, with thick-walled	1-13 rows	1-9 rows	4-15 rows, with very thick-walled	2-13 rows, with thick-walled
Vascular bundle	Collateral	Collateral	Collateral	Collateral	Collateral
Floem cells	3-7 rows, irregularly shaped, small	1-4 rows, irregularly shaped, small	1-3 rows are irregularly shaped, small	3-6 rows, irregularly shaped, small	3-6 rows, irregularly shaped, small
Xylem	Prominent trachea and tracheids	3-8 lines and 2-7 vessels in each line	3-7 lines and 2-5 vessels in each line	3-8 lines and 2-7 vessels in each line	Large cells in 3-7 rows
Pith	1/3 of the body. Thin-walled, large cells containing druses	2/3 of the body. Thin-walled, large parenchymatic cells.	2/3 of the body. Thin-walled, large cells containing prismatic crystals.	1/4 of the body. Thick-walled, parenchymatic cells.	2/3 of the body. Thin-walled, large parenchymatic cells.
Secretory channel	-	Small, located in the cortex and in the pith	Small, located above or below the sclerenchyma cells	Enlarged, small, thick-walled between sclerenchyma cells	Enlarged, rather large, above endoderm cells

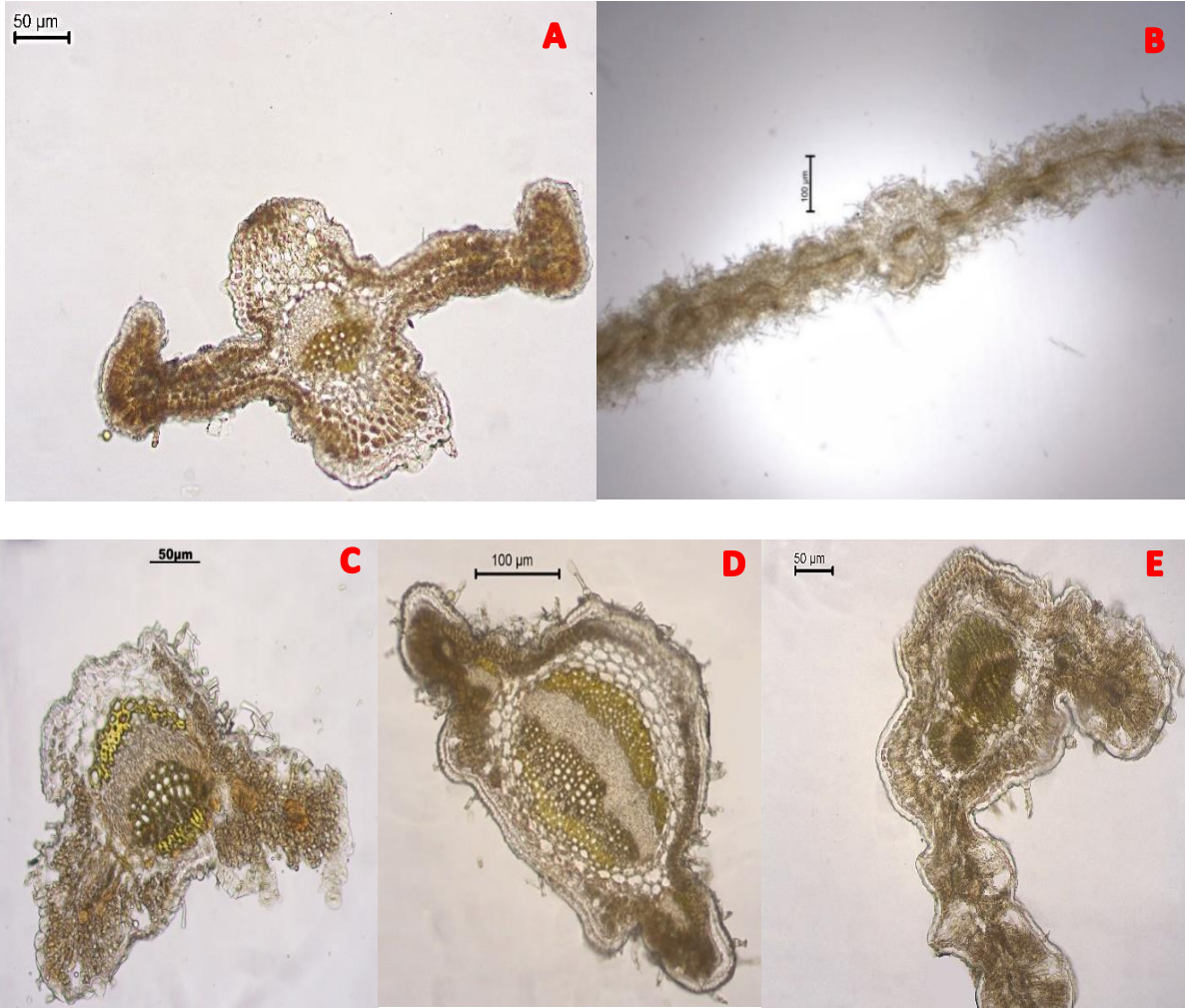


Figure 5. Cross section of leaf. A: *A. annua*, B: *A. absinthium*, C: *A. incana*, D: *A. abrotanum*, E: *A. tournefortiana*

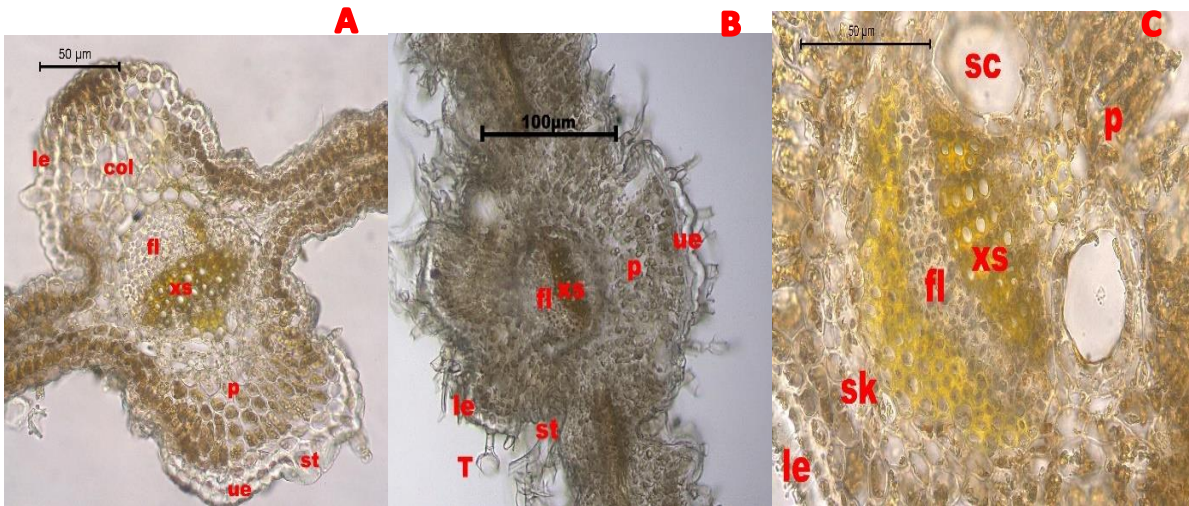


Figure 6. Cross sections of the leaf. A: *A. annua*, B: *A. absinthium*, C: *A. tournefortiana*, col: collenchyma, fl: phloem, le: lower epidermis, p: parenchyma, sc: secretory channel, st: stomata, T: T-shape non-glandular trichome, ue: upper epidermis, xs: xylem.

Table 3. Comparison of leaf anatomies of examined *Artemisia* species.

Anatomical character	<i>A. abrotanum</i>	<i>A. absinthium</i>	<i>A. annua</i>	<i>A. incana</i>	<i>A. tournefortiana</i>
Shape of the leaf	Triangular	Oblong-linear	Oblong-linear	Triangular	Oblong
Non-glandular trichomes (on the lower and upper surfaces)	T-shaped	Dense T-shaped	-	Very dense T-shaped	-
Asterace type glandular trichomes (on the lower and upper surfaces)	Sparse	Dense	Very sparse	Dense	Very sparse
Epidermis cells (on the lower and upper surfaces)	Single row, rounded, covered with cuticle	Single row, rounded/spherical, covered with cuticle	Single row, rounded/spherical, covered with cuticle	Single row, rounded, covered with cuticle	Single row, rounded, covered with cuticle
Mesophyll	Monofascial	Monofascial	Monofascial	Monofascial	Monofascial
Palisade parenchyma (above the lower epidermis and below the upper epidermis)	1-2 rows of long, tightly arranged cells	Single row of long, tightly arranged cells	2 rows of long, tightly arranged cells	1-2 rows of long, tightly arranged cells	Single row of long, tightly arranged cells
Spongy parenchyma (below the palisade parenchyma)	1-5 rows of cells with spaces between them		1-2 rows of cells with spaces between them	1-2 rows of cells with spaces between them	1-2 rows of cells with spaces between them
Secretory channel	-	Enlarged secretory ducts on the lower epidermis	-	-	Prominent large secretory ducts located on either side of the xylem
Midrib					
Protruding towards the lower and upper surface.	+	+	++	+	+
The xylem is towards the upper epidermis and the phloem is towards the lower epidermis.	+	+	+	+	+
Sclerenchyma cells on xylem and phloem	1-6 rows	-	-	1-3 rows	1-3 rows

Anatomical structure of leaves and stems of *A. tournefortiana* and *A. incana* growing in Turkey was demonstrated for the first time. Our results revealed that studied species are anatomically distinguishable between each other and also provided valuable features for better species identification and contribute to the anatomy of the genus *Artemisia*.

It has shown by studies that the differences and similarities between species are important in the correct diagnosis of plants. Although research on *Artemisia* species continues around the world, they mainly focus on the biological activities of plant extracts, essential oils or secondary metabolites obtained. Little is

known about the anatomical aspects and taxonomic value of the genus *Artemisia*. A comprehensive anatomical study is needed with all *Artemisia* taxa grown in Turkey.

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Contribution Rate Statement Summary of Researchers: The authors declare that they have contributed equally to the article.

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