

# LEAF ANATOMY OF ARTEMISIA (ASTERACEAE) IN IRAN AND ITS TAXONOMIC IMPLICATIONS

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Altogether 28 species of *Artemisia* were used in anatomical studies in order to introduce the most important anatomical features of them and their possible taxonomic applications. According to leaf sections, three groups are detectable which differ in their midrib shape, its position in relation to lamina and length and number of veins in each lamina half, which is in agreement with leaf morphology, in addition hygrophyte and xerophyte species are distinguishable. Using anatomical characters one can put some species in complexes which are close to each other in morphological features. Species of complexes of *A. aucheri*, *A. sieberi* and *A. kopetdaghensis* have common features which support the close relationship of them.

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مطالعه تشریحی برگ *Artemisia* L. در ایران و کاربرد تاکسونومیکی آن

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۲۸ گونه از جنس درمنه (*Artemisia* L.) بمنظور شناسایی مهمترین ویژگی های تشریحی این جنس و امکان کاربرد تاکسونومیکی آنها، مورد بررسی قرار گرفت. بر اساس مقطع برش عرضی برگ، سه گروه قابل تشخیص می باشند که از نظر شکل رگبرگ میانی، موقعیت آن نسبت به پهنک، طول پهنک و تعداد دستجات آوندی در هر نیمه پهنک، متفاوت می باشند. این تفاوتها منطبق با صفات ریخت شناسی بوده و گونه های نم پسند و خشکی پسند را بخوبی نشان می دهند. طبق صفات تشریحی، برخی گونه ها در کمپلکسهایی قرار می گیرند که از نظر ریخت شناسی نیز به هم نزدیک هستند. گونه های کمپلکسهای *A. aucheri*، *A. sieberi* و *A. kopetdaghensis* دارای صفات مشترکی هستند که ارتباط نزدیک بین آنها را تایید می کند.

## INTRODUCTION

*Artemisia* L. is the largest genus in the tribe *Anthemideae* and one of the largest genera in the *Asteraceae*. It contains 500 species, widely distributed all over the northern hemisphere, in Asia with two main speciation centers in west and central Asia, in the New World and few representatives in the South Africa and Hawaii Islands (Bremere & Humphries 1993). *Artemisia* is one of the most important genera of the Irano-Turanian steppes, having a wide and different

range of morphological features and a wide habitat (hygrophyte to xerophyte and from sea level to more than 3000 meter) which makes their diagnosis troublesome.

The importance of this genus has prompted many authors to undertake studies of the genus. In spite of differences in the proposed classifications of many authors, it is obvious that the main taxonomic divisions in the genus have persisted. The four large subgenera more or less present in all classifications of *Artemisia*

are: *A.* subgen. *Artemisia* has heterogamous capitula with female outer florets and hermaphroditic fertile central florets and a glabrous or hairy receptacle. *A.* subgen. *Dracunculus* has heterogamous capitula with female outer florets and functionally male central florets and a glabrous receptacle. *A.* subgen. *Seriphidium* Besser has homogamous capitula with all florets hermaphroditic and fertile and a glabrous receptacle (Podlech 1986). Among the subgenus *Seriphidium* some authors have separated a new group named as subgenus *Tridentatae* Rydb. which includes most of North American members of *Seriphidium* (Rydberg 1916).

Many studies considering morphological, palynological, cytogenetical, molecular and evolutionary features of the genus *Artemisia* has been done, but there are few data about anatomical aspects and its value in classification of the species of *Artemisia* specially those occurring in Iran.

Metcalfe & Chalk (1958) described some features of *Artemisia* as: nonglandular hairs, medulary canals, secretory cavities and clustered crystals. They did not detect latex canals and secretory cells. Botha & Evert (1981) studied the phloem in stem and leaf of *Artemisia afra* Jacq. Fahn (1990) discussed split axis and interxylary cork in *A. herba-alba* Asso. Ahmadi et al., (2002) in a survey considering volatile constituents of *A. marschaliana* Sprengel, also studied secretory elements in this species. They had shown glandular hairs and canals in different parts of leaf and in the cortex of stem. Naseri (2004) in a botanical and ecological study of the species of *Artemisia* in east Azerbaijan province in Iran studied anatomical features of seven species of that region. Marchese et al. (2005) studied leaf anatomy and carbon isotope composition of the useful herb, *A. annua* L. Rabie et al. (2006) also studied five *Artemisia* species in north Iran. Zarinkamar (2006) discussed the stomatal types of *Asteraceae* and notes the stomatal type of *Artemisia* as anomocytic to anisocytic.

In this paper 28 species of *Artemisias* are used for anatomical studies in order to introduce the most important anatomical features of them and their possible taxonomic applications.

## MATERIALS AND METHODS

28 species of three subgenera of *Artemisia* L. occurring in Iran were investigated. Dried material was obtained from specimens in Tehran University Central Herbarium (TUH) and Ferdousi University of Mashhad Herbarium (FUMH). Voucher specimens are listed in table 1, the species are arranged in the order of Flora Iranica (Podlech 1986), grouped in three subgenera.

Leaves in the middle of stem were fixed in alcohol-glycerin (1:1) for five month. Cross sections were made at the middle of leaves in mature plants with a razor, transverse sections were cleared with sodium hypochlorite, dehydrated and stained with Methyl Green and Bismarck brown colors (Mahdigholi 2002). Then they were studied and photographed by Olympus Light Microscope.

## RESULTS

The most prominent properties of sections are summarized in tables 2 and 3 and is as follows. Leaf symmetry is very variable. Bifacial symmetry is only seen in *A. vulgaris* which shows the best example of a hygrophyte species in the genus. Leaf symmetry of other species is isobilateral (palisade parenchymatous cells are present on both adaxial and abaxial surfaces) which can be homogenous (number of palisade cell rows on both surfaces are equal) or heterogenous (number of palisade cell rows on superior and inferior surfaces are not equal, e.g. *A. icana*, Fig. 3, no. 2) In the case of homogenous symmetry, number of palisade cells on superior and inferior surfaces could be one (*A. annua* Fig. 1, no. 4), two (*A. marschaliana* Fig. 4, no. 2) or three (*A. fragrans*, Fig. 7, no. 1) layers.

Epidermal cells are arranged in a single layer, varying from 10 to 43  $\mu\text{m}$ . Trichomes are glandular (in most species), with unicellular to uniseriate base with a long whip like terminal cell (in most species), having a uniseriate stalk with a unicellular head i.e. two armed or T-shaped (*A. persica*, Fig. 2, no. 4) or secretory (*A. armeniaca*, Fig. 3, no. 3). Where there is a glandular trichome usually there is a depression in epidermis. Stomata are at the same level with epidermal cells or are prominent.

In all of the species investigated, there are secretory canals, in some of species they are very large and prominent (*A. marschaliana*) and in some others small (*A. fragrans*). These canals are usually located near or attached to xylem tissues (*A. fragrans*) but in some cases they are around midrib (*A. marschaliana*). In some species especially xerophytes of deserts, there are a lot of large deposits mostly in palisade cells (*A. deserti* Fig. 4, no. 3 and *A. fragrans*) but in some others there are few and small particles (*A. incana*, Fig. 3, no. 2). Detection of crystals due to presence of a lot of deposits, is difficult but in some species prismatic crystals can be seen (*A. haussknechtii*, Fig. 2, no. 3). Palisade cell length can reach to 115  $\mu\text{m}$  and their walls are smooth to sinusal. Spongy parenchyma size varies from 16 to 60  $\mu\text{m}$  with spheroid to irregular walls. Spongy parenchyma around midrib is stretched toward epidermis and gradually would become lamellar

Table 1. Specimens examined (voucher specimens from which materials were sampled and deposited at the Central Herbarium of University of Tehran).

Subgen. *Artemisia*

*Artemisia vulgaris* L., Gilan: Bandar-e Anzali, alt. 25m, 21.9.1988, V. Mozaffarian, no. 7135.

A. *chamaemelifolia* Vill., 24.6.2001, alt. 2150m, B. Eslami, no. 33527.

A. *biennis* Willd., Mazandaran: Road of HARaz: Sangdeh, alt. 1800m, 20.10.1995, Ghahreman & Attar, no. 20233.

A. *annua* L., Mazandaran: Road of Haraz: Sngdeh, alt. 1800m, 20.10.1995, Ghahreman & Attar, no. 20232.

A. *splendens* Willd., Urumieh, Silvana, Mavana, Kuh-e Khizan, alt. 2600-3000m, 6.7.1991, V. Mozaffarian, no. 69904.

A. *austriaca* Jacq., Azarbaiejan: ca. 17km from Ahar to Tabriz, alt. 1400m, 30.8.1993, A. Ghahreman & V. Mozaffarian, no.17610.

A. *haussknechtii* Boiss., Khorramabad: Sefid-Kuh, alt. 2500m, 22.7.1999, Veiskarami, no. 23690.

A. *persica* Boiss., Kerman: 40 km to Baft, south of Gugher, alt. 2880m, 28.5.2002, Ghahreman, Attar & Mehdigholi, no. 28604.

A. *absinthium* L., Firuzkuh, pass. Kadook, alt. 2000m, 5.12.2001, B. Eslami, no. 33538.

A. *incana* (L.) Druce, Zanjan to Bijar, alt. 1840m, 20.10.1987, V. Mozaffarian no. 59471.

A. *armeniaca* Sam., Azarbaiejan: Arasbaran protected area Kuh-e Kallan, alt. 2500m, 26.8.1993, A. Ghahreman & V. Mozaffarian, no. 17572.

Subgen. *Dracunculus*

A. *tschernieviana* Besser, Mazandaran: Behshahr, Zaghmarz, alt. see level, 28.10.1987, V. Mozaffarian, no. 59623.

A. *scoparia* Waldst. & Kit., Gorgan: Bandar-e Turkman, Ashuradeh, alt. see level, 29.10.1987, V. Mozaffarian, no. 59640.

A. *marschalina*, Azarbaiejan: ca. 20 km from Ardabil to Khalkhal, Hir to Shibli to Gheshlagh, alt. 2400m, 30.9.1991, V. Mozaffarian, no. 70131.

Subgen. *Serephidium*

A. *deserti* Ktasch., Tehran: Firuzkuh. Mahmoudabad, 11.10.1990, A. Ghahreman & V. Mozaffarian, no.9833

A. *santolina* Schrenk., Semnan: Shahrud, 20 km from Mayamay to Jilan (Chehel Dokhtar), alt. 1100m, 27.10.1987, V. Mozaffarian, no. 59605.

A. *turanica* Krasch., Khorassan: 17 km from Shirvan to Sovaldi (12 km from Ziarat to Sovaldi), alt. 1380m, 31.10.1987, V. Mozaffarian, no. 59698.

A. *diffusa* Krasch. ex Poljak., Khorassan: 26 km from Bojnourd to Gilan, 31.10.1987, V. Mozaffarian, no. 59684.

A. *kopetdaghensis* Krasch., M. Pop. & Lincz. ex Poljak., Khorassan: N. of Shirvan, Sovaldi, alt. 1200m, 31.10.1987, V. Mozaffarian, no. 59694.

A. *turcomanica* Grand., Khorassan: 80km to Bojnurd from Gorgan, Zamansufi, alt. 1500m, 30.10.1987, V. Mozaffarian, no. 59665.

A. *sieberi* Besser, Semnan: 20 km from Shahrud to Mayamay, alt. 1390m, 26.10.1987, V. Mozaffarian, no. 59575.

A. *olivieriana* J.Gay ex DC., Semnan: road from Semnan to Firuzkuh, alt. 1900m, 27.9.1994, A. Ghahreman & V. Mozaffarian, no. 18113.

A. *gypsacea* Poljak., Semnan: 85 km to Azadshahr from Shahrud, alt. 700m, 27.10.1987, V. Mozaffarian, no. 59616.

A. *khorrassanica* Podl., Northwest Gha'en, Borzabad, alt. 1650m, 8.10.2003, Joharchi, No. 35224.

A. *aucheri* Boiss., Khorassan: Birjand: Razg, alt. 1811m, 31.10.1997, Aliyabadi, no. 22133.

A. *ciniformis* Krasch & M. Pop. ex. Poljak., Sarakhs, Shahan Garm'ab, alt. 1300m, 19.11.1992, Niavazangouii, No. 22547.

A. *fragrans* Willd., Tehran: Dmavand, Jaban, Garamasard, Gavij, 11.10.1990, A. Ghahreman & V. Mozaffarian, no. 9839.

A. *spicigera* C. Koch., Tehran: Damavand-Rineh, V. Mozaffarian, no. 11374.

Table 2. The most important anatomical characters of heterogamous species of *Artemisia*.

Heterogames	Leaf section shape	Leaf symmetry	Size of epidermal cells $\mu\text{m}$	Trichomes shape (in anthesis)	Stomata type	Amount of sediments	Palisade cell rows in superior face	Palisade cell rows in inferior face	Palisade cell length in superior face $\mu\text{m}$	Palisade cell length in inferior face $\mu\text{m}$	Palisade cell wall	Midrib souting tissue	Midrib size
<i>A. vulgaris</i>	Group 1	Bifacial	16	T-shape	Isoplanar (inferior face)	few-large	1	-	40-50	-	large depressions	medium	very large
<i>A. chamaemelifolia</i>	Group 2	Isobilateral-heterogeneous	13-16	uniseriate	Isoplanar (both faces)	few-small	2-3	1-2	20-33	16-23	smooth	medium	large
<i>A. biennis</i>	Group 1	Isobilateral 2 layered	16-26	uniseriate	Isoplanar (both faces)	medium-small	2	2	23-46	23-46	sinusoidal	medium	small
<i>A. annua</i>	Group 1	Isobilateral 1 layered	10-16	uniseriate	Isoplanar (superior)-prominent (inferior)	medium-large	1	1	23-40	23-40	smooth	a lot	large
<i>A. splendens</i>	Group 3	Isobilateral 2 layered	10-16	T-shape	Prominent (inferior face)	very few	2	2	33-40	23-40	smooth	none-few	small
<i>A. austriaca</i>	Group 2	Isobilateral 2 layered	16	T-shape	Prominent (both faces)	few-small	2	2	40-60	40-60	large depressions-sinusoidal	none-few	small
<i>A. haussknechtii</i>	Group 3	Isobilateral 2 layered	10-16	T-shape	Prominent (inferior face)	very few	2	2	33-50	33-50	large depressions	none-few	small
<i>A. persica</i>	Group 2	Isobilateral 2 layered	16	T-shape	Prominent (inferior face)	few-small	2	2	16-36	16-36	smooth	medium	large
<i>A. absinthium</i>	Group 1	Isobilateral-heterogeneous	16-20	T-shape	Prominent (inferior face)	very few	2	1	33-43	33-43	few depressions	a lot	very large
<i>A. incana</i>	Group 3	Isobilateral-heterogeneous	16-26	T-shape	Isoplanar (both faces)	medium-small	2	1	115	82-115	large depressions	none-few	small
<i>A. armeniaca</i>	Group 1	Isobilateral 2 layered	20-26	T-shape and secretary	Prominent (inferior face)	few-small	2	2	23-36	23-36	large depressions-sinusoidal	a lot	large
<i>A. tschernieviana</i>	Group 2	Isobilateral 3 layered	33-43	T-shape	Isoplanar (both faces)	very few	3	3	23-50	23-33	large depressions	none-few	small
<i>A. scoparia</i>	Group 2	Isobilateral 3 layered	21-31	-	Isoplanar (both faces)	very few	3	3	16-33	16-33	few depressions	none-few	small
<i>A. marschalina</i>	Group 2	Isobilateral 2 layered	20-23	secretary	Isoplanar (both faces)	very few	2	2	33-60	33-60	sinusoidal	medium	rather large

Table 3. The most important anatomical characters of Homogamous species.

Homogam sl	Leaf section shape	Leaf symmetry	Size of epider mal cells µm	Trichome s shape (in anthesis)	Stomata type	amount of sediments	Palisade cell rows in superior face	Palisade cell rows in inferior face	Palisade cell length in superior face µm	Palisade cell length in inferior face µm	Palisade cell walls	Midrib soutian tissue	Midrib size
<i>A. deserti</i>	Group 3	Isobilateral 2 layered	13-16	uniseriate	Isoplanar (both faces)	a lot- large or small	2	2	50-60	50-60	smooth- few depression s	few	small
<i>A. santolina</i>	Group 3	Isobilateral 2 layered	23	uniseriate	Isoplanar (both faces)	few-small	2	2	33-63	33-63	smooth- few sinusoidal	few	large
<i>A. turanica</i>	Group 2	Isobilateral 2 layered	16-20	-	Isoplanar (both faces)	few-small	2	2	33-43	30-40	smooth- sinusoidal	few	large
<i>A. diffusa</i>	Group 2	Isobilateral 2 layered	20-26	-	Isoplanar (both faces)	few-small	2	2	26-60	26-60	smooth- few depression s	a lot	large
<i>A. kopetdaghe nsis</i>	Group 2	Isobilateral 2 layered	20-26	-	Prominen t (both faces)	medium- small	2	2	40-46	40-46	smooth- sinusoidal	a lot	large
<i>A. turcomanic a</i>	Group 2	Isobilateral 2 layered	16-20	-	Prominen t (both faces)	medium- small	2	2	33-60	33-60	smooth- few sinusoidal	a lot	large
<i>A. sieberi</i>	Group 3	Isobilateral 2 layered	23-30	-	Isoplanar (both faces)	a lot- large or small	2	2	33-50	33-50	very depression s- sinusoidal	very much	small
<i>A. olivieriana</i>	Group 3	Isobilateral 2 layered	20	uniseriate	Isoplanar (both faces)	medium- small	2	2	40-66	40-66	very depression s- very sinusoidal	very much	large
<i>A. gypsacea</i>	Group 3	Isobilateral 2 layered	23-26	-	Isoplanar (both faces)	a lot- large or small	2	2	40-60	33-60	few depression s	medium	large
<i>A. khorassanic a</i>	Group 2	Isobilateral 3 layered	16-20	T-shape	Isoplanar (both faces)	medium- small	3	3	20-30	20-30	large depression s	none- few	small
<i>A. aucheri</i>	Group 2	Isobilateral 3 layered	16-23	-	Isoplanar (both faces)	a lot- large	3	3	33-40	33-40	smooth- few depression s	very much	large
<i>A. ciniformis</i>	Group 2	Isobilateral 3 layered	13-23	-	Isoplanar (both faces)	a lot-small	3	3	30-50	26-40	large depression s- few sinusoidal	very much	large
<i>A. fragrans</i>	Group 2	Isobilateral 3 layered	16-20	-	Isoplanar (both faces)	a lot- large	3	3	33-50	33-50	smooth- few depression s	none- few	small
<i>A. spicigera</i>	Group 2	Isobilateral - heterogene ous	13-16	T-shape	Isoplanar (superior) - prominen t (inferior)	few-small	2-3	1-2	33-50	23-56	smooth- few depression s	none- few	small

collenchyma under epidermis. This collenchymateous cell can be seen in superior or inferior epidermis (*A. korassanica*, Fig. 6, no 2) or both (*A. chamaemelifolia*, Fig. 1, no. 2) and in a much amount (*A. vulgaris*, Fig. 1, no. 1) or there can be no collenchymas (*A.*

*hausknechtii*). Central vascular bundle is mostly spheroid but in a few cases ovoid is also seen. Amount of soutian tissues in central vascular bundle is sclerenchyma which in some species there are many fibrous sclerenchyma that fills the bundle (*A. aucheri*,

Fig. 6, no. 3). Vascular bundle sheath cells in most species have a few or no differences from its neighboring spongy parenchyma but in *A. deserti* and *A. incana*, these cells are smaller.

In figures 1 to 7 leaf section shapes and in table 2 and 3, the most important anatomical features of investigated species are displayed. In figure 7-3 and 4 representatives of the three main anatomical groups are displayed. An identification key is prepared for 28 species according to anatomical characters.

### Leaf anatomy key in *Artemisia* L.

- 1- Midrib region volumetric and more than two folds of lamina thick, a lot of chollenchyma under epidermis, a little or no palisade cells in this region 2  
 -Midrib volume usually fewer than two folds of lamina thickness, a lot of palisade cells in midrib region and a little or no chollenchyma under epidermis, leaf section spheroid-ovoid, shortly linear or oblong-linear 6

### Midrib region volumetric and more than two folds of lamina

- 2- Leaf symmetry bifacial *A. vulgaris* L. 3  
 - Leaf symmetry isobilateral 3  
 3- Leaf symmetry isobilateral heterogenous, palisade cell rows in superior surface 2 and inferior 1, stomata in the inferior surface prominent, no stomata the superior surface *A. absinthium* L. 4  
 - Leaf symmetry isobilateral homogenous 4  
 4- Leaf symmetry isobilateral one layer *A. annua* L. 5  
 - Leaf symmetry isobilateral two layers 5  
 5- Epidermis with secretory and T-shaped trichomes, stomata in the inferior surface prominent, no stomata on the superior surface *A. armeniaca* Lam. 6  
 - Epidermis without secretory and T-shaped trichomes, stomata at the same plane with epidermis *A. tournefortiana* Willd. 6

- 6- Number of veins in each half of lamina one or two, leaf section shape spheroid-ovoid or shortly linear 7  
 - Number of veins in each half of lamina three or more, leaf section shape oblong-linear 20

### Number of veins in each lamina half one or two, leaf section shape spheroid-ovoid or shortly linear

- 7- Leaf symmetry isobilateral heterogeneous, palisade cell rows in superior surface 2 to 3 and inferior 1 to 2 8  
 - Leaf symmetry isobilateral homogenous 9  
 8- Epidermis with densely T-shaped trichomes, stomata at the inferior surface prominent and at the same plane with epidermis in the inferior surface

- A. spicigera* C. Koch  
 - Epidermis having uniseriate sparse trichomes and no T-shaped trichomes, stomata at the same plane with epidermis  
*A. chamaemelifolia* Vill. 10  
 9- Epidermis with T-shaped trichomes 10  
 - Epidermis without T-shaped trichomes 13  
 10- Leaf symmetry isobilateral with three palisade cell rows (three layers), stomata at the same plane with epidermis 11  
 - Leaf symmetry isobilateral with two palisade cell rows (two layers), stomata prominent 12  
 11- Epidermis surface with a sparse indumentum, palisade cell length at the superior surface up to 50  $\mu\text{m}$ , no chollenchyma or a little under inferior surface  
*A. tcherieviana* Besser  
 - Epidermis surface with a dense indumentum, palisade cell length at the superior surface up to 30  $\mu\text{m}$ , with chollenchyma under inferior or superior surface  
*A. khorassanica* Podl. 12  
 12- With short lamina half, palisade cell length up to 36  $\mu\text{m}$ , palisade cell shape spheroid, with chollenchyma under inferior and superior surface, central vascular bundle large with some sclerenchymatous tissues  
*A. persica* Boiss.  
 - With longer lamina half, with two deep depressions around midrib, palisade cell length up to 60  $\mu\text{m}$ , palisade cell shape irregular, with no chollenchyma, central vascular bundle small with almost no sclerenchymatous tissues *A. austriaca* Jacq. 13- With secretory canals near midrib, leaf section shape almost spheroid, with a little or no sediments in palisade tissues, no chollenchyma, a little or no sclerenchyma in central vascular bundle  
*A. scoparia* Waldst. & Kit. 14  
 - Characters otherwise 14  
 14- Leaf symmetry isobilateral two layers 15  
 - Leaf symmetry isobilateral three layers 18  
 15- Stomata at the same plane with epidermis, under inferior epidermis with chollenchyma 16  
 - Stomata prominent, under inferior and superior epidermis with chollenchyma 17  
 16- Palisade cell length up to 60  $\mu\text{m}$ , epidermal cell size up to 26  $\mu\text{m}$  *A. diffusa* Krasch. Ex Poljak.  
 - Palisade cell length up to 43  $\mu\text{m}$ , epidermal cell size up to 20  $\mu\text{m}$  *A. turanica* Krasch.  
 17- Epidermis under midrib with three convexities, palisade cell length up to 46  $\mu\text{m}$ , palisade cell shape spheroid, epidermal cell size up to 26  $\mu\text{m}$   
*A. kopetdaghensis* Krasch.  
 - Epidermis under midrib with two convexities, palisade cell length up to 60  $\mu\text{m}$ , palisade cell shape spheroid to irregular, epidermal cell size up to 20  $\mu\text{m}$   
*A. turcomanica* Gand.

18- Central vascular bundle with no or little soutian tissue, with a small size and spheroid

*A. fragrans* Willd.

- Central vascular bundle with a lot soutian tissue composed of fibrous sclerenchyma, with a large size and ovoid 19

19- Epidermis under midrib with two convexities, with many large sediments in palisade tissues, palisade cell shape spheroid

*A. aucheri* Boiss.

- Epidermis under midrib with three convexities, with many small sediments in palisade tissues, palisade cell shape spheroid to irregular

*A. ciniformis* Krasch & M. Pop. ex Pljak.

### Number of veins in each lamina half three or more, leaf section shape oblong linear

20- Leaf symmetry isobilateral heterogeneous, palisade cell rows in superior surface 2 and inferior and tip of lamina 1 layer, palisade cell length up to 115  $\mu$ m

*A. incana* (L.) Druce

- Leaf symmetry isobilateral two layers 21

21- Epidermis with T-shape trichomes 22

- Epidermis without T-shape trichomes 23

22- Epidermis with dense indumentum, palisade cell length up to 50  $\mu$ m, with prismatic crystals

*A. haussknechtii* Boiss.

- Epidermis with rather sparse indumentum, palisade cell length up to 40  $\mu$ m, without prismatic crystals

*A. splendens* Willd.

23- Epidermis with secretory trichomes and large secretory canals around central vascular bundle

*A. marschaliana* Sprengel

- Epidermis without secretory trichomes, small secretory canals around xylem or secretory canals not detectable 24

24- Soutian tissues in central vascular bundle little and without fibrous sclerenchyma 25

- A lot soutian tissues in central vascular bundle with fibrous sclerenchyma 27

25- Little and small amount of sediments in palisade cells

*A. santolina* Schrenk.

- Many large sediments in palisade cells 26

26- Number of veins in each lamina half more than 4 (up to 6 or 7), vascular bundle sheath cells smaller than their neighboring parenchymatous cells, little collenchyma under inferior epidermis, vascular bundle small

*A. deserti* Krasch.

- Number of veins in each lamina half 3 to 4, vascular bundle sheath cells with no differentiation to neighboring parenchymatous cells, some collenchyma under inferior and superior epidermis, vascular bundle large

*A. gypsacea* Krasch.

27- Sediments many, with different sizes in palisade cells, vascular bundle small, epidermal cell length up to 30  $\mu$ m

*A. sieberi* Besser

- Sediments few, with small size in palisade cells, vascular bundle large, epidermal cell length up to 20  $\mu$ m

*A. oliveriana* J. Gay ex DC.

## DISCUSSION

According to leaf sections three groups are detectable which differs by their midrib shape and its position in relation to lamina which is in agreement with leaf morphology. In the first group due to the presence of a lot of collenchymatous tissues under epidermis, the midrib is voluminous and each half of lamina is long. All species in this group belongs to the subgenus *Artemisia* and their leaf morphology is similar to hygrophyte species rather than xerophytes. In the second group there are a few amounts of collenchymatous tissues or there is no collenchymatous tissue at all, there are one to two veins in each half of lamina and the leaf section shape (as compared to the other species) is oval-spheroid to short linear. In the third group amount of collenchymatous tissues are similar to the second group but there are more than three or more veins in each half of lamina and the leaf section shape is oblong-linear. All species in these groups belong to the three subgenera (*Artemisia*, *Dracunculus* and *Seriphidium*) and their leaf anatomy shows xerophytic or semi-hygrophytic species.

In the first group *A. vulgaris* is the best example of a hygrophyte. It has a large leaf with bifacial symmetry. This species with *A. absinthium* and *A. armeniaca* are the only species which their stomata are present only on the inferior surface. *A. absinthium* has T-shaped trichomes, a very large midrib and heterogeneous leaf symmetry while *A. armeniaca* has both secretory and T-shaped trichoms, a smaller midrib and isobilateral two layers leaf symmetry.

*A. annua* is the only species with isobilateral one layer leaf symmetry but there is two layers of palisade cells in lamina tip. Large trichomes are seen especially in midrib region which contains antimalaria substance named artemisinin (Ahmadi et al. 2002). Stomata at the inferior surface are prominent and at the same plane with epidermis in the inferior surface (also seen in *A. spicigera* from the third group). *A. biennis* leaf anatomy is similar with *A. armeniaca* but lacks any secretory or T-shaped trichomes and has a smaller midvein. Uniseriate trichomes of this species are very similar to *A. annua*.

In the second group *A. spicigera* and *A. chamaemelifolia* have heterogeneous leaf symmetry. Epidermis surface is covered by T-shape trichomes and

stomata are the same as *A. annua*. *A. chamaemelifolia* has a large midrib but because of short lamina, little collenchyma in comparison with the first group and leaf morphology which is very similar to *A. persica* and *A. khorassanica* (both from the second group), this species is placed in the second group. Regular leaf divisions, numbers of divisions (three to four) and leaf section width and shape of these species are similar while *A. spicigera* has irregular leaf divisions (up to two divisions). In *A. khorassanica* leaf symmetry is isobilateral three layered and stomata are at the same plane with epidermis which is similar to *A. chamaemelifolia* stomata, while in *A. persica* leaf symmetry is isobilateral two layered and stomata are prominent. In *A. austriaca* like *A. persica* has isobilateral three layered leaf symmetry and prominent stomata, but by having two deep epidermal depressions on both sides of midrib and little or no collenchymatous cells it differs from *A. persica*. *A. haussknechtii* and *A. splendens* are morphologically close to *A. austriaca*. Leaf sections of these two species have irregular divisions with wider segment width, while in *A. austriaca*, it has a regular divisions with narrower segment width. Also *A. haussknechtii* and *A. splendens* are small shrubs with a small synflorescence and capitula little aggregated at top of synflorescence, while *A. austriaca* is a taller shrub with a large synflorescence with many capitula. All of these three species belong to subgen. *Artemisia*, have T-shape trichomes and similar stomata and midrib structure, but because of different lamina veins, *A. haussknechtii* and *A. splendens* are placed in the third group and *A. austriaca* in the second. From subgen. *Dracunculus*, *A. scoparia* and *A. tschernieviana* belong to the second group with a small rather spheroid leaf section, isobilateral three layered leaf symmetry and obvious secretory canals with no or little sicutian tissues in midrib and no collenchyma. In *A. tschernieviana* there are T-shaped trichomes and stomata are at the same plane with epidermis surface but due to very thick cuticle, they are seen a bit depressed. In *A. scoparia* stomata are prominent and no trichoms are seen. Other species in this subgenus is *A. marschaliana* which belongs to the third group.

Anatomical characters put some species in complexes which are close to each other considering morphological features. A morphological complex composed of *A. aucheri* and *A. ciniformis* is also shown by anatomical features. Their leaves are tripinnatisect with regular divisions. From anatomical point of view, their leaf section shape is very similar, their leaf symmetry is isobilateral three layered; central vascular bundle is large and ovoid with a lot of fibrous sclerenchyma which nearly completely fill the bundle.

Deposit amount is very high and most of palisade cells are filled by large (*A. aucheri*) or small (*A. ciniformis*) particles. There is not any strong anatomical character to distinguish these two species that shows very close relation between them. *A. fragrans* is a very close species with the same leaf features, e.g. isobilateral three layered and it can be apart of this complex. Its deposits are very large the same as *A. aucheri*, but there are differences especially in smaller leaf section shape and sicutian tissues of midrib which is very low in *A. fragrans*. Another complex is composed of *A. diffusa*, *A. turanica*, *A. kopetdaghensis* and *A. turcomanica*. They share common life form and habitat and also leaf characters, their leaves are bipinnatisect with rather irregular divisions. Their leaf section shape is very similar and short with isobilateral two layered symmetry. *A. diffusa* and *A. turanica* are closer together and both have their stomata at the same plane with epidermis while *A. kopetdaghensis* and *A. turcomanica* have prominent stomata. However there is not any strong character to separate these four species from each other.

In the third group, *A. incana* is the only species with heterogeneous leaf symmetry (palisade cell rows in superior surface two and inferior and tip of lamina one layered) while others have isobilateral two layered leaf symmetry. Palisade cell length is up to 115  $\mu\text{m}$  which is the longest in Iranian species. This shrub has its capitula aggregated in the tip of synflorescence like corymbs. Two close species, *A. haussknechtii* and *A. splendens* have T-shape trichomes and small central vascular bundle with little sicutian tissues. Small characters separate these species since their habitat, life form and irregular leaf divisions are similar. Trichome density on epidermal surface of *A. haussknechtii* is denser and prismatic crystals (also visible in some other species) are easily seen. These two species have a small difference in their palisade cell length. *A. marschaliana* has large secretory canals around midrib and in addition to secretory trichomes, is well characterized. *A. armeniaca* has the same features but presence of both secretory and T-shape trichomes and a lot of collenchymatous tissues characterize this species. Their leaf morphology is different. Rather large, tripinnatisect leaf and ovoid segments with dentate margins in *A. armeniaca* and small, bipinnatisect leaf and oblong segments with smooth margins in *A. marschaliana*, distinguishes them in addition that capitula size is large (width 5mm) in *A. armeniaca* compared to small ones (width 3mm) in *A. marschaliana*. *A. deserti* has a different leaf in the genus; its leaf is not divided and is completed with two to three (five) terminal teeth. Number of veins in each lamina half is six or more, there are many large sediments in palisade cells that



occupies much of the cell space and small midrib with a little sclerenchymateous and collenchymatous tissues, are its diagnostic characters. *A. santolina* has a complete or divided leaf with some convexities on its margins. There are three veins in each lamina half, small and sparse sediments in palisade cells and large midrib with some sclerenchymateous and collenchymatous tissues. There is a morphological complex of species in this group mostly known by *A. sieberi*. *A. olivieriana* and *A. gypsecea* are also close species. They share common anatomical features as:

three to four veins in each lamina half, rather large central vascular bundle which is filled by fibrous sclerenchyma or there are fewer sclerenchymateous tissues (*A. gypsecea*). Central vascular bundle is ovoid and full of fibrous sclerenchyma in *A. sieberi* and *A. olivieriana* and in *A. gypsecea* there is an orbicular bundle with sclerenchymateous soutil tissues. *A. sieberi* and *A. gypsecea* have much sediment in their palisade cells which are very large in *A. gypsecea*. In *A. olivieriana* there is a few small sediments.

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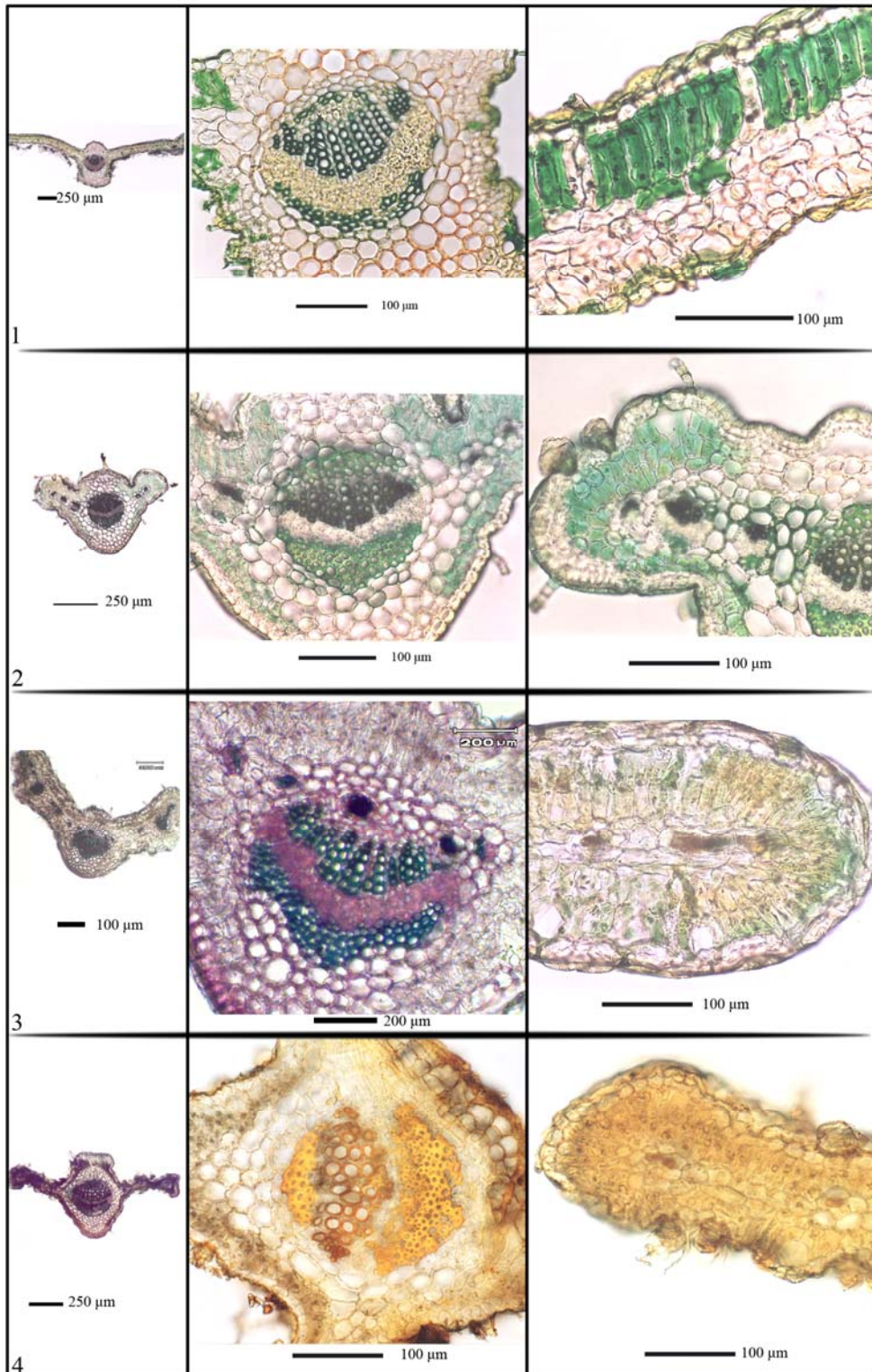


Fig. 1. Leaf sections. Left to right section shape, midvein and lamina. 1- *Artemisia vulgaris*, 2- *A. chamaemelifolia*, 3- *A. biennis* and 4- *A. annua*.

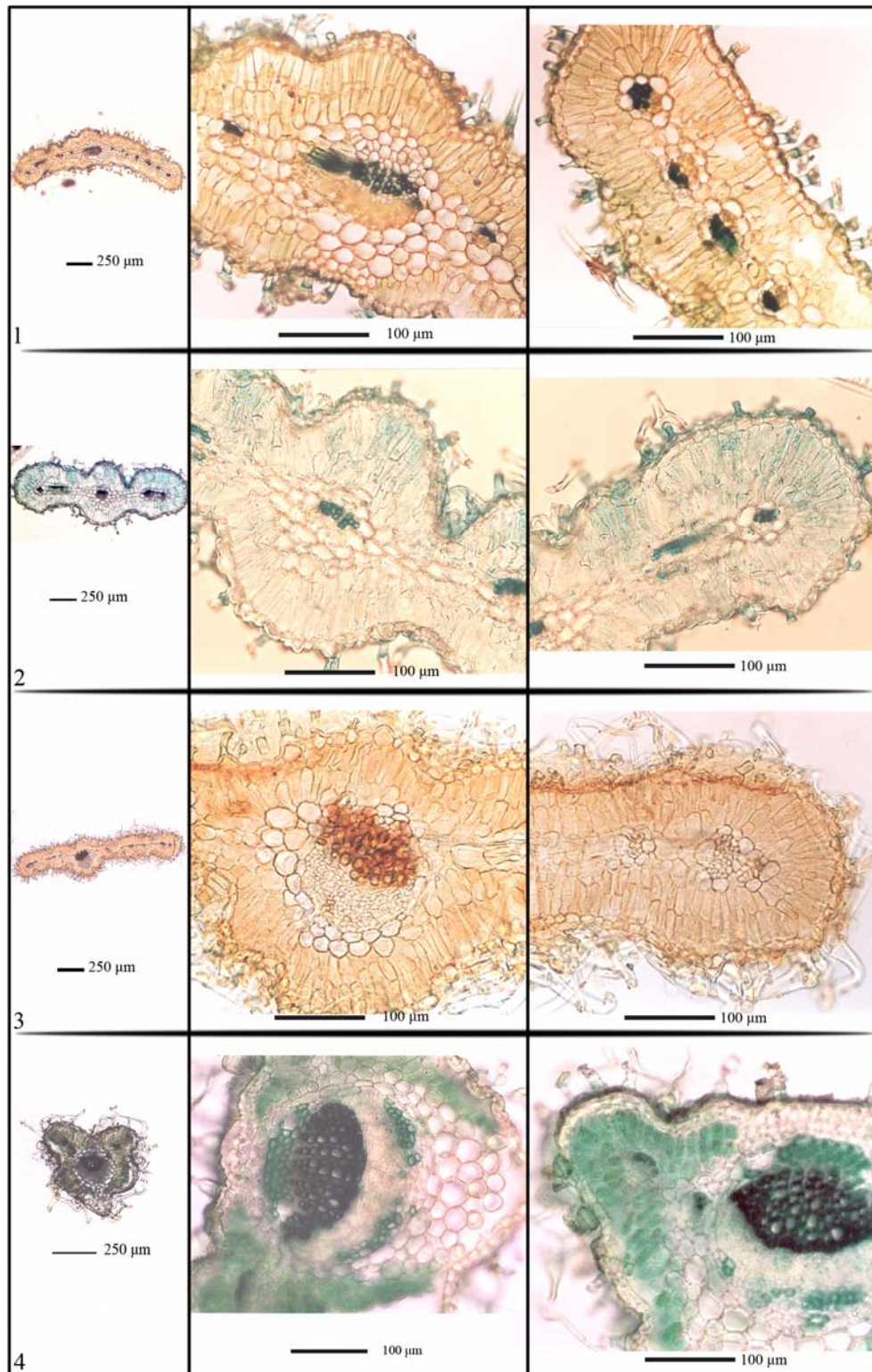


Fig. 2. Leaf sections. Left to right section shape, midvein and lamina. 1- *Artemisia splendens*, 2- *A. austriaca*, 3- *A. haussknechtii* and 4- *A. persica*



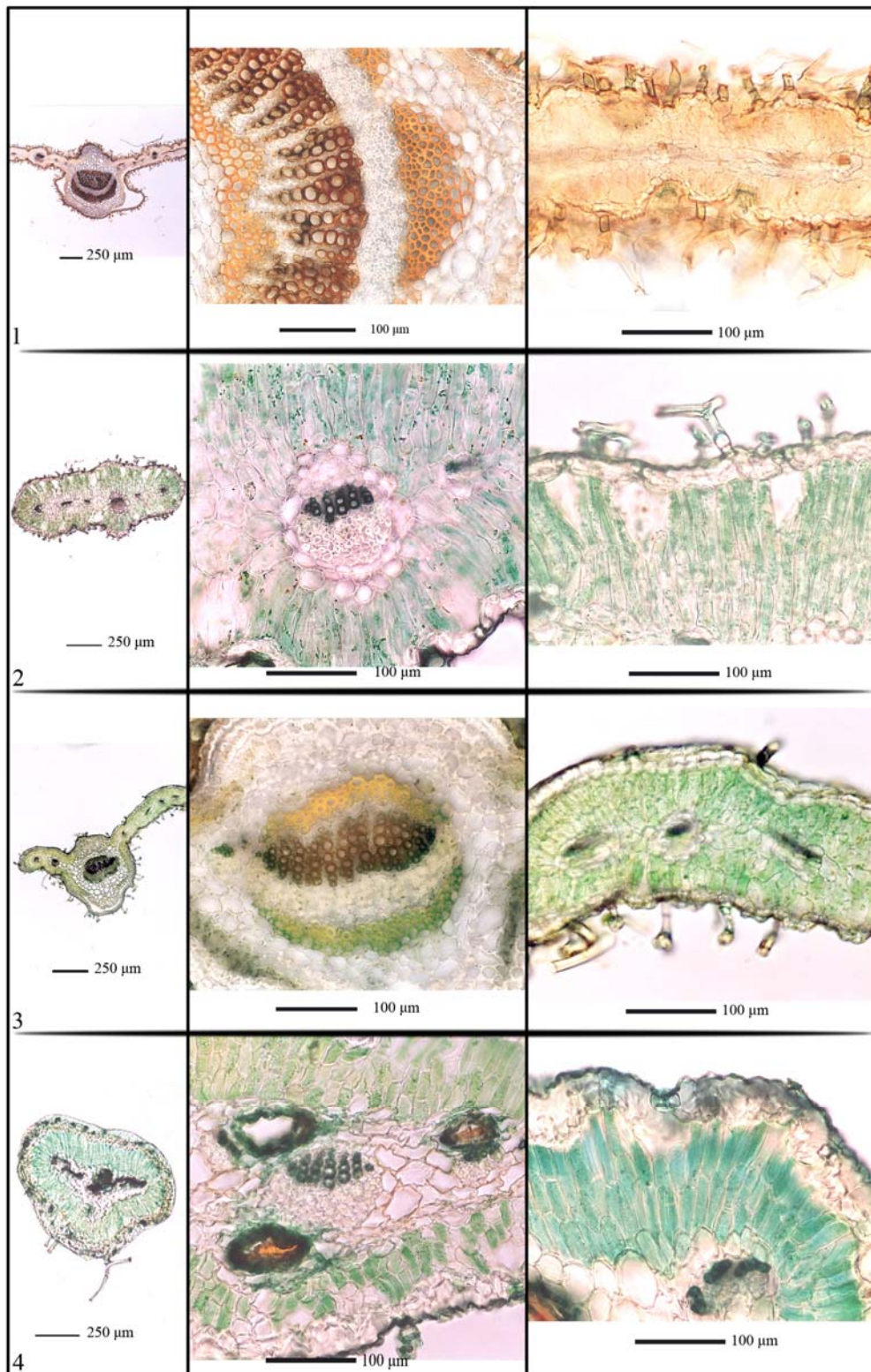


Fig. 3. Leaf sections. Left to right section shape, midvein and lamina. 1- *Artemisia absinthium*, 2- *A. incana*, 3- *A. armeniaca* and 4- *A. tschernieviana*.

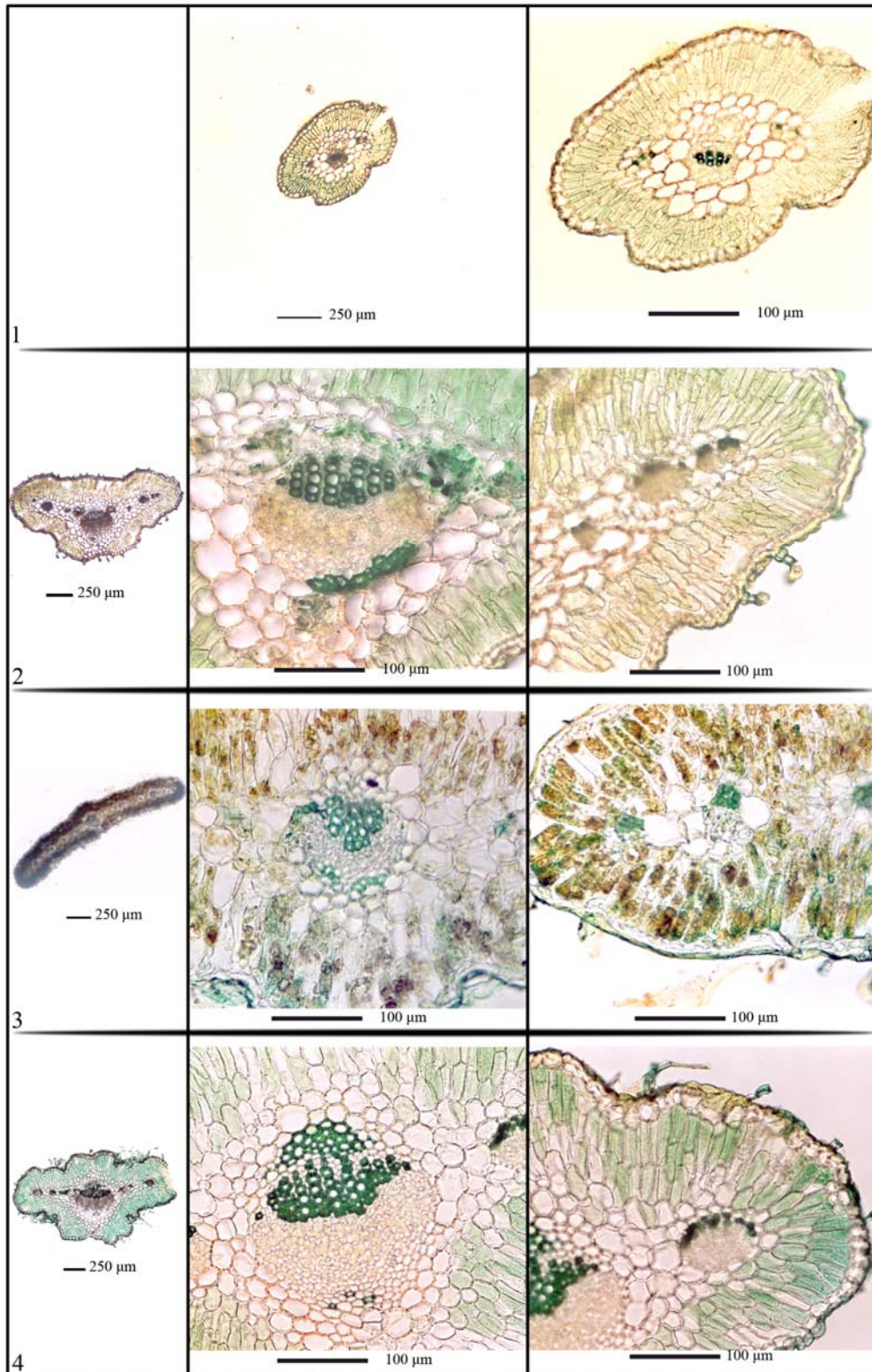


Fig. 4. Leaf sections. Left to right section shape, midvein and lamina. 1- *Artemisia scoparia*, 2- *A. marschaliana*, 3- *A. deserti* and 4- *A. santolina*.



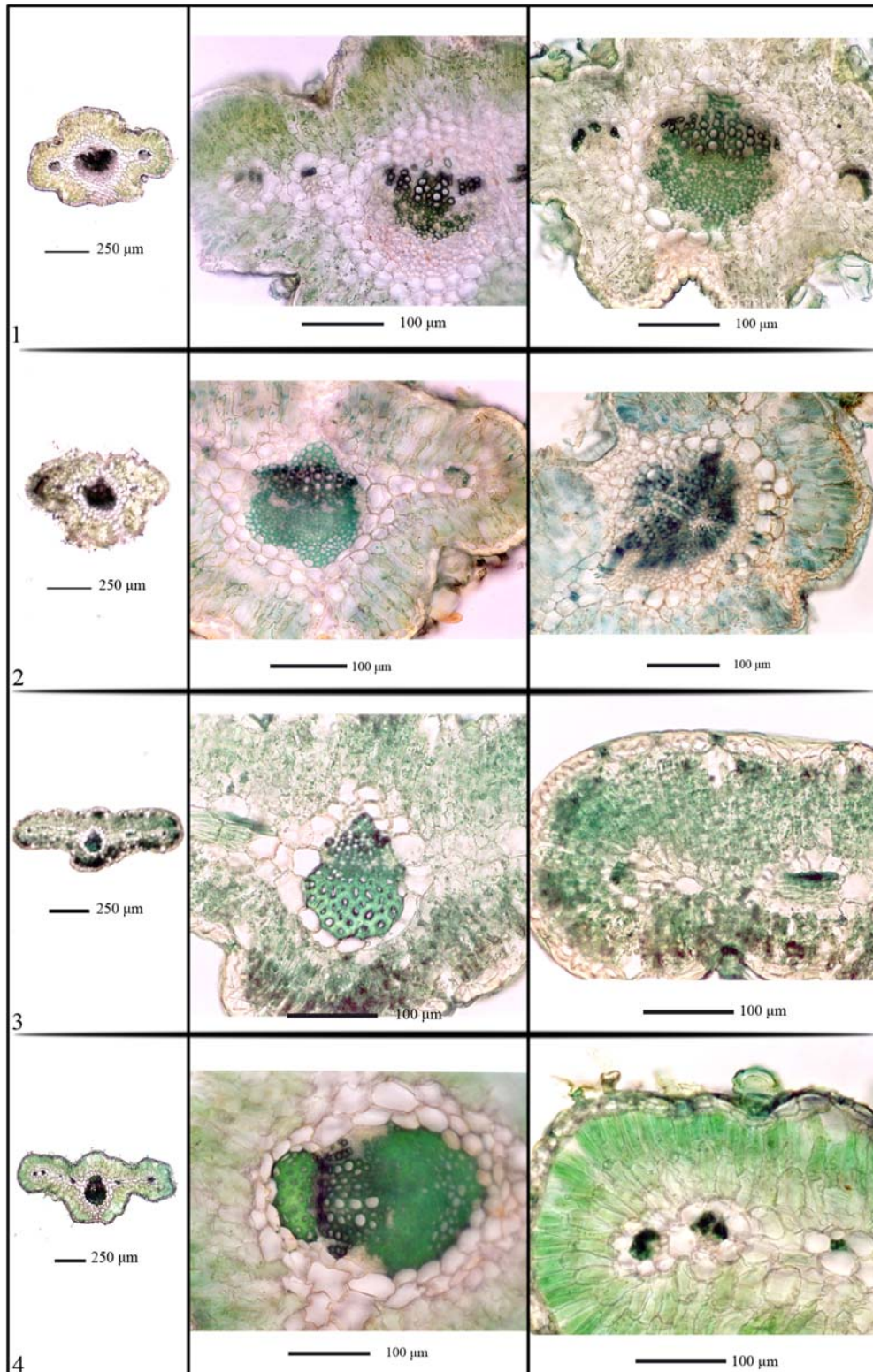


Fig. 5. Leaf sections. Left to right section shape, midvein and lamina. 1- *Artemisia turanica*, right *A. diffusa*, 2- left and center *A. kopetdaghensis*, right *A. turcomanica*, 3- *A. olivieriana* and 4- *A. sieberi*.

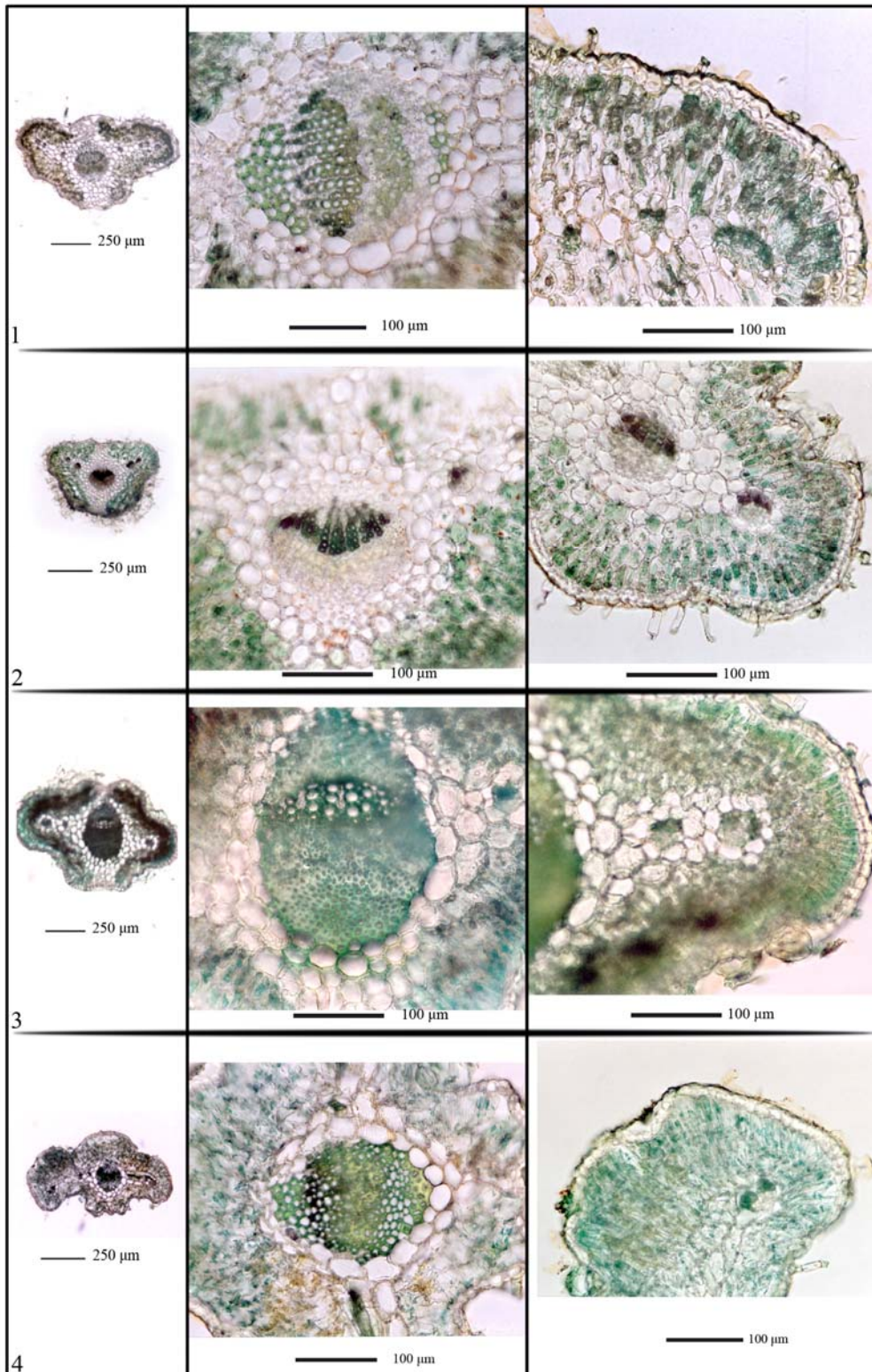


Fig. 6. Leaf sections. 1- *Artemisia gypsacea*, 2- *A. khorassanica*, 3- *A. aucheri* 4- *A. ciniformis*.



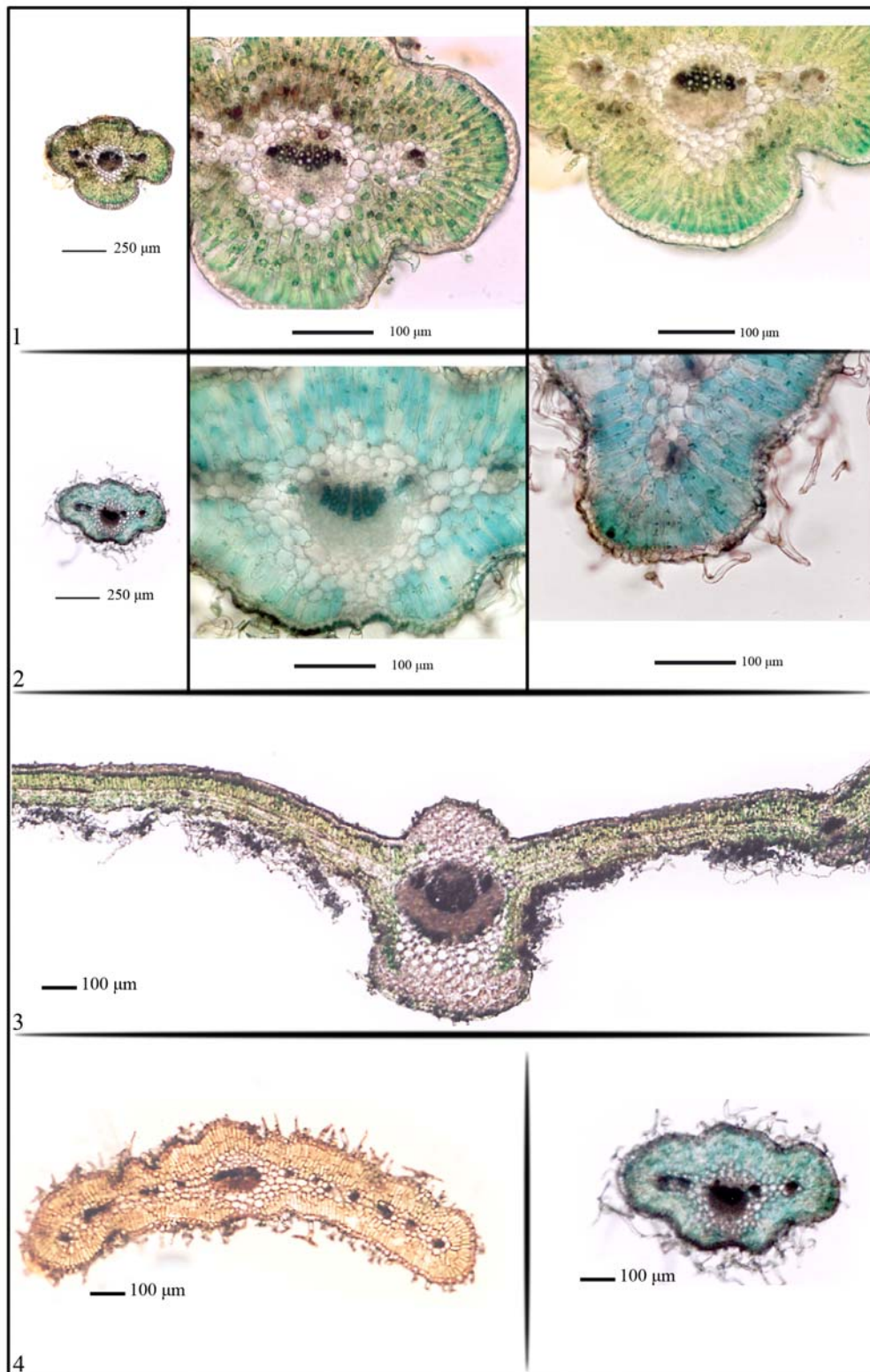


Fig. 7. Leaf sections. 1- *Artemisia fragrans*, 2- *A. spicigera*, 3 and 4 representatives of the three main anatomical groups are displayed. 3- *A. vulgaris*, 4- left *A. splendens* and right *A. spicigera*.