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Foliar micromorphology of some selected species of the genus *Artemisia* and its taxonomic implications

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Abstract

Artemisia is a polymorphic and widespread genus from the *Asteraceae* family. This genus includes more than 500 species out of which 37 species have been reported from Iran. Widely distributed in the desert area of Iran that makes this genus as one of the main elements of the Irano-Turanian region. In this study, eight species of *Artemisia* from different parts of Isfahan province were analyzed using scanning electron microscopy (SEM) and light microscopy (LM) techniques based on both qualitative and quantitative features of foliar epidermal cells. Type of epidermal cells varied from polygonal to irregular and elongated in shape with wavy to smooth in margins. This study, defined three different types of stomata: anemocytic, anisocytic, and paracytic on both the adaxial and the abaxial sides of *Artemisia* leaves species. Six different types of both glandular and non-glandular trichomes including capitate, peltate, aduncate, tectorial, conical type, and stinging hair types were observed in these species.

Keywords: *Asteraceae*, epidermal cells, Isfahan, microscopy study, stomata, trichome

ریزریخت‌شناسی برگ برخی از گونه‌های انتخابی جنس *Artemisia* و کاربردهای آرایه‌شناختی آن*

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خلاصه

Artemisia L. از نظر ریخت‌شناسی یک جنس متنوع و گسترده از تیره کاسنیان (*Asteraceae*) است. این جنس بیش از ۵۰۰ گونه دارد که ۳۷ گونه از آن در ایران گزارش شده و پراکندگی گسترده این جنس در مناطق بیابانی ایران، آن را یکی از عناصر اصلی منطقه ایرانو-تورانی ساخته است. در این مطالعه، هشت گونه *Artemisia* از مناطق مختلف استان اصفهان با استفاده از روش‌های میکروسکوپ الکترونی روبشی (SEM) و میکروسکوپ نوری (LM) و براساس ویژگی‌های کمّی و کیفی سلول‌های اپیدرمی برگ، مورد تجزیه و تحلیل قرار گرفت. شکل سلول‌های اپیدرمی از نوع چندضلعی تا نامنظم متفاوت بوده و شکل آن در حاشیه سلول به صورت موجدار تا صاف است. این مطالعه، سه نوع مختلف روزنه anemocytic، anisocytic و paracytic را در هر دو سطح رویی و زیرین برگ‌های گونه‌های *Artemisia* مشخص نمود. شش نوع کرک غده‌ای و غیرغده‌ای شامل رأسی (capitate)، سپری‌شکل (peltate)، قلابی‌شکل (aduncate)، پوششی (xc)، مخروطی‌شکل (conical) و نیش‌دار (stinging) در این گونه‌ها مشاهده شد. در نهایت، با وجود این که تنوع ویژگی‌های ریزریخت‌شناسی برگ در بین گونه‌ها برای شناسایی گونه‌های این جنس مفید بود، اما برای طبقه‌بندی قابل اتکاء و دقیق‌تر، نیاز به ادغام داده‌های ریزریخت‌شناسی با سایر اطلاعات است.

واژه‌های کلیدی: اصفهان، تیره آفتابگردان، روزنه، سلول‌های اپیدرمی، کرک، مطالعه میکروسکوپی

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Introduction

Artemisia L. belongs to the *Asteraceae* family is a cosmopolitan genus that is distributed world-widely in temperature areas of mid- to high latitude of the northern hemisphere but uncommon in the southern hemisphere (not more than 10 species) (Valles & McArthur 2001, Hayat *et al.* 2009, Emami *et al.* 2012). It contains ca. 500 species of both herbs and shrubs (Janackovic *et al.* 2021), almost all are perennial with less than annual species (Haghighi *et al.* 2014). West and central Asia are two important speciation centers of the genus, while the Mediterranean region and North America are two secondary specification areas (Ghahreman *et al.* 2007, Mahboubi & Farzin 2009). For the ability of this genus to adapt and inhabit many different ecosystems, it has representatives ranging from desert to humid area and sea level to high mountains (Valles & McArthur 2001, Pellicer *et al.* 2011). Many species of *Artemisia* have economic value as food, medicine, forage, ornamental, or other effective uses. Some species are aromatic and allergenic (Tan *et al.* 1998, Hayat *et al.* 2009), and occasionally some of them have allelopathy effects (Li *et al.* 2010). *Artemisia* has a wide distribution in the desert area of Iran that makes it one of the main elements of the Irano-Turanian region. It has a representative in the lowest land of Iran, the border of the Caspian Sea, to the top of Jebal Barez mountains (southeast of Iran at 4000 m) (Rabie *et al.* 2006). Some species such as *A. kulbadica* Boiss. are restricted to particular regions, which is restricted to Golestan province and *A. melanolepis* Boiss. limited to Semnan province (Naghavi *et al.* 2014) of Iran. There are 64 species of *Artemisia* in the Flora Iranica and 37 species in Iran of which two species are endemics (Podlech 1986, Mozafferian 1998) known for traditional medicine, namely, "Dermaneh" (Mojarab *et al.* 2009). Eight species of *Artemisia* are growing in the Isfahan province, including *A. vulgaris* Burm. f., *A. haussknechtii* Boiss., *A. persica* Boiss. of the subgenus *Artemisia*, *A. scoparia* Rich. ex Besser of the subgenus *Dracunculus* Besser Rydb. and *A. deserti* Krasch., *A. oliveriana* Bunge, *A. sieberi* Besser, and *A. aucheri* Boiss. from the subgenus *Seriphidium* Besser ex Less. Among these taxa, *A. sieberi* and *A. aucheri* are widely distributed in this province that as a total of 6.25 million hectares of rangelands in the province, these two species occur since more than 4 million hectares (Yaghmaei *et al.* 2009).

The micromorphological diversity of epidermal cells is one of the main sources of data in taxonomy and plays a fundamental role in systematics (Metcalfe 1988). The foliar epidermal cells of *Artemisia* are remarkable characters for the distinction of different species (Hayat *et al.* 2009). These characters include the size and shape of epidermal cells, distribution of stomata, size of guard cells, number of subsidiary cells, and types of glandular and non-glandular trichomes (Kumar & Murugan 2015). The use of foliar anatomical attributes in plant systematics has been over the last 100 years (Bahadur *et al.* 2019). The foliar epidermal morphology of *Artemisia* species has been studied by many researchers and taxonomists all over the world. Metcalfe and Chalk (1950) explained some features of *Artemisia* like non-glandular hairs. Lodari *et al.* (1989) analyzed carefully the foliar surface of 12 *Artemisia* species with the SEM technique and validated the species delimitation and their close relationships based on foliar epidermis anatomical features. Floral morphology of *Artemisia annua* investigated with LM and SEM technique (Ferreira & Janick 1995). Although, previous studies offered two different trichomes as capitate glandular trichomes and T-shaped non-glandular trichomes Hayat (2011) in 24 taxa studied foliar morphology of *Artemisia* using light microscopy (LM) and scanning electron microscopy (SEM), so that the glandular and non-glandular trichomes classified into 16 main types, and also identified six stomata types in the genus *Artemisia*.

In the Azerbaijan province of Iran, Naseri (2004) suggested a pattern for the ecology of *Artemisia* with documentation of anatomical traits of seven *Artemisia* species. Rabie *et al.* (2006) analyzed carefully five species of *Artemisia* for their foliar anatomical characteristics in the North of Iran. Nourbakhsh *et al.* (2008) investigated the foliar anatomy of 28 *Artemisia* species and confirmed three different groups. In addition, they suggested that, anatomical traits are important for the identification of species of the genus. Recently in an anatomical investigation from the Northeast region of Pakistan by Hussain *et al.* (2019), reported 10 types of glandular and non-glandular trichomes and four different types of stomata in *Artemisia* species. Their investigation confirmed that the foliar anatomical characteristics of *Artemisia* with other traits are useful for resolving taxonomic issues in the genus *Artemisia.* In a study by Karbalaie *et al.* (2021) quantitative and qualitative characters of 64 specimens of 8 species of *Artemisia* in Isfahan province were analyzed and expressed the morphological traits that are very much effective in the identification of *Artemisia* species. Therefore, due to the widespread distribution of this economic genus and for its complex taxonomy, the micro-morphological characteristics of foliar epidermal in some selected species of *Artemisia* were monitored with scanning electron microscopy (SEM) techniques to better understanding the taxonomic value of micro-morphological traits among different species of *Artemisia* and determine the influence these traits to resolving taxonomic conflicts of this genus.

Materials and Methods

- Sampling

All leaf samples were obtained from 24 specimens belonging to eight species (on average 3–4 individuals per species) deposited at the herbarium of the Isfahan Agricultural and Natural Resources Research Center (SFAHAN) and Herbarium of the University of Isfahan (HUI), Isfahan (Iran), listed in table 1. Plants were identified based on morphological data and the *Artemisia* taxa based on the intensive review of different flora including Flora Iranica (Podlech 1986), Flora of Iran (Mozaffarian 2008), Flora of China (Ling *et al.* 2006), and Flora of Pakistan (Ghafoor 2002).

- Light Microscopy (LM)

For light microscopy, a dried specimen of each plant from herbarium was taken. Primarily, some parts of the leaf were kept in the test tube filled with 70% ethanol and 30% acetic acid solution for 48–72 h. After that, the leaves were washed with distilled water (2–3 min) and the whole specimens were transferred to a solution of hydrogen peroxide and acetic acid in a ratio of 1:1 for 3–4 h (among species, *A. deserti* was kept in solution for a longer time). Finally, peeling of the epidermis was performed and after washing with distilled water, the samples were stained for 2–3 min with Zaji Carmen and were transferred to glass slides prepared for LM investigation.

- Scanning Electron Microscopy (SEM)

For SEM investigation dried leaves were used. Leaves segments from both upper and lower surfaces were fixed on double- sided tape mounted on tubs. The specimens were sputter-coated with gold-palladium with a thickness of 10 nm and then observed under SEM (Model SERON; AIS2300C) installed in the Materials Engineering Laboratory of Isfahan University of Technology (IUT), Isfahan (Iran).

Table 1. Information of examined *Artemisia* species in Iran

Taxon	Voucher No.	Locality	Altitude (m)	Collector
A. aucheri	**SFAHAN-6488	Isfahan prov.: Meymeh, Mutche golden mine	2100	Janighorban & Feyzi
	**SFAHAN-6493	Isfahan prov.: Daran, Takht-e Mirzahassan	2500	Feyzi
	*SFAHAN-10943	Isfahan prov.: Naein, Gardaneh Molla-Ahmad	2200	Shams
A. deserti	**SFAHAN-11198	Isfahan prov.: Najaf abad, Dehagh to Tikan	1900	Feyzi & Shams
	**SFAHAN-17302	Isfahan prov.: Ardestan, Marshenan mountain	1650	Feyzi
	*SFAHAN-7878	Isfahan prov.: Ardestan, Marshenan mountain	2200	Feyzi
A. haussknechtii	**HUI-17178	Isfahan prov.: Semirom, Vanak		Parishani
	**SFAHAN-14656	Isfahan prov.: Khansar to Fereydan	1850	Shams & Janighorban
	*SFAHAN-15469	Isfahan prov.: Khansar, Khansar mountain	3000	Feyzi,
	*SFAHAN-17158	Isfahan prov.: Semirom, Nesar Abbas mountain	3000 2350	Akhavan & Bagheri
A. oliveriana	**SFAHAN-15102	Isfahan prov.: Tiran, Sadegh abad	2050	Feyzi
	*SFAHAN-11199	Isfahan prov.: Najaf abad, Dehagh to Tikan	1900	Feyzi & Shams
	*SFAHAN-11195	Isfahan prov.: Najaf abad, Dehagh to Tikan	1900	Feyzi & Shams
A. persica	**SFAHAN-14998	Isfahan prov.: Fereydunshahr, Affus mountain	3300	Nekookhoo
	**SFAHAN-15181	Isfahan prov.: Semirom, Padena, Bijan Pass	2900	Feyzi
	*SFAHAN-17473	Isfahan prov.: Khansar, Golestan-kooh	2300	Akhavan & Bagheri
A. scoparia	**SFAHAN-11194	Isfahan prov.: Najaf abad, Dehagh to Tekan	1900	Feyzi & Shams
A. sieberi	**SFAHAN-10927	Isfahan prov.: Ardestan to Naein, Faran		Janighorban & Shams
	**SFAHAN-4673	Isfahan prov.: Varzaneh, Gav-khooni, Kuh-e Seyah	1880	Shams
	**SFAHAN-5722	Isfahan prov.: 15 km Chaleh Seyah to Isfahan	1300	Nowroozi & Heydari
	*SFAHAN-137	Isfahan prov.: Najaf abad, Ghameshloo pasture	1600	Asef,
	*SFAHAN-8992	Isfahan prov.: Between Segzi & Ghehi	2000 1500	Nowroozi & Asadi
A. vulgaris	**HUI-6921	Isfahan prov.: Isfahan	1550	Khosravi
	**HUI-6920	Isfahan prov.: Isfahan	1500	Haj Mohammadi

* = Specimens used for LM
** = Specimens used for SEM and LM

- Qualitative and Quantitative traits

According to the LM and SEM photographs, 17 different quantitative and qualitative traits were defined that are listed in tables 2 & 3. Quantitative traits were an average of three or four specimens that were measured with Digimizer Ver. 5.4.6 software.

Results

Different foliar epidermal features and the results of qualitative and quantitative analysis of eight species under LM and SEM are summarized in tables 2–4.

- Morphology of epidermal cell

Based on LM and SEM observation, epidermal cells were varied from polygonal to elongate and irregular and with smooth and wavy margins that listed in table 2, epidermal cells of *Artemisia* species were classified into the following three groups:

Group I: Irregular epidermal cells with wavy margins that are presented in the abaxial side of *A. sieberi* (Figs 1G, 5I), the adaxial side of *A. persica* (Figs 1E, 5F), *A. haussknechtii* (Figs 1D, 5D), *A. vulgaris* (Figs 1H, 5J) and *A. scoparia* (Figs 1G, 5H), and the adaxial side of *A. aucheri* (Figs 1A, 5B).

Group II: Elongated cell with smooth wall that presented in *A. oliveriana* (Figs 1E, 5E), the adaxial of *A. aucheri* (Figs 1B, 5B), and the adaxial side of *A. sieberi* (Fig. 5J).

Group III: Polygonal cell with smooth wall that presented in *A. deserti* (Figs 1B, 5C), and the abaxial side of *A. persica* (Fig. 5G).

The largest and smallest length of epidermal cells existed in the adaxial sides of *A. oliveriana* and *A. persica*, respectively. Also, the largest width of epidermal cells was measured in the adaxial side of *A. vulgaris*, and the smallest one was measured in the abaxial side of *A. persica*.

- Trichome morphology

According to the observations obtained by using LM and SEM, the foliar trichomes in eight species of *Artemisia* were classified into six main types of trichome (glandular and non-glandular trichome) (Table 3).

Type I. Capitate trichome: these trichomes are glandular. They have ellipsoid-like appearance and are found in *A. aucheri* (Fig. 2A), *A. deserti* (Fig. 2B), *A. haussknechtii* (Fig. 2C), *A. oliveriana* (Fig. 2D), *A. scoparia* (Fig. 2F), *A. sieberi* (Fig. 2G), and *A. vulgaris* (Fig. 2H).

Type II. Peltate trichome: these trichomes are also glandular. They are similar to a ball that was found in *A. persica* (Fig. 2E).

Type III. Aduncate trichome: non-glandular trichomes that are long with hook-like apex and found in *A. vulgaris* (Fig. 3H).

Type IV. Tectorial: these trichomes are non-glandular that look like clusters and fiber forms and observed in *A. aucheri* (Fig. 3A), *A. oliveriana* (Fig. 3D), *A. persica* (Fig. 3E), and *A. sieberi* (Fig. 3G).

Type V. Conical trichome: non-glandular trichomes that have the shape of a cone. This type was observed in *A. deserti* (Fig. 2B).

Type VI. Stinging trichome: non-glandular trichomes that have a sting like apex that is presented in *A. haussknechtii* (Fig. 2C).

In addition, based on the number of trichome's branches, species were classified into two main groups: T-shaped that were observed in *A. sieberi*, *A. deserti*, *A. aucheri*, *A. haussknechtii*, *A. vulgaris*, and star-shaped that were presented in *A. persica* and *A. oliveriana*.

- Morphology of stomata

Basic terminology used in stomata classification was that suggested by Metcalf & Chalk (1950), stomata classified into different types on the basis of structure and shape of subsidiary cells. In this study, three types of stomata were observed on both foliar surfaces including anemocytic, anisocytic, and paracytic. Qualitative and quantitative traits of foliar epidermal cells are summarized in table 4 and to better recognize the type of stomata, the results of light microscopy were simply painted (Fig. 6).

Type I: Anemocytic type: were observed in *A. aucheri* (Figs 4B, 5A, 5B), *A. deserti* (Figs 4C, 5C), *A. hausknechtii* (Figs 4D, 5D), *A. oliveriana* (Figs 4E, 5F), *A. scoparia* (Figs 4I, 5G), *A. sieberi* (Fig. 5H, I), and *A. vulgaris* (Fig. 4H) that in this types the subsidiary cells are indiscernible from other epidermal cells.

Type II: Anisocytic type: of stomata were found in the adaxial side of *A. persica* (Fig. 4H), and the adaxial side of *A. deserti* (Fig. 5C) that in this type guard cells are between two larger subsidiary cells and one is smaller.

Type III: Paracytic type: were observed in *A. persica* (Figs 4G, 5F) that in this type stomata have one or more subsidiary cells parallel to opening the guard cells.

The average length of the adaxial sides of *A. aucheri*, *A. deserti*, *A. haussknechtii*, *A. persica*, and *A. sieberi* were longer, in *A. oliveriana* and *A. scoparia*, the abaxial sides were longer, while in *A. vulgaris*, both sides were identical in length. The largest and smallest length of stomata existed in *A. aucheri* and *A. sieberi*, respectively.

Table 2. Qualitative and quantitative traits of foliar epidermal cells in examined *Artemisia* species

Taxon	Surface/side	Shape	Margin	Length (µm)	Width (µm)
A. aucheri	AB	Irregular	Wavy	44.75	11.3
	AD	Elongated	Smooth	58.2	7.2
A. deserti	AB	Polygonal	Smooth	41.13	10.85
	AD	Polygonal	Smooth	55.5	14.05
A. haussknechtii	AB	Irregular	Wavy	29.6	8.66
	AD	Irregular	Wavy	47.3	7.63
A. oliveriana	AB	Elongated	Smooth	65.25	14.51
	AD	Elongated	Smooth	73.35	10.65
A. persica	AB	Polygonal	Smooth	20.9	6.56
	AD	Irregular	Wavy	15. 45	8.52
A. scoparia	AB	Irregular	Wavy	35.25	14.4
	AD	Irregular	Wavy	45.6	13.9
A. sieberi	AB	Irregular	Wavy	35.95	10.55
	AD	Elongated	Smooth	50.75	13.55
A. vulgaris	AB	Irregular	Wavy	52.1	20.0
	AD	Irregular	Wavy	48.0	12.5

AB = Abaxial, AD = Adaxial

Table 3. Quantitative and qualitative traits of glandular and non-glandular foliar trichomes in examined *Artemisia* species

Taxon	Non-glandular trichome	Height width (µm)	Shape of branch	Type of glandular trichrome	Height width (µm)
A. aucheri	Capitate	18.56 × 14.9	T-shaped	Tectorial	311.1 × 16.4
A. deserti	Capitate	97.65 × 68.1	T-shaped	Conical type	367. 18 × 14.5
A. haussknechtii	Capitate	75.6 × 38.95	T-shaped	Stinging hair	272.1 × 9.4
A. oliveriana	Capitate	26.96 × 10.18	Star-shaped	Tectorial	756.18 × 7.5
A. persica	Peltate	21.25 × 21.7	Star-shaped	Tectorial	281.1 × 7.3
A. scoparia	Capitate	27.35 × 15.15	Absent	Absent	Absent
A. sieberi	Capitate	64.9 × 49.0	T-shaped	Tectorial	288.1 × 11.4
A. vulgaris	Capitate	45.2 × 30.1	T-shaped	Aduncate	182.55 × 10.45

Table 4. Quantitative and qualitative traits of stomata in examined *Artemisia* species

Taxon	Surface/side	Stomata type	Guard cell		Stomata aperture		No. of subsidiary cells	Stomata complex	
			Length (µm)	Width (µm)	Length (µm)	Width (µm)		Length (µm)	Width (µm)
A. aucheri	AB	Anemocytic	11.46	4.44	6.6	1.8	4	21.84	10.34
	AD	Anemocytic	31.38	7.44	25.1	9.36	4	38.88	24.2
A. deserti	AB	Anemocytic	16.04	4.98	12.56	1.88	4	25.1	17.46
	AD	Anisocytic	29.08	9.8	17.8	2.54	4	31.75	19.55
A. haussknechtii	AB	Anemocytic	12.22	3.42	9.5	1.62	4–5	16.9	11.28
	AD	Anemocytic	21.2	9.12	14.68	3.56	4–5	27.0	25.08
A. oliveriana	AB	Anemocytic	30.78	8.04	17.56	2.56	4–5	33.52	19.36
	AD	Anemocytic	28.12	8.72	14.14	4.14	4	26.98	21.12
A. persica	AB	Paracytic	11.6	4.2	10.34	1.8	2	27.0	19.15
	AD	Anisocytic	19.3	7.08	17.18	1.98	2–3	21.34	14.34
A. scoparia	AB	Anemocytic	16.2	3.24	11.34	0.82	4–5	17.94	9.68
	AD	Anemocytic	14.68	5.78	11.54	2.14	4	20.76	14.54
A. sieberi	AB	Anemocytic	13.54	4.1	9.36	3.16	3–5	18.86	11.04
	AD	Anemocytic	30.76	10.84	12.44	2.14	4	17.38	22.14
A. vulgaris	AB	Anemocytic	20.0	8.1	21.4	1.4	4–5	23.0	15.01
	AD	Anemocytic	19.7	6.7	10.9	3.4s	N	24.8	13.7

N = Not recognizable

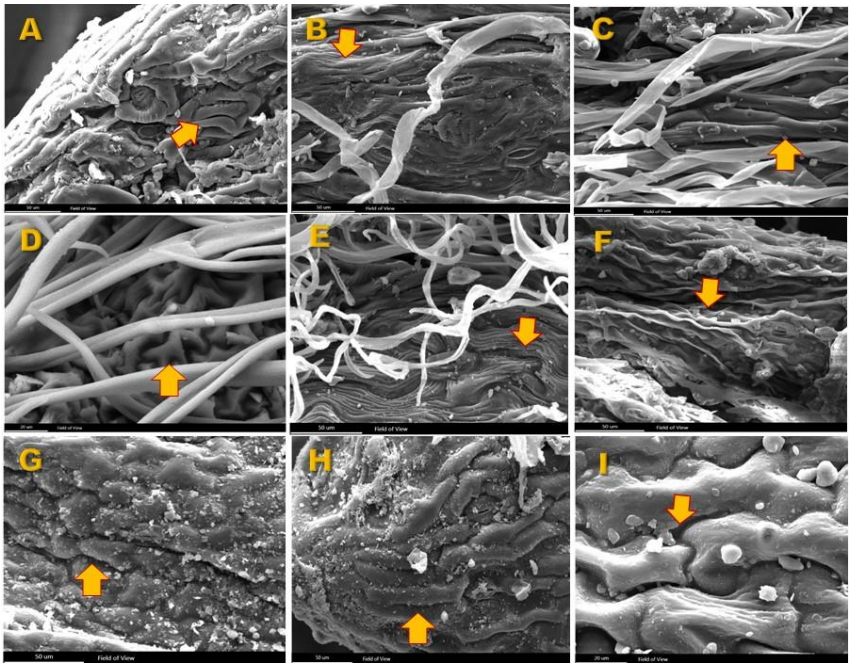

Fig. 1. SEM of foliar epidermal cells of different *Artemisia* species: A. The abaxial side of *A. aucheri*, B. The adaxial side of *A. aucheri*, C. *A. deserti*, D. *A. haussknechtii*, E. *A. oliveriana*, F. *A. persica*, G. *A. scoparia*, H. *A. sieberi* (Bar = 50 µm), I. *A. vulgaris* (Bar = 20 µm).

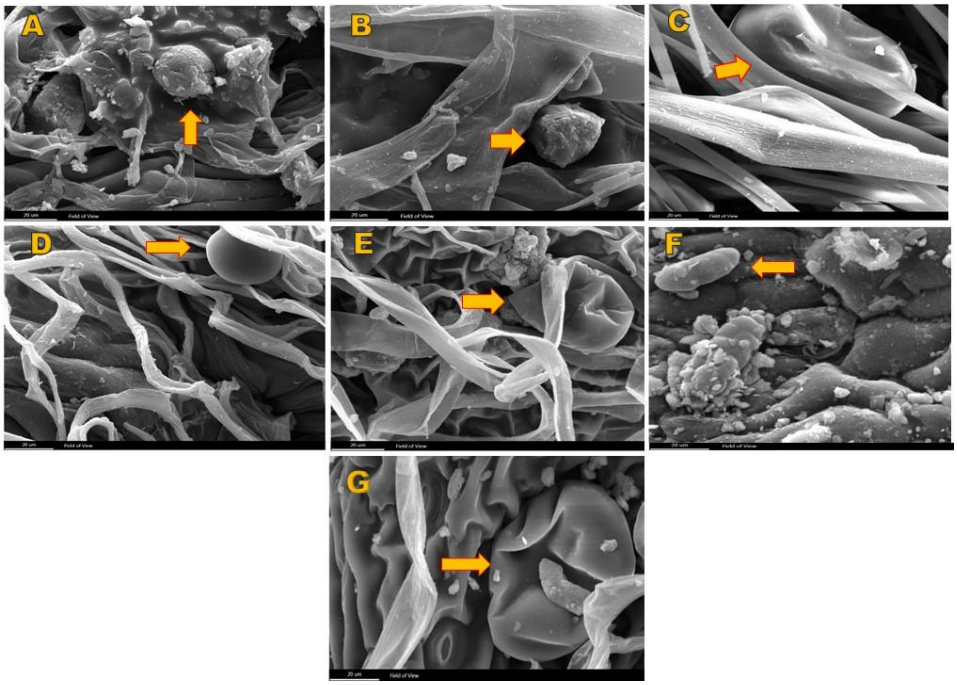

Fig. 2. SEM of glandular trichomes in different *Artemisia* species: *A. aucheri*, B. *A. deserti*, C. *A. haussknechtii*, D. *A. oliveriana*, E. *A. persica*, F. *A. scoparia*, G. *A. sieberi*, H. *A. vulgaris* (Bar = 20 µm).

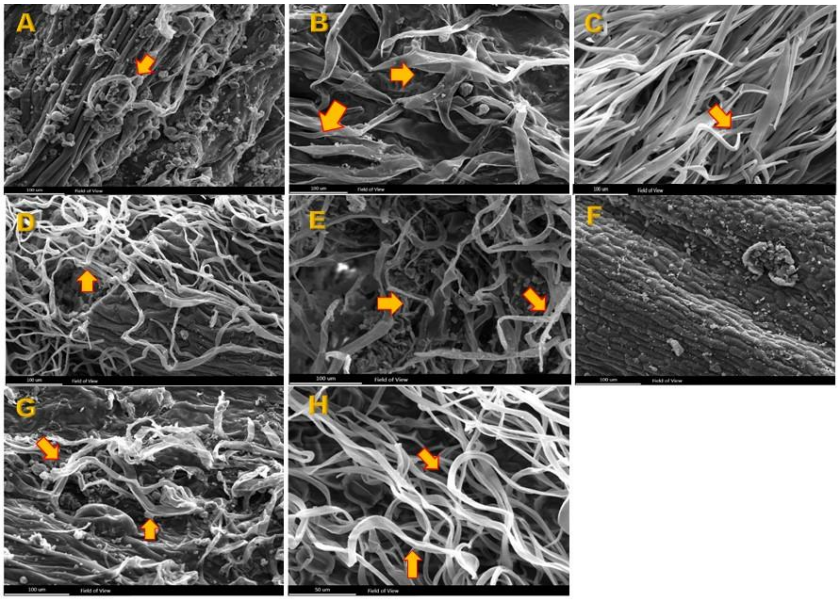

Fig. 3. SEM of non-glandular trichomes in different *Artemisia* species: A. *A. aucheri*, B. *A. deserti*, C. *A. haussknechtii*, D. *A. oliveriana*, F. *A. persica*, G. *A. scoparia*, H. *A. sieberi* (Bar = 100 µm), I. *A. vulgaris* (Bar = 50 µm).

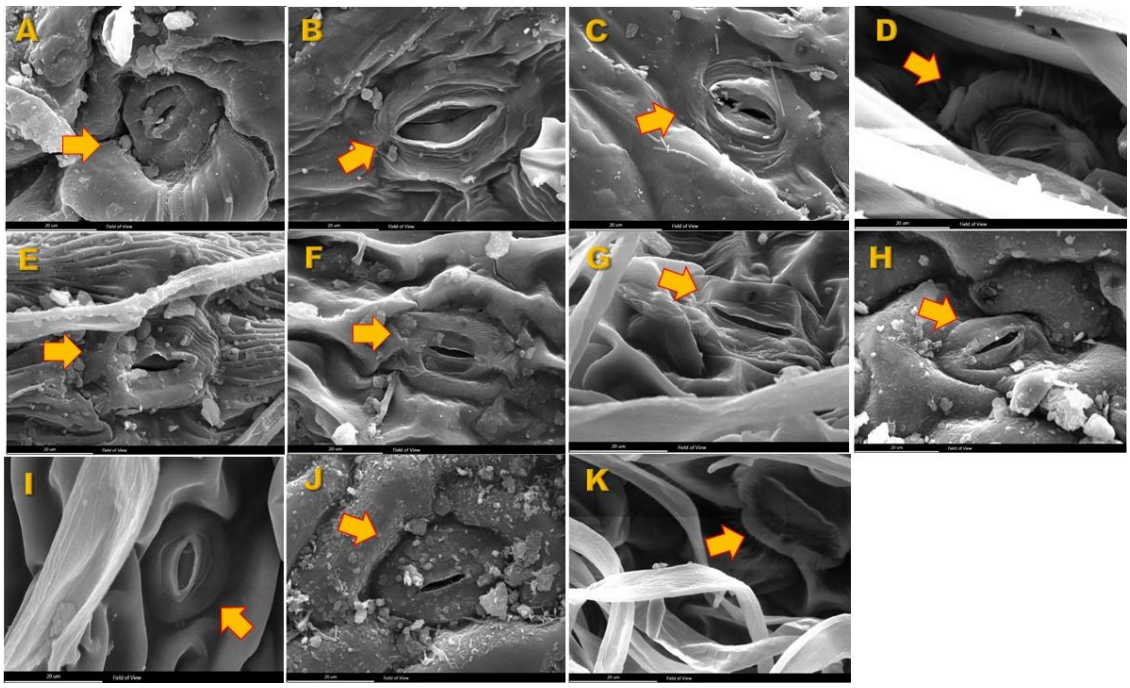

Fig. 4. SEM of stomata in different species *Artemisia*: A. The abaxial side of *A. aucheri*, B. The adaxial side of *A. aucheri*, C. *A. deserti*, D. *A. haussknechtii*, E. *A. oliveriana*, F. The abaxial side of *A. persica*, G. The adaxial side of *A. persica*, H. *A. scoparia*, I. The abaxial side of *A. sieberi*, J. The adaxial side of *A. sieberi*, K. *A. vulgaris* (Bar = 20 µm).

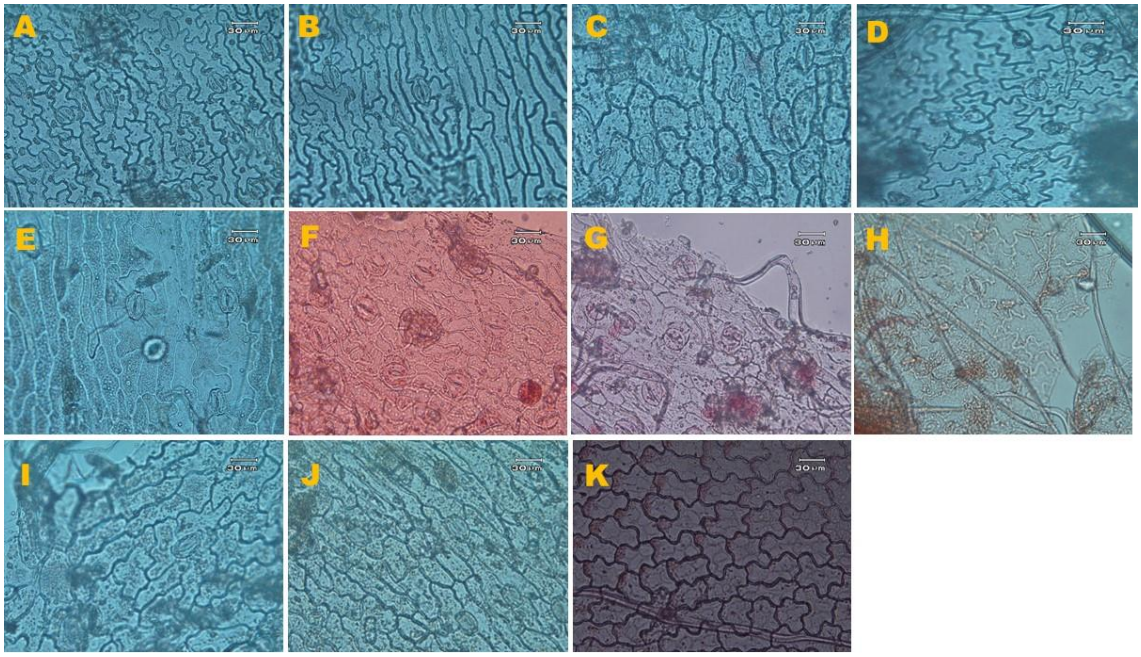

Fig. 5. LM of the adaxial and the abaxial sides of foliar epidermal cells of different *Artemisia* species: A. The abaxial side of *A. aucheri*, B. The adaxial side of *A. aucheri*, C. *A. deserti*, D. *A. haussknechtii*, E. *A. oliveriana*, F. The adaxial side of *A. persica*, G. The abaxial side of *A. persica*, H. *A. scoparia*, I. The abaxial side of *A. sieberi*, J. The adaxial side of *A. sieberi*, K. *A. vulgaris* (Bar = 30 µm).

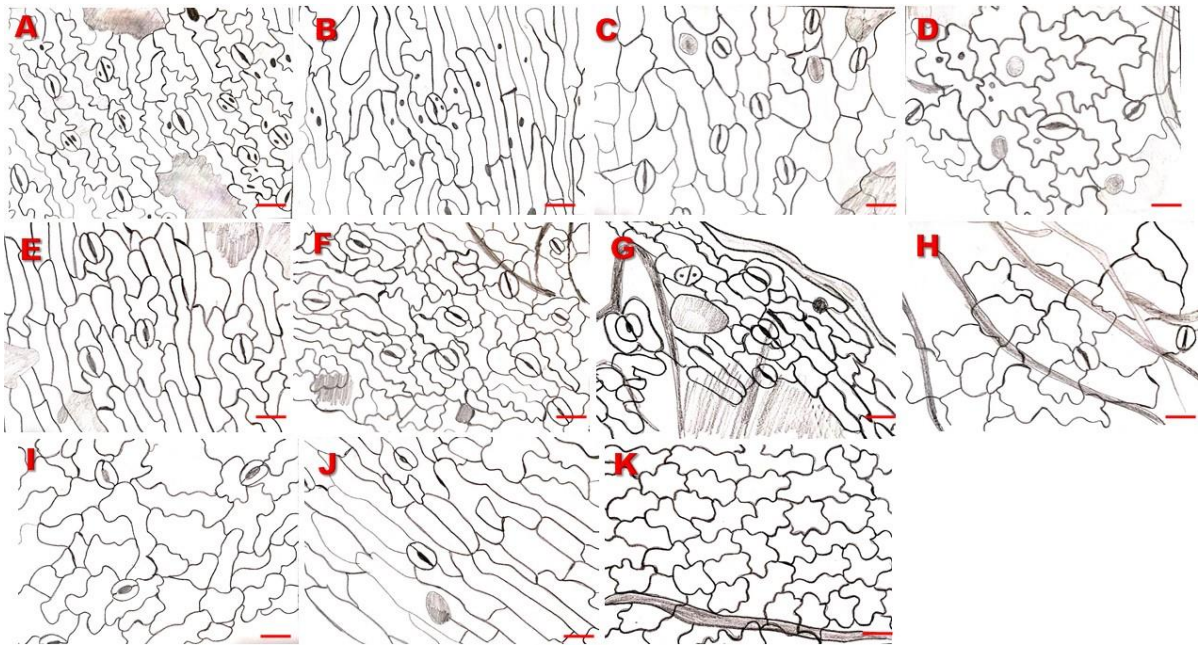

Fig. 6. Foliar epidermal cells hand-drawings of different species of *Artemisia*: A. The abaxial side of *A. aucheri*, B. The adaxial side of *A. aucheri*, C. *A. deserti*, D. *A. haussknechtii*, E. *A. oliveriana*, F. The adaxial side of *A. persica*, G. The abaxial side of *A. persica*, H. *A. scoparia*, I. The abaxial side of *A. sieberi*, J. The adaxial side of *A. sieberi*, K. *A. vulgaris* (Bar = 30 µm).

Discussion

As global research on *Artemisia* taxa is continuing, it is mainly focused on phytochemistry and still, little is known about anatomical features and their value in the taxonomy of the genus *Artemisia* (Janackovic *et al*. 2019). The foliar epidermal anatomy of *Artemisia* species has been reported by many researchers worldwide. Davis and Heywood (1963) emphasized the use of anatomical characters as a reliable constant within a taxon. SEM is a better choice compared with other traditional microscopic techniques because it gives a distinct structure and helps

to solve taxonomic ambiguities in the genus *Artemisia* (Hayat *et al*. 2010).

Hussain *et al*. (2019) reported three different types of epidermal cells in *Artemisia* species including, irregular with wavy walls, and polygonal with smooth margins, and elongated cells with smooth walls. They also reported four different types of stomata in *Artemisia* species including, anemocytic, anomotetracytic, diacytic, and anisocytic types. Our results about these species agreed with their findings regarding the type and arrangements of the epidermal cells. Examination of epidermal cell shape and stomatal type determination by LM was more specific than electron microscopy. However, SEM is better to determine quantitative traits and measure the length and width of epidermal cells and stomata, as well as the type of trichomes. In this study, we noticed three different types of the epidermal cells that the most of *Artemisia* species like *A. sieberi*, *A. haussknechtii*, *A. vulgaris*, and *A. scoparia* showed irregular epidermal cells shape with wavy margins. Among them, *A. haussknechtii*, *A. vulgaris*, and *A. persica* from subgenus *Artemisia* are very similar to each other and also, *A. deserti*, *A. sieberi*, and *A. aucheri* from subgenus *Seriphidium* are similar. Therefore, the shape of foliar epidermal cells is a useful tool to distinct species at the subgenus level. In *A. aucheri* and *A. sieberi*, the adaxial sides showed elongated type while in the abaxial side, showed irregular type that these variations can reflect environmental effects (Stenglein *et al*. 2005, Srilakshmi & Naidu 2014).

Based on interpretation of LM and SEM photographs, three different stomata including anemocytic, anisocytic, and paracytic were observed. The type of stomata in most species were anemocytic. In *A. persica* and *A. deserti* both foliar surfaces showed two different types of stomata. Bano *et al.* (2015) discussed different stomata types in the *Asteraceae* family that introduced anisocytic stomata and polygonal shape of epidermal cells in *A. persica*. Rabie *et al.* (2006) analyzed five *Artemisia* species from north of Iran and their investigations showed a variation of epidermal cells in some species of *Artemisia*. In *A. haussknechtii* and *A. vulgaris* due to the higher density of trichomes on the abaxial side, the detailed study of stomata is not possible. No trichome was seen in the adaxial side of *A. vulgaris*. Prominent stomata are present in *A. deserti*, *A. sieberi*, and the adaxial side of *A. scoparia*. The distribution and density of stomata in the adaxial and the abaxial sides of different species of *Artemisia* are very variable that reflect environmental changes (Mirhaji *et al*. 2001, Azarnivand, 2004). In addition, quantitative traits, that which averages a few specimens, due to the great impact of ecological and climatic conditions, have a lot of overlap with qualitative traits and cannot be considered as diagnosis traits in terms of taxonomy (Gurcharan, 2004).

Trichomes were divided into two general types: non-glandular trichomes and glandular trichomes. These two types of trichomes are defined to serve different functions. Normally, non-glandular trichomes function in water absorption and seed dispersion while glandular trichomes are the main place for biosynthesis and secretion of plant secondary metabolites (Wu *et al*. 2012). Two types of glandular trichomes including peltate and capitate were observed. Capitate trichomes found in the majority of studied species that in previous investigations also were confirmed (Kelsey 1984, Lodari *et al*. 1989, Hayat *et al.* 2009). In previous studies, the type of trichomes of *A. scoparia* was mentioned unicellular and T- shaped (Naseri 2004, Rabie *et al*. 2006, Saedi *et al*. 2009) while in the present study, *A. scoparia* have no non-glandular trichomes and only observed glandular trichomes. Hayat *et al*. (2009), in addition to capitate and T-shaped trichomes, unveiled eight main types of trichomes of *Artemisia* and also Hussain *et al*. (2019) added and introduced two new types of trichome in *Artemisia*. Based on their definition of trichome types, our results about this species, with a little different, agree with their findings. Accordingly, various glandular and non-glandular foliar trichomes help resolve taxonomic conflicts but it is necessary to do more detailed studies of these micromorphological traits and develop better terminology. In our previous study of the genus *Artemisia* in Isfahan province based on morphological features

related to reproductive and vegetative structures, three subgenera of *Artemisia* were placed in separate clusters (Karbalaie *et al*. 2021), also the molecular phylogenetic data have confirmed this clustering and results (Haghighi *et al*. 2014). In conclusion, although foliar micro-morphological features are useful to clarify the taxonomic issues of the genus *Artemisia*, the present investigation also shows that, epidermal cell variations are not reliable when utilized alone. They can be important when they serve as supportive features with other traits. Therefore, there is a need to integrate this data with other information, especially with molecular data.

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