



Full Length Article

Unveiling the Foliar Epidermal Anatomical Characteristics of Genus *Artemisia* (Asteraceae) from Northeast (Gilgit-Baltistan), Pakistan

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Abstract

This study for the first time examined foliar epidermal anatomical characteristics (Epidermal cells, types of stomata and trichomes) of thirteen *Artemisia* species (Including rare ones) with Light microscopy (LM) and Scanning electron microscopy (SEM) from the Northeast (Gilgit-Baltistan) region of Pakistan. The epidermal cells varied from polygonal to irregular and elongate in shape, while wavy to smooth in margins. This investigation revealed four different types of stomata viz; Anomocytic, diacytic, anomotetracytic and anisocytic, which were unequally scattered on both the adaxial and abaxial surfaces of studied *Artemisia* species. Ten different types of trichomes were observed in the studied species. Four types of glandular trichomes including peltate, pluricellular, capitate and thin necked were observed, whereas six types of non-glandular trichomes including, Aduncate, unicellular calavate, conical type, stinging hair type, unicellular tector and unicellular filiform were observed. This study authenticated that the morphology of these foliar anatomical characteristics in amalgamation with other features are helpful for the species delimitation within the genus *Artemisia*. © 2019 Friends Science Publishers

Keywords: *Artemisia*; Epidermal cells; Stomatal diversity; Trichomes variation; Gilgit-Baltistan

Introduction

Artemisia L. (family Asteraceae; tribe Anthemideae) is well-known for having antimalarial properties in its species. This genus is taxonomically complex comprising ~500 herbs and shrubs (Martin *et al.*, 2003), many of which have significant economic importance because they possess antiseptic, antispasmodic, antimicrobial, antitumor, hepatoprotective and antirheumatic properties (Terra *et al.*, 2007). The genus is distributed mostly in the northern hemisphere's temperate sectors. A limited number of *Artemisia* species are found in the southern hemisphere (Oberprieler *et al.*, 2009). Central Asia is recognized as the center of diversity for *Artemisia*. Ancient microfossil of *Artemisia* are known from the Eocene end (Zaklinskaja, 1957) and the Miocene radiation (Wang, 2004).

For a long time, the infrageneric classification of this diverse genus has presented a challenge for taxonomists. These historical developments were well documented in the former studies (Torrell *et al.*, 1999; Valles and McArthur, 2001). Tournefort (1700), Bremer (1994) and Ghafoor (2002), all the studies concerning the taxonomy and classification of *Artemisia* were based on capitulum morphology. They recognized four sections or subgenera in

the genus *Artemisia* (*sensu lato*) i.e., *Artemisia*, *Absinthium*, *Dracunculus* and *Seriphidium*. Throughout this period, the status of *Seriphidium* as a separate genus or a subgenus of the *Artemisia* remained a topic of debate among taxonomists. For example, the former treatment was adopted by Ling (1982), Bremer and Humphries (1993), Bremer (1994), Ling (1995) and Ghafoor (2002) while the latter was adopted by Kornkven *et al.* (1998, 1999), Torrell *et al.* (1999), Watson *et al.* (2002) and D'Andrea *et al.* (2003).

In the light of recent studies based on microscopic and molecular approaches, the delimitations of classic subgenera have been rearranged (Sanz *et al.*, 2008). Researchers strongly agreed that the critical anatomical enquiry of rarer morphological attributes with molecular phylogenies is fruitful and this union could be a power tool to resolve taxonomic conflicts (Scotland *et al.*, 2003).

The leaf epidermal cells features play significant role in the identification of different plant genera and species (Scatena *et al.*, 2005; Taia, 2005; Yang and Lin, 2005). These features holds numerous beneficial diagnostic properties like shape and size of stomata, no of subsidiary cells, guard cells profile, and structural particularities of cell walls and trichomes types (Dickison, 2000). That's why the

taxonomists are paying attention towards foliar epidermal anatomical examinations with the aim of resolving conflicts associated with taxonomy and classification (Fang and Fan, 1993). The differences of epidermal attributes between species might be due to their different genetic makeup or due to some environmental effects (Stenglein *et al.*, 2005).

Trichomes are very specialized structures present in the foliar epidermal cells of plants and divided in to two main categories (glandular and non-glandular). An important aspect of the glandular trichomes is their ability to store, synthesize and secrete different types of metabolites. These metabolites include organic acids, terpenoids and alkaloids, polysaccharides, polyphenols and proteins (Hallahan *et al.*, 2000). Consequently, they are the significant foundations of essential chemical compounds employed in the pharmaceutical industry, cosmetics/perfumes and the food additives (Callow, 2000; Wagner *et al.*, 2004).

Although, the diversity of foliar characters of some *Artemisia* species have previously been reported from Pakistan (Hayat *et al.*, 2009a, b), but little is known about the importance of these foliar anatomical attributes present in rare *Artemisia* species from the Northeast (Gilgit-Baltistan) region of Pakistan. In this research we scrutinized the foliar epidermal anatomical features of *Artemisia* species with LM and SEM techniques. This study will narrate the utilization of foliar attributes for their conceivable practical role in the taxonomy of genus and for the future inquiries based on medicinal significance.

Materials and Methods

Study Area

Gilgit-Baltistan (Formerly called Northern Areas) is a Northeastern region of Pakistan situated between 74°–77°E and 34°–37° N, covering an area of about 45224 km². The altitude of this region ranges from ±1400 to 8611 m. The area is divided into seven main districts *i.e.*, Gilgit, Skardu, Hunza-Nagar, Astore, Diamer, Ghizer and Ghanche. This region includes world-renowned mountain ranges like the Karakorum, Hindu Kush and the Himalayas. There are several peaks with heights above 7000 m, including Godwin Austin (K-2, 8611 m), Rakaposhi (7788 m) and Deran peak (7268 m). The world's largest glaciers are also found in this region, such as Baltoro Glacier, which extends for about 62 km with an area of 529 km² (Anonymous, 2003). This area is well known for a great diversity of plants (Shinwari, 2010) and is a center for traditional medicinal herbs (Shinwari and Gilani, 2003).

Plant Collection and Sampling

Extensive field surveys were conducted over a period of two years (2016–2017) and *Artemisia* species were collected from their natural habitat from different regions of Northeast

(Gilgit-Baltistan) region of Pakistan (Fig. 1). The collected plant specimens were primarily authenticated and recognized by a specialist at the Botanical Science Division, Pakistan Museum of Natural History Islamabad. The source and collection details along with GPS data of studied *Artemisia* species are provided in Table 1.

Herbarium Preparation and Plant Identification

Thirteen species of *Artemisia* collected from different parts of Northeast (Gilgit-Baltistan) of Pakistan were included in the study and the voucher specimens were submitted to the herbarium of Pakistan Museum of Natural History (PMNH) Islamabad Pakistan. Plants were identified on the basis of morphological data, molecular data and Flora of Pakistan (Ali and Qaiser, 1993–2010).

Light Microscopy (LM)

For light microscopy dried specimen of each plant from herbarium was taken. Primarily, 30% nitric acid and about 1.5 g of potassium chloride was taken in a test tube, the leaves were boiled for few min (2–3 min) in the solution. After boiling, the leaves were washed with deionized water. Peeling of epidermis was performed and 60% potassium hydroxide solution was used to keep the peel for 2 h. Finally, these peels were transferred to lactic acid and glass slides were prepared for LM investigation (Hayat *et al.*, 2009b).

Scanning Electron Microscopy (SEM)

From the dried herbarium specimen, 4–5 mm of top and bottom leaf was taken and then it was fixed in glutaraldehyde (6%) with 0.05 M sodium cacodylate and kept for 24 h. The specimen was then washed with distilled water for 2–3 times by means of the pasture pipette. Ethanol (10–100%) was used to dehydrate the samples for 20 min per wash. The specimens were then kept in 100% ethanol in the refrigerator for further use. Each dried specimen was mounted on aluminum specimen stub with carbon on both sides. Sputter coating was done with gold particles in the sputtering chamber of Pelco Auto sputter Coater (SC-7, Ted Pella Inc).

Surface of the leaves was scrutinized at variable magnifications using Philips XL30 TMP (FEI Company) scanning electron microscope, operated at 10–20 kV accelerated voltage, at the electron microscopy core laboratory, Tupper Hall, University of California Davis USA. All the demonstrative structures observed were taken digitally with the help of Microsoft image programmed for windows. The quantitative measurements of each characteristic were recorded for 4 to 5 times and averaged.

For the identification of epidermal cells and stomata, the basic criterion was adapted from Hayat *et al.* (2010). Glandular and non-glandular trichomes were identified on

Table 1: Collection details of *Artemisia* species from Gilgit-Baltistan region of Pakistan with voucher specimen number

<i>Artemisia</i> Spp.	Latitude	Longitude	Locality	Voucher specimen number	Collectors
<i>A. annua</i>	N-35°54.949	E-74°18.508	Barmas paen Gilgit	PMNH-41582	Adil Hussain and Tanseer
<i>A. austriaca</i> *	N-36°01.609	E-74°33.255	Bagrote valley Gilgit	PMNH-41643	Adil Hussain and Tabeer
<i>A. dubia</i>	N-35°54.491	E-74°23.867	Danyore Oshkandas Gilgit	PMNH-41608	Adil Hussain and Tanseer
<i>A. chinensis</i> *	N-35°26.585	E-75°27.011	Shangrilla Skardu	PMNH-41722	Adil Hussain and Tanseer
<i>A. chamaemelifolia</i> *	N-36°09.622	E-74°11.622	Naltar valley Gilgit	PMNH-41630	Adil Hussain and Tanseer
<i>A. argyi</i> *	N-35°54.951	E-74°18.503	Barmas paen Gilgit	PMNH-41583	Adil Hussain and Tabeer
<i>A. indica</i>	N-36°15.250	E-73°24.240	Yasin Ghizer	PMNH-41694	Adil Hussain and Tanseer
<i>A. gmelinii</i>	N-36°08.967	E-74°12.112	Naltar valley Gilgit	PMNH-41621	Adil Hussain and Tanseer
<i>A. herba alba</i>	N-35°54.061	E-74°12.762	Kargah nala Gilgit	PMNH-41599	Adil Hussain and Tanseer
<i>A. montana</i> *	N-35°30.883	E-75°40.115	Hashupi Shigar Skardu	PMNH-41708	Adil Hussain and Tanseer
<i>A. tournefortiana</i>	N-35°25.493	E-75°44.507	Shigar valley Skardu	PMNH-41704	Adil Hussain and Tanseer
<i>A. verlotiorum</i> *	N-36°08.543	E-73°51.721	Bubar Ghizer	PMNH-41684	Adil Hussain and Amar
<i>A. vulgaris</i>	N-36°20.508	E-74°52.277	Shishkat Hunza-Nagar	PMNH-41646	Adil Hussain and Amar

The voucher numbers have been obtained from Pakistan Museum of Natural History and its code is PMNH. * Represents rare *Artemisia* species from the Northeast (Gilgit-Baltistan) region of Pakistan

the basic criterion given by Hayat *et al.* (2009a, b), Popa and Sipos (2009) and Shaheen *et al.* (2009). For some specific kind of epidermal cells, stomata and trichomes, simple self-explanatory terms were also used and results are presented in the form of Micrographs and Table.

Results

Epidermal Cells

This study on *Artemisia* species showed variations in their leaf epidermal anatomical attributes, such as the epidermal cells structure, stomata and trichomes. Epidermal cells investigated were varied from polygonal to elongate and irregular in shape, while smooth and wavy in margins (Fig. 2 and 3). Majority of *Artemisia* species like *A. annua* (Fig. 2A and 3A), *A. chamaemelifolia* (Fig. 2C), *A. tournefortiana* (Fig. 2D and 3E), *A. verlotiorum* (Fig. 2E and 3F), *A. indica* (Fig. 2F and 3D), *A. chinensis* (Fig. 2G and 3B), *A. austriaca* (Fig. 2H), *A. gmelinii* (Fig. 2I), *A. vulgaris* (Fig. 2K and 3G) and *A. dubia* (Fig. 3C) showed irregular epidermal cells shape with wavy walls. *A. herba alba* showed elongated shape of cells with smooth walls (Fig. 2J). *A. argyi* and *A. montana* showed polygonal shape with smooth walls (Fig. 2B and 2L). The qualitative and quantitative attributes of all the epidermal cells of studied *Artemisia* species based on SEM and LM investigations (Table 2).

Stomatal Diversity

In this study, total 4 types of stomata were noticed in 13 different species of *Artemisia*. For all species, stomata were observed on both foliar surfaces, predominantly on the abaxial surface. These types of stomata were: Anomocytic, anisocytic, anomotetracytic and diacytic. The details based on SEM and LM observations of the stomata types are provided in Table 3.

Anomocytic type of stomata was found in *A. annua* (Fig. 4A), *A. argyi* (Fig. 4B), *A. indica* (Fig. 4F) *A. austriaca* (Fig. 4H), *A. herba alba* (Fig. 4J) and *A. montana*

Table 2: Qualitative and quantitative foliar epidermal cells characteristics of different *Artemisia* species

<i>Artemisia</i> spp.	Surface	Shape	Margin	Length μ m	Width μ m
<i>A. annua</i> L.	AB	Irregular	Wavy	36.11	14.21
	AD	Irregular	Wavy	41.87	15.00
<i>A. austriaca</i>	AB	Irregular	Wavy	40.30	11.62
	AD	Irregular	Wavy	41.27	13.28
<i>A. chinensis</i>	AB	Irregular	Wavy	30.23	9.84
	AD	Irregular	Wavy	36.94	14.24
<i>A. dubia</i>	AB	Irregular	Wavy	42.19	13.55
	AD	Irregular	Wavy	56.11	22.64
<i>A. chamaemelifolia</i>	AB	Irregular	Wavy	75.38	21.79
	AD	Irregular	Wavy	79.00	22.60
<i>A. argyi</i>	AB	Polygonal	Smooth	32.78	33.69
	AD	Polygonal	Smooth	23.73	15.59
<i>A. gmelinii</i>	AB	Irregular	Wavy	20.44	12.53
	AD	Irregular	Wavy	28.04	10.89
<i>A. herba alba</i>	AB	Elongated	Smooth	64.02	7.58
	AD	Elongated	Smooth	71.27	8.87
<i>A. indica</i>	AB	Irregular	Wavy	47.40	14.51
	AD	Irregular	Wavy	58.13	23.60
<i>A. montana</i>	AB	Polygonal	Smooth	30.69	17.10
	AD	Polygonal	Smooth	34.41	34.74
<i>A. tournefortiana</i>	AB	Irregular	Wavy	29.70	12.17
	AD	Irregular	Wavy	39.43	16.64
<i>A. verlotiorum</i>	AB	Irregular	Wavy	46.37	31.97
	AD	Irregular	Wavy	58.22	29.30
<i>A. vulgaris</i> L.	AB	Irregular	Wavy	36.26	15.44
	AD	Irregular	Wavy	44.02	15.50

AB= Abaxial, AD= Adaxial

(Fig. 4L). Diacytic types of stomata were found in *A. chamaemelifolia* (Fig. 4C). The abaxial side of *A. tournefortiana* (Fig. 4D) showed anomocytic while the adaxial surface showed anomotetracytic type of stomata. *A. chinensis* (Fig. 4G) and *A. gmelinii* (Fig. 4I) showed Anomotetracytic type of stomata. Anisocytic type of stomata was noticed in *A. vulgaris* (Fig. 4K) and *A. verlotiorum* (Fig. 4E). The qualitative and quantitative attributes of all stomata types observed in *Artemisia* species based on SEM investigations are given in Table 3.

Variation in Trichomes

For the first time in this study, distribution of foliar

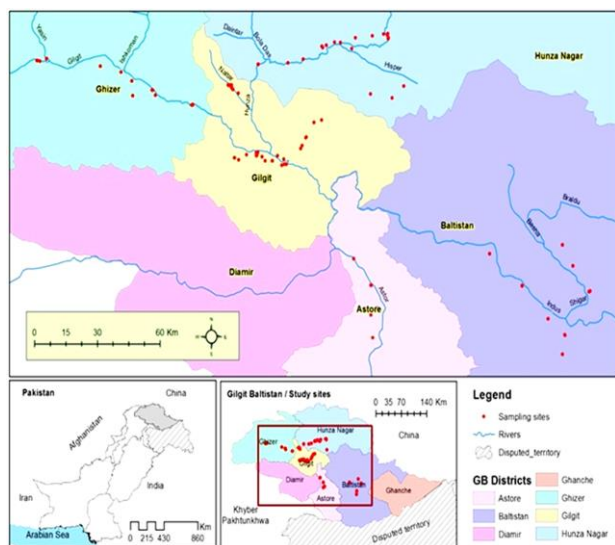


Fig. 1: Map showing the study area. Red dots specifies samples of *Artemisia* from different localities of Northeast (Gilgit-Baltistan) region of Pakistan

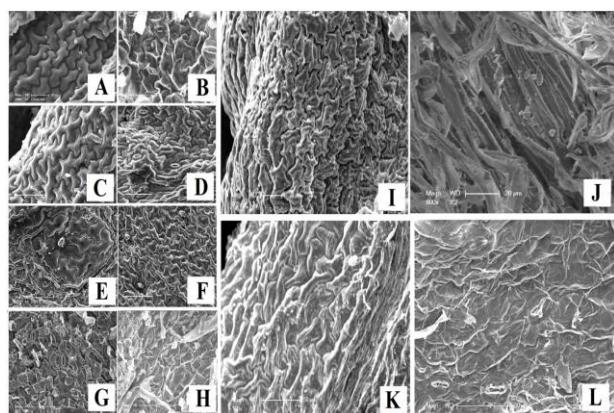


Fig. 2: Scanning electron micrographs of foliar epidermal cells of different *Artemisia* species: (A) *A. annua*, (B) *A. argyi*, (C) *A. chamaemelifolia*, (D) *A. tournefortiana*, (E) *A. verlotiorum*, (F) *A. indica*, (G) *A. chinensis*, (H) *A. austriaca*, (I) *A. gmelinii*, (J) *A. herba alba*, (K) *A. vulgaris*, (L) *A. montana*. Scale bar = 20, 50 μm

trichomes in 13 rare *Artemisia* species collected from the Northeast (Gilgit-Baltistan) region of Pakistan were assessed. A total of 10 main types of trichomes (glandular and non-glandular) were observed using light microscopy and scanning electron microscopic observations (Fig. 5, 6 and 7). Four types of glandular trichomes including peltate, pluricellular, capitate and thin necked were observed, while six types of nonglandular trichomes including, aduncate, unicellular calavate, conical type, stinging hair type, unicellular tector and unicellular filiform were observed. The qualitative and quantitative attributes of all glandular and non-glandular trichomes observed in *Artemisia* species based on SEM and LM are provided (Table 4 and 5). The

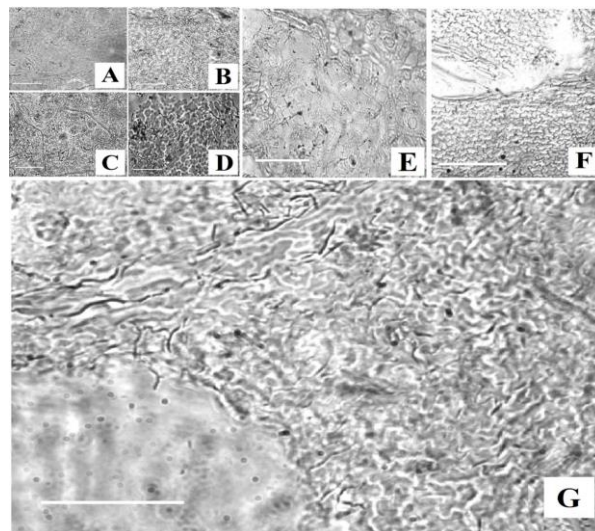


Fig. 3: Epidermal cells arrangement in *Artemisia* by means of LM: (A) *A. annua*, (B) *A. chinensis*, (C) *A. dubia*, (D) *A. indica*, (E) *A. tournefortiana*, (F) *A. verlotiorum*, (G) *A. vulgaris* (Scale bar = 50-100 μm)

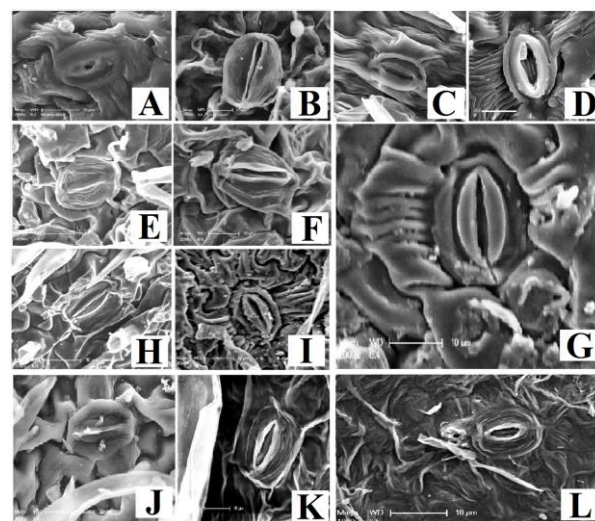


Fig. 4: Scanning Electron micrographs showing variations in stomata of different *Artemisia* species: (A) *A. annua*, (B) *A. argyi*, (C) *A. chamaemelifolia*, (D) *A. tournefortiana*, (E) *A. verlotiorum*, (F) *A. indica*, (G) *A. chinensis*, (H) *A. austriaca*, (I) *A. gmelinii*, (J) *A. herba alba*, (K) *A. vulgaris*, (L) *A. montana*. Scale bar = 10 μm

details of all the investigated trichomes are given below:

(I). Capitate trichomes: These glandular trichomes have ellipsoid like appearance. In this study we found these types of trichomes in *A. argyi* (Fig. 5B), *A. dubia* (Fig. 7C), *A. verlotiorum* (Fig. 5E), *A. tournefortiana* (Fig. 5D) and *A. vulgaris* (Fig. 5J).

(II). Peltate trichomes: These trichomes are non-glandular that looks like a ball with multicellular structures and are common in *austriaca* (Fig. 5F), *A. montana* (Fig. 5G) *A. indica* (Fig. 7D) and *A. chinensis* (Fig. 5H and 7B).

Table 3: Qualitative and quantitative characteristics of stomata in different *Artemisia* species

Artemisia spp.	Shape Stomata Type	Guard cell				Stomata aperture		No. of subsidiary cells	Stomata complex	
		Length μm	Width μm	Length μm	Width μm	Length μm	Width μm		Length	Width
<i>A. annua</i> L.	AB Anomocytic	20.24	16.61	17.52	6.47	9.52	4.42	5	43.69	42.39
	AD Anomocytic	23.39	17.18	20.79	6.76	12.18	2.79	5	53.01	33.25
<i>A. austriaca</i>	AB Anomocytic	25.59	29.19	22.36	12.11	19.63	4.70	---	Not differentiated	
	AD Anomocytic	17.32	10.36	14.57	4.13	11.92	2.77	---	Not differentiated	
<i>A. chinensis</i>	AB Anomotetracytic	26.98	19.76	18.28	8.5	10.49	1.87	4-5	65.06	43.27
	AD Anomotetracytic	25.48	18.69	17.05	7.07	14.39	2.49	4	46.97	50.29
<i>A. chamemelifolia</i>	AB Diacytic	32.28	21.52	26.60	8.95	18.77	4.13	2	65.80	51.00
	AD Diacytic	40.71	23.17	35.47	9.34	21.80	4.87	2	52.60	40.92
<i>A. argyi</i>	AB Anomocytic	34.29	20.86	28.65	9.75	23.60	3.03	---	Not differentiated	
	AD Anomocytic	32.44	20.65	25.42	10.60	24.40	6.01	---	Not differentiated	
<i>A. gmelinii</i>	AB Anomotetracytic	39.92	25.48	24.76	9.14	17.04	4.57	---	Not differentiated	
	AD Anomotetracytic	17.32	10.36	14.57	4.13	11.92	2.77	5-6	48.51	42.71
<i>A. indica</i>	AB Anomocytic	20.84	19.06	26.10	9.12	23.98	4.24	4	60.12	45.52
	AD Anomocytic	30.17	22.52	27.86	19.44	15.5	5.56	5-6	50.42	41.81
<i>A. montana</i>	AB Anomocytic	25.41	23.73	8.37	10.34	4.89	3.30	5	35.10	36.40
	AD Anomocytic	25.41	23.73	23.84	8.37	10.34	4.89	5	36.01	37.38
<i>A. herba alba</i>	AB Anomocytic	29.35	18.39	21.65	9.76	13.40	4.86	3	34.53	28.62
	AD Anomocytic	23.39	17.52	21.58	8.38	13.67	6.77	5-6	35.67	29.57
<i>A. tournefortiana</i>	AB Anomocytic	30.80	19.23	22.65	6.53	16.35	5.62	4	61.76	56.98
	AD Anomotetracytic	29.92	20.78	26.46	7.12	15.33	3.65	4	59.50	49.59
<i>A. verlotiorum</i>	AB Anisocytic	25.70	15.90	23.30	10.11	17.30	4.10	---	Not differentiated	
	AD Anisocytic	24.90	15.75	21.31	8.28	16.57	2.58	---	Not differentiated	
<i>A. vulgaris</i> L.	AB Anisocytic	19.20	14.30	14.70	7.7	4.12	2.31	3	54.60	50.15
	AD Anisocytic	18.24	14.48	15.03	6.24	10.69	1.51	3	55.30	52.40

AB= Abaxial, AD= Adaxial

Table 4: Quantitative attributes of glandular foliar trichomes in *Artemisia* species

Artemisia spp.	Capitate trichome		Peltate trichome		Pluricellular trichome		Thin neck trichome	
	Presence /Absence	Height x width (μm)	Presence /Absence	Diameter (μm)	Presence /Absence	Height x width (μm)	Presence /Absence	Height x width (μm)
<i>A. annua</i>	Absent	---	Absent	---	Present	50.02-52.06x17.58-19.20	Absent	---
<i>A. austriaca</i>	Absent	---	Present	81.38-83.85	Absent	---	Absent	---
<i>A. chinensis</i>	Absent	---	Present	61.22-63.72	Absent	---	Absent	---
<i>A. chamemelifolia</i>	Absent	---	Absent	---	Present	48.12-50.30x18.57-24.37	Absent	---
<i>A. argyi</i>	Present	44.19-46.49x31.60-31.87	Absent	---	Absent	---	Absent	---
<i>A. dubia</i>	Present	---	Absent	---	Absent	---	Absent	---
<i>A. gmelinii</i>	Absent	---	Absent	---	Absent	---	Present	41.81-42.26x8.09-10.15
<i>A. indica</i>	Absent	---	Present	---	Absent	---	Absent	---
<i>A. montana</i>	Absent	---	Present	34.13-40.31	Absent	---	Absent	---
<i>A. tournefortiana</i>	Present	39.84-41.20x24.14-27.74	Absent	---	Absent	---	Absent	---
<i>A. verlotiorum</i>	Present	47.30-49.56x36.28-38.31	Absent	---	Absent	---	Absent	---
<i>A. vulgaris</i>	Present	65.24-66.30x35.52-41.97	Absent	---	Absent	---	Absent	---

(III). Pluricellular trichomes: These glandular trichomes are long with 2 to 5 cells. They look broader from the base and tapering towards the top. These type of trichomes are found in *A. annua* (Fig. 5A and 7E) and *A. chamaemelifolia* (Fig. 5C and 7A).

(IV). Thin neck trichomes: They have short neck with large head and these are glandular trichomes are found in *A. gmelinii* (Fig. 5I).

(V). Conical type trichomes: These are non-glandular trichomes present in conical shape having a circular base tapering towards the apex. In this study we found these types of trichomes in *A. chinensis* (Fig. 6E).

(VI). Stinging hair trichomes: These trichomes are sharp with a sting like apex. These trichomes are non-glandular and are found in *A. argyi* (Fig. 6A).

(VII). Aduncate: These types of trichomes are non-glandular. They are long with curved or hook like apex and are present in *A. verlotiorum* (Fig. 6C), *A. indica* (Fig. 6D and 7F) and *A. dubia* (Fig. 7G).

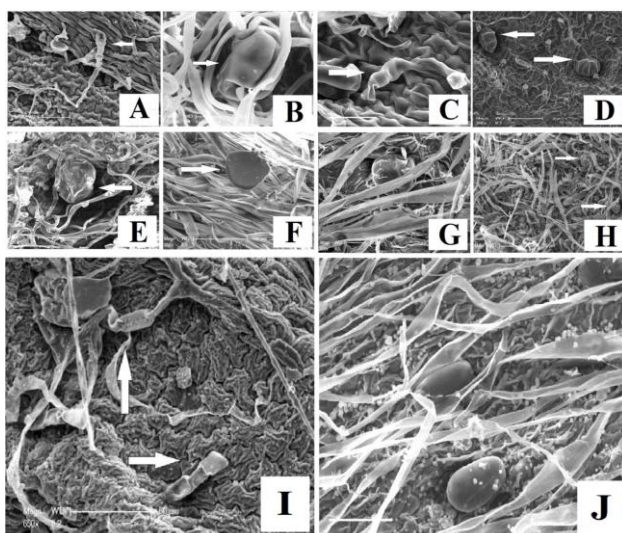
(VIII). Unicellular calavate: These trichomes are also non-glandular. They are short and narrow from the base and thicker at the apex. These types of trichomes were seen in *A. tournefortiana* (Fig. 6B).

(IX). Unicellular filiform: These trichomes are sharper at the apex. These trichomes are non-glandular and are found in *A. austriaca* (Fig. 6F).

(X). Unicellular tector: They are non-glandular. They are present in clusters or thread like. These types of trichomes are found in *A. herba alba* (Fig. 6G).

Table 5: Quantitative attributes of non-glandular foliar trichomes in *Artemisia* species

<i>Artemisia</i> spp.	Aduncate		Unicellular Clavate		Conical trichome	
	Presence/ Absence	Height x width (μm)	Presence/Absence	Length x width (μm)	Presence/Absence	Height x width (μm)
<i>A. austriaca</i>	Absent	---	Absent	---	Absent	---
<i>A. argyi</i>	Absent	---	Absent	---	Absent	---
<i>A. dubia</i>	Present	---	Absent	---	Absent	---
<i>A. herba alba</i>	Absent	---	Absent	---	Absent	---
<i>A. indica</i>	Present	189.55-199.30x7.87-10.90	Absent	---	Absent	---
<i>A. chinensis</i>	Absent	---	Absent	---	Present	47.95-50.51x15.21-23.76
<i>A. tournefortiana</i>	Absent	---	Present	68.80-70.65x16.70-21.12	Absent	---
<i>A. verlotiorum</i>	Present	130.46-127.30x9.94-12.64	Absent	---	Absent	---
<i>Artemisia</i> spp.	Stinging hair trichome		Unicellular filiform		Unicellular tector	
	Presence/ Absence	Height x width (μm)	Presence/Absence	Height x width (μm)	Presence/Absence	Height x width (μm)
<i>A. austriaca</i>	Absent	---	Present	133.89-135.30x7.64-12.09	Absent	---
<i>A. argyi</i>	Present	159.03-161.30x11.40-13.24	Absent	---	Absent	---
<i>A. herba alba</i>	Absent	---	Absent	---	Present	90.11-94.20x2.30-3.25
<i>A. indica</i>	Absent	---	Absent	---	Absent	---
<i>A. chinensis</i>	Absent	---	Absent	---	Absent	---
<i>A. tournefortiana</i>	Absent	---	Absent	---	Absent	---
<i>A. verlotiorum</i>	Absent	---	Absent	---	Absent	---

**Fig. 5:** Scanning electron micrographs showing glandular trichomes of different *Artemisia* species: (A) *A. annua*, (B) *A. argyi*, (C) *A. chamaemelifolia*, (D) *A. tournefortiana*, (E) *A. verlotiorum*, (F) *A. austriaca*, (G) *A. montana* (H) *A. chinensis*, (I) *A. gmelinii*, (J) *A. vulgaris*. (Scale bar = 20, 200 μm)

Discussion

The foliar epidermal characteristics are considered generally as a fruitful tool for determining taxonomic affiliations within the genus *Artemisia*. SEM is a better option concerning taxonomic resolutions in the genus *Artemisia* because; it gives a clear structure than other traditional microscopic techniques by showing distinct aspects of important micromorphological characters (Hayat *et al.*, 2010). With the help of microscopic techniques, Bano *et al.*

(2015) revealed polygonal shaped epidermal cells with straight pattern walls in *A. persica*. Wang *et al.* (2016) recently studied drought adaptive characteristics of leaf epidermal cells of different *Artemisia* species. Ivashchenko and Ivanenko (2017) studied the foliar epidermal cells of *A. abrotanum* and found thick walled epidermal cells. In the present study, three major types of epidermal cells among *Artemisia* species were observed: one with irregular shape and wavy margins, second with elongated shape and smooth margins and third with polygonal shape and smooth margins (Fig. 2 and 3). These types of epidermal cells in *Artemisia* species were also reported in previous studies of Rabie *et al.* (2006) and Hayat *et al.* (2010). But the quantitative measurements of epidermal cells recorded here are somewhat different from the values reported by Rabie *et al.* (2006) and Hayat *et al.* (2010). It might be due to some environmental fluctuations faced by *Artemisia* species. For example, the variations in epidermal cells of both abaxial and adaxial surfaces, also explained that *Artemisia* species (*A. herba alba*), which had elongated epidermal cells with smooth walls. Some species including *A. argyi*, and *A. montana* have polygonal type cells with smooth walls. Other species like, *A. annua*, *A. austriaca*, *A. chamaemelifolia*, *A. chinensis*, *A. dubia*, *A. gmelinii*, *A. indica*, *A. tournefortiana*, *A. verlotiorum* and *A. vulgaris* showed irregular shape with wavy margins.

A majority of studied rare *Artemisia* species including *A. argyi*, *A. chamaemelifolia*, *A. chinensis*, *A. montana* and *A. austriaca* were not examined by other researchers since many years for their foliar anatomical attributes. Hayat *et al.* (2010) disclosed the foliar epidermal anatomy of some *Artemisia* species and resolved the subgeneric classification of genus *Artemisia* based on these features. The utilization of foliar anatomical characteristics for taxonomic purpose in

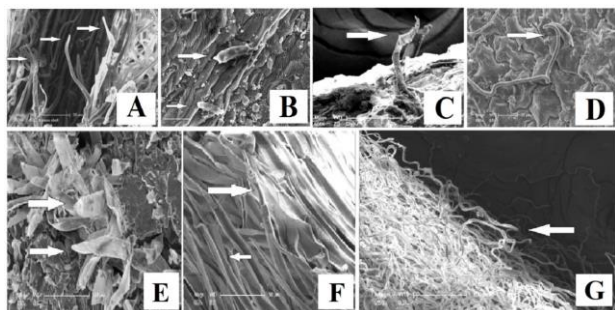


Fig. 6: Scanning electron micrographs showing non-glandular trichomes of different *Artemisia* species: (A) *A. argyi*, (B) *A. tournefortiana*, (C) *A. verlotiorum*, (D) *A. indica*, (E) *A. chinensis*, (F) *A. austriaca*, (G) *A. herba alba*. (Scale bar = 20, 100 μ m)

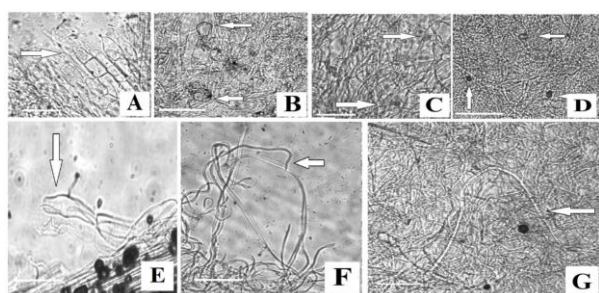


Fig. 7: LM monographs of glandular and non-glandular trichomes of different *Artemisia* species: (A) *A. chamaemelifolia*, (B) *A. chinensis*, (C, G) *A. dubia*, (D, F) *A. indica* (E) *A. annua*. (Scale bar = 50–100 μ m)

Artemisia species were revealed by Rabie *et al.* (2006), Noorbakhsh *et al.* (2008) and Saedi *et al.* (2009). The anatomical changes and frequencies of stomata in leaves of few *Artemisia* species were revealed by Nautiyal and Purohit (1980) and Hayat *et al.* (2010). This study confirmed four different stomata types viz; Anisocytic, diacytic, anomocytic and anomotetracytic (Fig. 4). These findings regarding the types of stomata are in agreement with the previous findings (Rabie *et al.*, 2006; Noorbakhsh *et al.*, 2008; Saedi *et al.*, 2009; Hayat *et al.*, 2010). Investigation of the characteristics of stomata suggests that these features can be served as crucial taxonomic traits in resolving taxonomic clashes within the genus *Artemisia*. For instance, Anomocytic type of stomata is common in a majority of *Artemisia* species. *A. chinensis* and *A. gmelinii* can be easily distinguished from the rest of species by their Anomotetracytic type of stomata. Similarly, Anisocytic stomata are prominent feature of *A. vulgaris* and *A. verlotiorum*. Diacytic types of stomata are only present in *A. chamaemelifolia*, respectively (Fig. 4).

This study also reported distribution of foliar trichomes in rare *Artemisia* species from Northeast (Gilgit-Baltistan) region of Pakistan. A lot of taxonomic studies based on foliar trichomes characters have gained the attention of systematists and plant morphologists to resolve

taxonomic conflicts (Fang and Fan, 1993; Hayat *et al.*, 2009a, b; Saedi *et al.*, 2009). Similarly, for the better description of taxonomic relationship within the genus *Artemisia*, the foliar trichomes features are very crucial (Hall and Clements, 1923). More so, multiple types of trichomes including both glandular and non-glandular have been documented in different *Artemisia* species (Ferreira and Janick, 1995; Hayat *et al.*, 2009a, b). In this study, majority of *Artemisia* species showed capitate type trichomes (Fig. 5). Previous studies by Kelsey (1984), Ascensao and Pais (1987) Lodari *et al.* (1989) Ferreira and Janick (1995) and Hayat *et al.* (2009a, b) also confirmed that the capitate glandular trichomes are most common in species of *Artemisia* genus.

Moreover, huge variations have been observed in capitate type trichomes in the studied *Artemisia* species. For example, the capitate type of trichomes in *A. argyi*, *A. dubia*, *A. verlotiorum*, *A. tournefortiana* and *A. vulgaris* were present in both upper and lower surface of the leaves. These types of trichomes in *Artemisia* were also reported in the previous studies of Hayat *et al.* (2009a, b) confirming their ellipsoidal shape with the division of two halves. The capitate trichomes of few species showed ellipsoid shape with two halves and few species did not show the two halves division. In few species, the shape of trichome was same and division of halves was not clear.

The capitate types of trichomes observed in this study were different from those reported previously in *A. tridentate* (Slone and Kelsey, 1985), *A. annua* (Ferreira and Janick, 1995), *A. ludoviciana* (Smith and Kreitner, 1983), *A. compestris* (Ascensao and Pais, 1987), *A. nova* (Kelsey, 1984) and *A. princeps* (Lodari *et al.*, 1989). In addition to the capitate trichomes, we also noticed some other types of trichomes which may be important from the taxonomic point of view. Among those trichomes, pluricellular trichomes of *A. annua* and *A. chamaemelifolia* were observed. Peltate trichomes that look like a ball with multicellular structures were important feature of *austriaca*, *A. montana*, *A. indica* and *A. chinensis*. Thin neck trichomes were appeared in *A. gmelinii*. Conical type trichomes with a circular base tapering towards the apex were found in *A. chinensis*. Stinging hair sharp non-glandular trichomes with a sting like apex were present in *A. argyi*. Aduncate with long curved or hook like apex trichomes were observed in *A. verlotiorum*, *A. indica* and *A. dubia* (Fig. 6 and 7).

Unicellular calavate trichomes, short and narrow from the base and thicker at the apex were found in *A. tournefortiana*. Unicellular filiform trichomes with sharper apex were observed in *A. austriaca*. Similarly, the unicellular tector non-glandular trichomes in clusters form were the characteristic feature of *A. herba alba*. The majority of non-glandular trichomes observed in this study were not previously been reported in *Artemisia* species except for the aduncate and unicellular tector non-glandular trichomes. However, we found aduncate curly trichomes in *A. indica* and *A. dubia* whereas other studies showed these types of

trichomes in *A. roxburgiana* (Hayat *et al.*, 2009b). Moreover, this study found unicellular tector trichomes in *A. herba alba* whereas, other studies disclosed these types of trichomes in *A. dubia* (Hayat *et al.*, 2009a). It clearly indicates that the aduncate curly and unicellular tector trichomes are also common in the foliar surface of different *Artemisia* species.

Conclusion

This study authenticates foliar epidermal anatomical characteristics as valuable traits in resolving taxonomic issues and may essentially enlighten taxonomist's perceptions in the delimitation of *Artemisia* species. Nevertheless, integrating this type of data with molecular phylogenetic approaches and investigations of foliar micromorphological attributes in other closely associated *Artemisia* species would be more convenient to resolve taxonomic issues within the genus *Artemisia*.

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