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MICROMORPHOLOGICAL CHARACTERIZATION OF SELECTED SCROPHULARIACEAE SPECIES FROM PAKISTAN: A COMPARATIVE FOLIAR EPIDERMAL STUDY

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Abstract
Foliar epidermal anatomical features provide reliable taxonomic markers that are largely unaffected by short-term environmental fluctuations. In the present study, twelve species of Scrophulariaceae from Pakistan were examined using light microscopy and a nail-polish impression technique was used to evaluate the systematic value of epidermal characters. Qualitative observations revealed that all species possess anomocytic stomata; diacytic stomata were not encountered. Trichome presence and density varied markedly: Mazus pumilus and all three Scrophularia species (Scrophularia sp., S. calycina, S. scopolii) completely lacked trichomes, whereas Torenia sp. and Veronica anagallis exhibited the highest trichome densities. Quantitative analysis showed that Penstemon hartwegii had the largest adaxial epidermal cell area, while Veronica anagallis had the smallest. Stomatal density was generally higher on the abaxial surface. ANOVA revealed highly significant differences (P < 0.05) among species for all measured traits, and principal component analysis (PCA) separated the taxa based on epidermal cell dimensions, stomatal area, and trichome characters. The study demonstrates that foliar epidermal micromorphology provides significant diagnostic features for identifying species in Scrophulariaceae, Epidermal features such as stomata type, numbers of stomata and number and types of trichomes, stomatal cell area, epidermis cell thickness, epidermal cell area were measured from systematic point of view. The collected data then was statistically analyzed through multivariate cluster analysis.

Introduction

The family Scrophulariaceae, commonly known as the snapdragon family, comprises approximately 220 genera and 3,000 species distributed across three subfamilies: Antirrhinoideae, Digitalidoideae, and Gratioloideae (Fischer, 2004). While members of this family are found in both the New and Old Worlds, their primary distribution is centered in the Holarctic region (Lersten & Curtis, 2007). In Pakistan, the family exhibits remarkable diversity, with the greatest concentration of species occurring in the Khyber Pakhtunkhwa province of northern Pakistan (Ali & Qaiser, 2009). Among the most prominent genera within this family, *Scrophularia* and *Verbascum* stand out as the most abundant, collectively comprising over 630 species and serving as primary subjects for phylogenetic analyses (Valtueña et al., 2017; Scheunert & Heubl, 2016; Ghahremaninejad et al., 2015; Attar et al., 2011). Notably, *Scrophularia* species demonstrate a preference for seaside to high mountain environments, rarely inhabiting hot desert regions (Scheunert et al., 2014).

The phylogenetic placement of Scrophulariaceae within the Lamiid clade has been well-established, with the family comprising numerous tribes including Hemimerideae, Limoselleae, Leucophylleae, Teedieae, Buddlejeae, Aptosimeae, Myoporeae, and Scrophularieae (Oxelman et al., 2005; Olmstead et al., 2001). Despite well-documented intertribal relationships, molecular phylogenetic studies indicate persistent inconsistencies in intratribal relationships, underscoring the need for continued taxonomic investigation (Tank et al., 2006). The genus *Buddleja* (Buddleia), a relatively recent addition to Scrophulariaceae comprising over 100 species distributed across Asia, Africa, and the Americas, exhibits significant molecular affinities with the family (Thompson & Abbott, 2013). The traditional medicinal applications of *Buddleja* species as laxatives and antiseptics in various parts of the world further highlight the family's pharmacological significance (Cortés et al., 2006).

The medicinal importance of Scrophulariaceae cannot be overstated. Species within this family demonstrate a diverse array of biological and pharmacological activities, including antioxidant, anti-inflammatory, anticancer, antipyretic, analgesic, and immunosuppressive properties (Zhang et al., 2010). Additionally, hepatoprotective, antidiabetic, antibacterial, antiviral, and anticancer activities have been documented (Lee et al., 2010). *Verbascum thapsus* (Linn.), commonly found throughout Asia and Europe in swamps, wooded clearings, and along roadsides on rough stony soils, exhibits remarkable pharmacological properties. Its leaves, roots, and flowers possess antioxidant, analgesic, antimicrobial, anticancer, antiviral, fungicidal, sedative, and narcotic properties, with dried leaves occasionally inhaled to relieve respiratory irritation (Ali et al., 2012; Chitnis et al., 2012).

Digitalis purpurea L., another medicinally significant species, yields cardiac glycosides such as digitoxin used in treating cardiac insufficiency, with its leaves characterized by sinuous epidermal cells and anomocytic stomata (Alipieva et al., 2014; Sotoodeh et al., 2015). *Bacopa monnieri* (L.) Wettst., an important medicinal herb traditionally used as a memory booster and locally known as brahmi or water hyssop, exhibits both anomocytic and diacytic stomata (Gohil & Patel, 2010). The ornamental significance of Scrophulariaceae is equally noteworthy, with genera such as *Antirrhinum*, *Veronica*, *Linaria*, *Mimulus*, *Cymbalaria*, *Russelia*, *Torenia*, and *Digitalis* being widely cultivated for their aesthetic appeal (Hamayun et al., 2006). These ornamental species also contribute to homeopathy and medicine, with *Digitalis* being particularly valued for cardiac treatment, *Verbascum thapsus* for respiratory ailments, *Torenia asiatica* for gonorrhea, *Lindenbergia indica* for bronchitis, *Anticharis* for diabetes, and *Cymbalaria muralis ramosissima* and *Linaria dalmatica* for liver function enhancement (Youssef et al., 2018).

Foliar epidermal anatomical characteristics have proven invaluable in pharmacognostic studies of Scrophulariaceae, providing essential diagnostic traits for species identification and quality control of medicinal materials (Sangeetha & Dharasurkar, 2015; Santhan, 2014). Leaf epidermal traits, being largely equivalent from one group to another and relatively unaffected by environmental fluctuations, serve as reliable taxonomic markers for identifying plant species and genera (Scatena et al., 2005). Among morphological features, leaf anatomical attributes, along with leaf venation, petal length, corolla tube shape, and life form, represent distinguishing characteristics for *Scrophularia* species (Scheunert et al., 2014). Intercellular inclusions such as cellular cavities and idioblasts function as significant systematic and taxonomic microcharacters, while leaf epidermal tissues including guard cells, trichomes, and subsidiary cells provide essential taxonomic information (Makbul et al., 2006; Munir et al., 2011).

Recent investigations have further demonstrated the value of foliar epidermal anatomy in species-level taxonomy. Aljowaie et al. (2024) utilized scanning electron microscopy to examine nine species across four Lamiales families, including Scrophulariaceae, documenting extensive trichome diversity and epidermal cell variability that proved significant for taxonomic delimitation. Similarly, Zaman et al. (2022) demonstrated that micromorphological features of Lamiaceae species provide taxonomically significant identification information, with trichome diversity differing across adaxial and abaxial surfaces and anomocytic stomata being most prevalent. Trichomes, specialized epidermal structures on plant leaf surfaces, play crucial roles in plant development and growth by serving as physical barriers against biotic and abiotic stresses (Jiwei et al., 2018). These structures exhibit significant variation in form, function, and density across species and populations of Scrophulariaceae (Dalin et al., 2008).

Glandular trichomes, found on approximately one-third of all vascular plants, frequently coexist with non-glandular trichomes and excrete secondary compounds with defensive and physiological functions that enhance plant survival (Huchelmann et al., 2017; Holeski et al., 2010; Liu et al., 2019; Tissier et al., 2017). Additionally, trichomes shield tissues from extreme temperatures, regulate transpiration, enhance freeze-tolerance, and protect against UV radiation damage (Mershon et al., 2015; Huttunen et al., 2010). Quantitative foliar epidermal features, including stomatal size and density, epidermal cell dimensions, and trichome characteristics, exhibit significant variation across adaxial and abaxial leaf surfaces (Bayat & Attar, 2016). Parameters such as stomatal cell area, cuticular ornamentation, epidermal cell thickness, wax coating, and trichome characteristics are instrumental in regulating gas exchange and determining species-specific adaptive capacities (Blanusa et al., 2015; Grodzinskiy, 2013; Shvets, 2013).

Leaf micro-roughness, which determines particle deposition and resistance to removal by rain or wind, further contributes to environmental adaptation (Mori et al., 2015). Morphological analysis encompassing size, surface characteristics, shape, appearance, color, taste, fracture, odor, and fracture surface, combined with eco-physiological features, reflects species-specific activities and environmental adaptation (Evans, 2009; Wallis, 2009; Grote et al., 2016). Previous studies have documented the taxonomic significance of foliar epidermal anatomy across diverse plant families. Kaplan and Inceoglu (2003) investigated the morphology and leaf anatomy of 14 Turkish Rhinantheae species from Scrophulariaceae, revealing diverse anatomical and physiological traits. Makbul et al. (2006) emphasized the importance of anatomical features over morphological characters in differentiating *Scrophularia* subspecies. Studies on trichome diversity by Khan et al. (2013) identified ten distinct types of glandular and non-glandular trichomes across 51 Scrophulariaceae species. Investigations on stomatal anomalies by Lahari and Hanumantha (2019) documented five major stomatal types across 60 Scrophulariaceae species from 39 genera.

Recent anatomical studies on endemic species have contributed significantly to the systematic understanding of the family. The anatomical investigation of *Scrophularia lepidota*, a narrowly distributed endemic Turkish species, revealed amphistomatic leaves with equifacial mesophyll and idioblastic cells containing druse crystals (Anatomical Investigations on Turkish Vulnerable Scrophularia Species, 2025). Özerkan et al. (2024) documented anomocytic stomata, stellate and glandular trichomes, and idioblast cells in *Verbascum stenostachyum*, reinforcing the taxonomic importance of foliar epidermal features in the genus *Verbascum*. Kılıç and Kılıç (2025) examined the comparative anatomy of seven *Verbascum* taxa from southeastern Anatolia, demonstrating that the presence of idioblasts in leaves serves as a valuable taxonomic character.

Despite significant taxonomic research conducted on Scrophulariaceae over the past decade, comprehensive foliar epidermal studies integrating morphological and anatomical characteristics remain incomplete, particularly for Pakistani species. Recent investigations by Ullah et al. (2021) examined foliar epidermal appendages of selected Scrophulariaceae species from northern Pakistan, documenting the presence of glandular trichomes and diacytic stomata in *Anticharis glandulosa*, while other species exhibited dendroid trichomes and anomocytic stomata on both surfaces. The family's extensive diversity and medicinal importance necessitate detailed investigation of foliar epidermal features to establish reliable taxonomic markers and pharmacognostic standards. Aljowaie et al. (2024) emphasized that the exploration of Lamiales epidermal micromorphology and their antimicrobial potential has significant implications for multidisciplinary fields including pharmacology and sustainable agriculture. Furthermore, the diversity of trichome morphometry and epidermal microscopic architecture across species underscores the value of foliar epidermal studies in understanding interspecies variability (Aljowaie et al., 2024; Zaman et al., 2022). The current investigation addresses this research gap by conducting a comprehensive foliar epidermal anatomical study of Scrophulariaceae species from Pakistan.

Objectives

1. To study the foliar epidermal characters of family Scrophulariaceae.
2. To study the variation in shapes of stomata and stomatal density present among species.
3. To study the variation in epidermal appendages such as trichome type, stomata type, number and length.
4. To study the inter and intraspecific similarities and dissimilarities among species of family Scrophulariaceae.

Materials and Methods

Twelve species of the family Scrophulariaceae were investigated (Table 1). Herbarium specimens were obtained from the Herbarium of the Department of Botany, University of Agriculture, Faisalabad (UAF), Pakistan. Healthy, fully expanded leaves were selected from each specimen. For anatomical examination, foliar epidermal impressions were prepared using the transparent nail-polish technique. A thin layer of transparent nail polish was applied separately to the adaxial and abaxial surfaces of each leaf and allowed to dry completely at room temperature. A piece of transparent adhesive tape was pressed over the dried film and gently peeled off to obtain the epidermal impression. The tape was then mounted on a clean glass microscope slide. Two slides—one for the adaxial and one for the abaxial surface—were prepared for each leaf. Three leaves per species were sampled.

5. The prepared slides were examined under a compound light microscope, and digital photomicrographs were captured for documentation. Qualitative characters recorded included epidermal cell shape, anticlinal wall configuration, stomatal type and distribution, trichome type and morphology, and the presence of cuticular ornamentation or epicuticular wax. Standard anatomical terminology was used (Makbul et al., 2006; Munir et al., 2011; Bayat & Attar, 2016; Ullah et al., 2021).
6. Quantitative measurements were taken from five non-overlapping microscopic fields per leaf surface. The parameters measured were: epidermal cell area (µm²), stomatal density (number per mm²), stomatal area (µm²), trichome density (number per mm²), trichome cell area (µm²), number of stomata per field (adaxial and abaxial), number of trichomes per field (adaxial and abaxial), and stomatal index. All measurements were averaged per leaf and then per species.
7. Data were analyzed using one-way analysis of variance (ANOVA) to test for significant differences among species, with significance set at $P < 0.05$. Principal component analysis (PCA) was performed on the correlation matrix of the measured traits to explore patterns of similarity among the taxa and to identify the characters most responsible for species discrimination.

Table 1. List of Scrophulariaceae species examined, with herbarium voucher information.

Vernacular name	Scientific name	Genus
Bird's eye, speedwell	*Veronica lasca*	Plantaginaceae
Himalayan Wulfenia	*Wulfenia amherstiana*	Plantaginaceae
Monkey flower, monkey musk	*Mimulus luteus*	Scrophulariaceae
Scarlet beard tongue	*Penstemon hartwegii*	Penstemon
Figwort	*Scrophularia* sp.	Scrophularia
Sepal Figwort	*Scrophularia calycina*	Scrophularia
Garden speedwell, long leaf speedwell	*Veronica longifolia*	Plantaginaceae
Bluewings, wishbone flower	*Torenia* sp.	Linderniaceae
Gidar Tambaku	*Celsia* sp.	Scrophulariaceae
Japanese Mazus	*Mazus pumilus*	Mazus (Scrophulariaceae)
Italian Figwort	*Scrophularia scopolii*	Scrophularia
Water speedwell	*Veronica anagallis*	Plantaginaceae

RESULT

Anatomical characteristics

The data concerning anatomical features showed significant modifications in various cells and tissues of all foliar epidermis of family Scrophulariaceae. Environmental variations caused changes in the plant anatomy. Following parameters were observed and recorded:

- Abaxial stomatal density
- Abaxial stomatal area
- Adaxial stomatal density
- Adaxial stomatal area
- Abaxial number of trichomes
- Abaxial size of trichomes
- Number of Adaxial trichomes
- Size of Adaxial of trichomes
- Abaxial Number of epidermal cells
- Abaxial epidermal cell size
- Adaxial epidermal cell area
- Abaxial epidermal cell area
- Adaxial number of epidermal cells
- Adaxial epidermal cells size
- Abaxial stomatal index
- Adaxial stomatal index

Adaxial epidermal cell area

The area of epidermal cells of all species was measured. The optimum epidermal cell area was measured in *Penstemon Hartwegii.* While minimum cell area was recorded in *Veronica Anagalis*. The adaxial cell area was also observed as medium size in the remaining species.

Abaxial epidermal cell area

Measurements were recorded for different plant specimens collected from UAF. Data indicated that *Veronica Anagalis* had larger cell area. In *Torenia*, the smallest abaxial cell area was measured. The abaxial cell area of the rest of the species was moderate.

Adaxial trichome cell area

Values for trichome cell area were noted. The maximum adaxial trichome cell area was observed in *Veronical Anagalis* obtained from UAF. While minimum trichome cell area was recorded in *Wulfenia Amherstiana.* Similarly, the other had medium adaxial trichome cell area.

Abaxial trichome cell area

Highest values of abaxial trichome cell area was observed in Torenia. While minimum abaxial trichome cell area was recorded in *Scrophularia Calycina.* Whereas other species showed moderate abaxial trichome cell area.

Stomatal cell area at adaxial

The maximum adaxial stomatal cell area was recorded in *Penstemon Hartwegii* and *Veronica Anagalis*. While minimum stomatal cell area was noted in *Veronica longifolia* collected from department of botany,UAF.

Stomatal cell area at abaxial

The results showed that maximum abaxial stomatal area was recorded in *Mimulus leutus* and *Scrophularia*. Meanwhile, minimum abaxial stomatal cell area was recorded in Torenia and *Wulfenia Amherstiana.* While rest of the species exhibited moderate stomatal cell area.

Number of trichomes at adaxial

Maximum number of adaxial trichomes were present in *Torenia*. While moderate number of trichomes were present in *Scrophularia scopoli*, *Mazus*, *Celsia* and *Veronica anagalis* respectively. While minimum number of trichomes were present in *Scrophularia calycina*, *Scrophularia* and *Penstemon Hartwegii*.

Abaxial number of trichomes

Highest number of trichomes at abaxial side were present in *Veronica anagalis*. In *Mazus* and *Scrophularia* trichomes were absent. While minimum number of trichomes were present in *Mimulus leutus* and *Penstemon Hartwegii*. Likewise, rest of the species had moderate number of trichomes.

Adaxial number of stomata

In adaxial leaf surface of *Celsia* maximum stomata were recorded. While minimum number of adaxial stomata were recorded in *Veronica lasca* and *Veronica anagalis*. Rest of the species exhibit non-significant variations.

Abaxial number of stomata

Maximum number of stomata were recorded at abaxial side of *Veronica lasca* and *Veronica anagalis*. While minimum number of abaxial stomatal density was recorded in *Veronica longifolia*. Meanwhile, rest of species showed significant variations.

Thickness of adaxial epidermal cell

Adaxial epidermal cell thickness was recorded in different plant species for foliar epidermal anatomy of family Scrophulariaceae. *Mazus* has the highest adaxial epidermal cell thickness. While, minimum adaxial epidermal cell thickness was measured in *Veronica anagalis*. Meanwhile, rest of the species exhibited non- significant variations in thickness of epidermal cell.

Thickness of abaxial epidermal cell

Maximum abaxial epidermal cell thickness was recorded in *Veronica anagalis*. While minimum abaxial epidermis cell thickness was reported in *Torenia*. Likewise, remaining species showed non-significant abbreviation in abaxial epidermal cell thickness.

Analysis of Variance of epidermal cell area of adaxial side

Note, Significant at $P<0.05$ level

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	3497099	249793	9.85	0.000***
Error	21	532664	25365		
Total	35	4029763			

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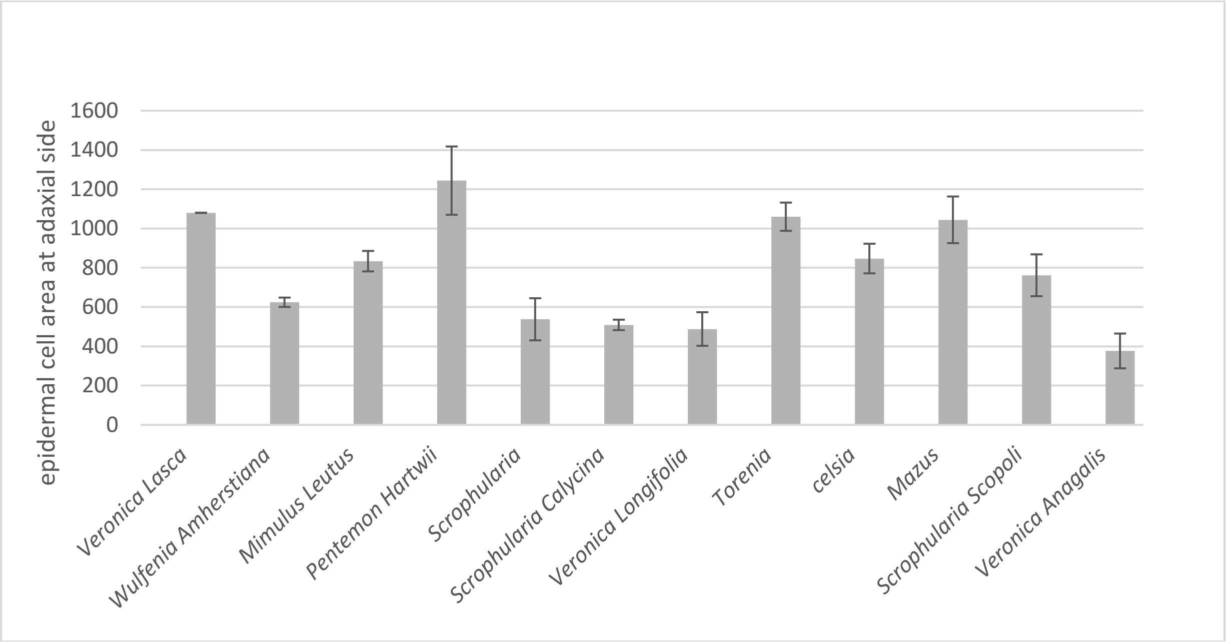
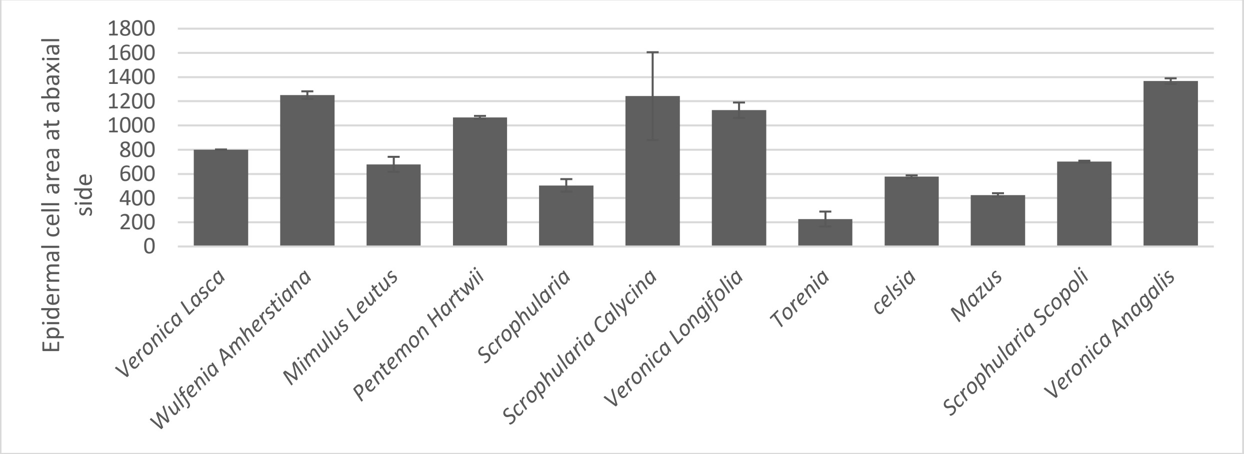


Graphical representation of adaxial epidermis cell area of different plant specimens from UAF

Analysis of Variance of epidermal cell area of Abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	5628370	402026	74.49	0.000***
Error	21	113333	5397		
Total	35	5741703			

Significant at P<0.05 level



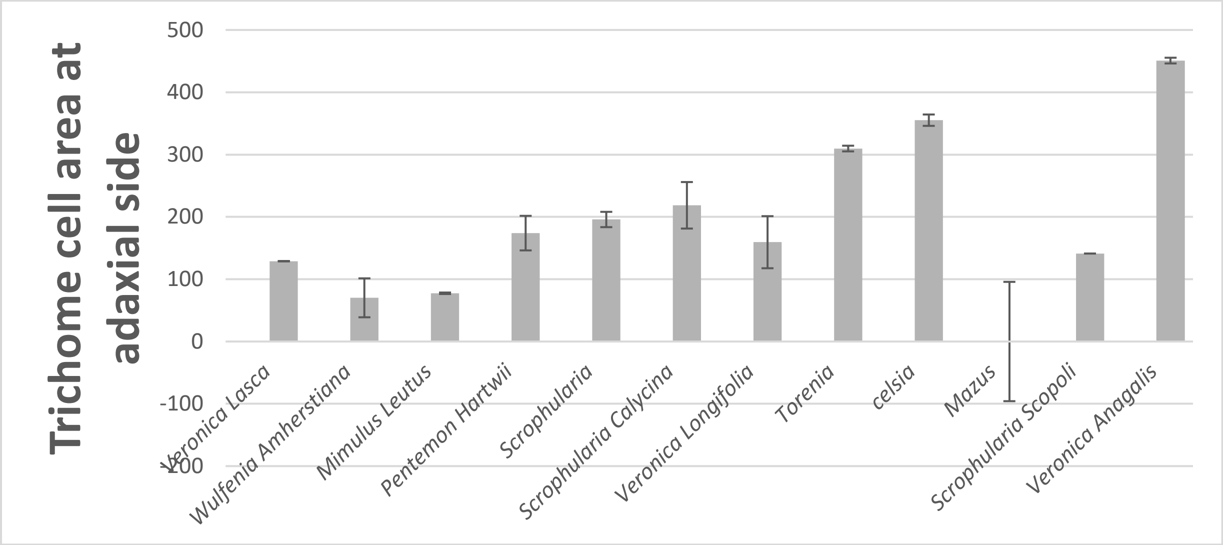
Graphical representation of abaxial epidermis cell area of different plant specimens from UAF

Analysis of Variance of trichome cell area of adaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	550054	39290	10.20	0.000***
Error	21	80862	3851		

Total	35	630916

Note; Significant at P<0.05 level

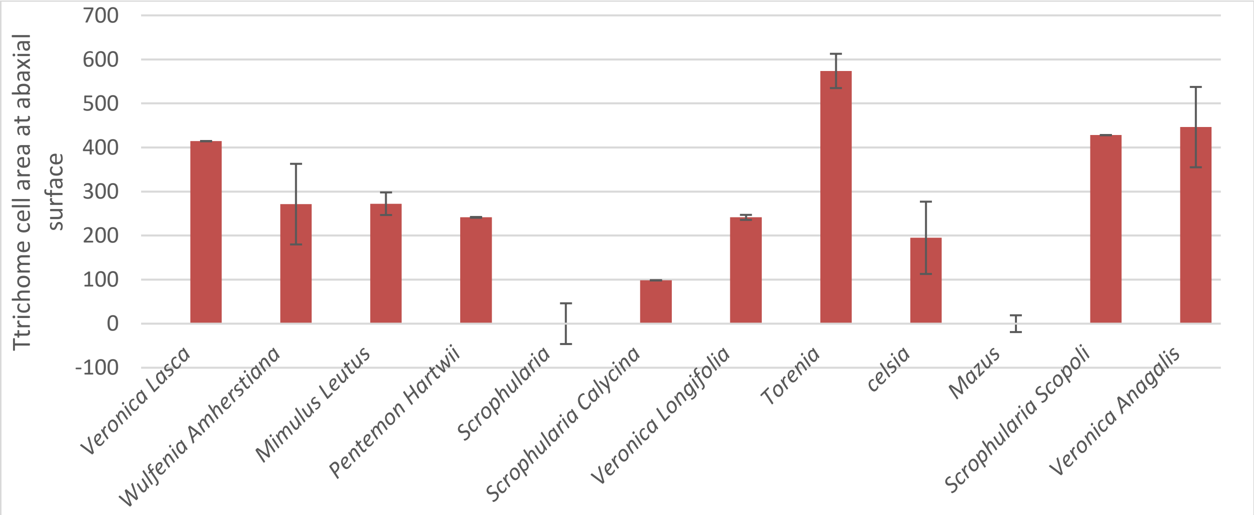


Graphical representation of adaxial trichome cell area of different plant specimens from UAF

Analysis of Variance of trichome cell area at abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	1062532	75895	7.74	0.000***
Error	21	205802	9800		
Total	35	1268334			

Note; Significant at P<0.05 level



Graphical representation of abaxial trichome cell area of different plant specimens from UAF

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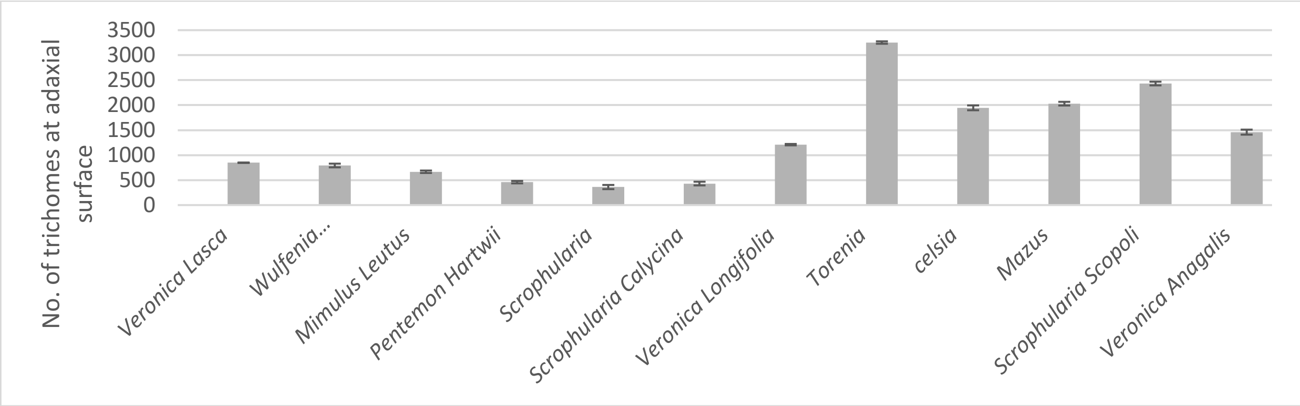
Analysis of Variance of number of trichomes at adaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	27849048	1989218	507.53	0.000
Error	21	82307	3919		
Total	35	27931355			

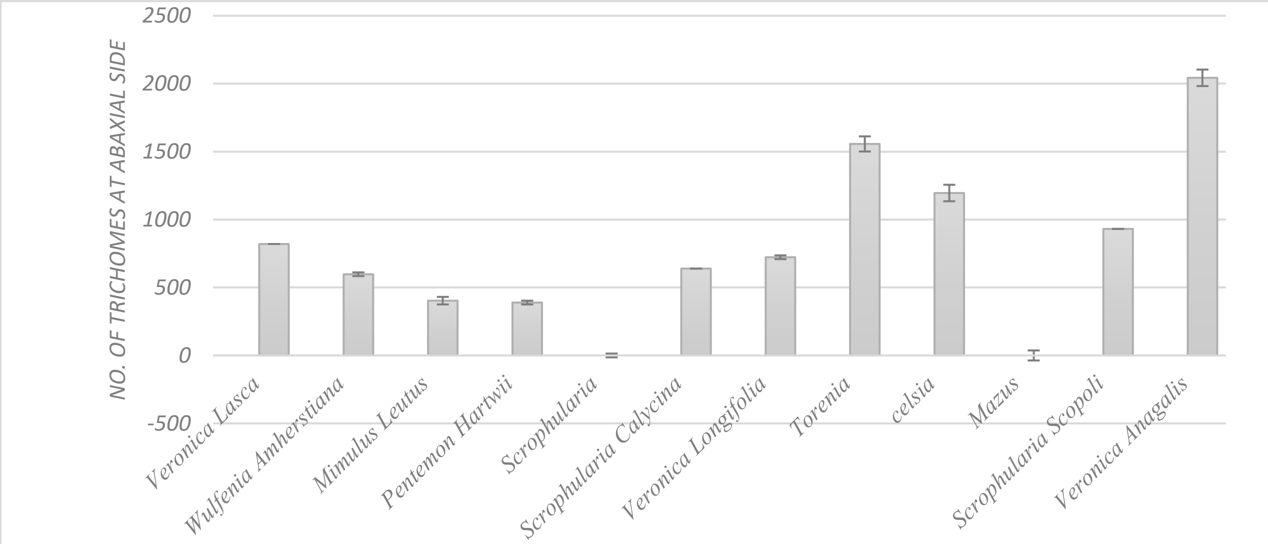


Graphical representation of number of trichomes at adaxial side of different plant specimens from UAF

Analysis of Variance number of trichomes at abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	11892124	849437	214.46	0.000***
Error	21	83177	3691		
Total	35	11975301			

Significant at P<0.05 level

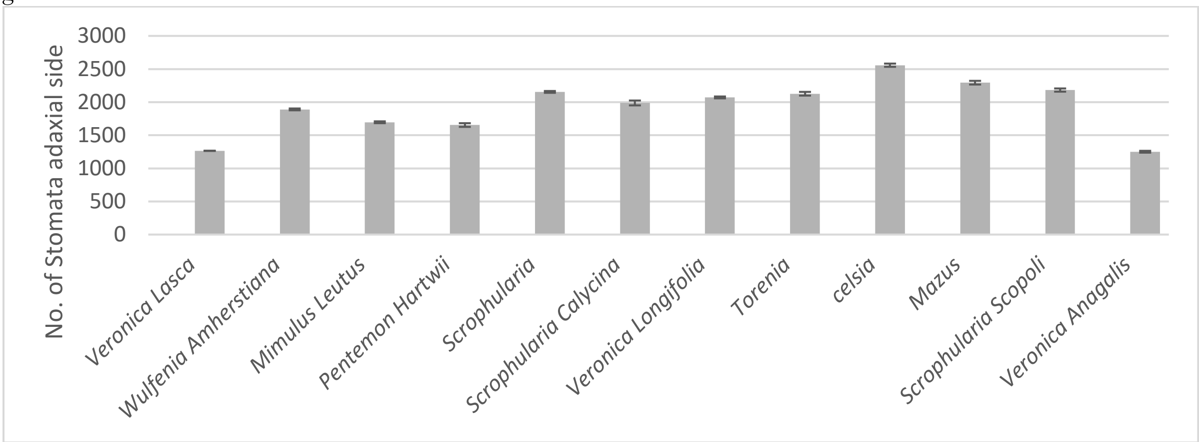


Graphical representation of number of trichomes at abaxial side of different plant specimens from UAF

Analysis of Variance number of stomata of adaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	5225461	373247	297.20	0.000***
Error	21	26373	1256		
Total	35	5251834			

Significant at P<0.05 level

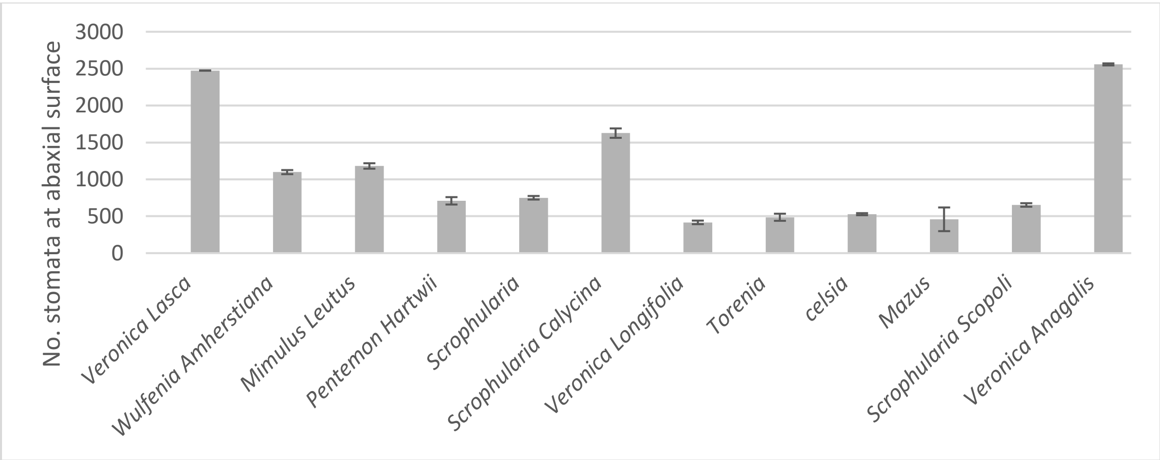


Graphical representation of number of stomata of adaxial side of different plant specimens from UAF

Analysis of Variance number of stomata at abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	19310415	1379315	37.88	0.000***
Error	21	764735	36416		
Total	35	20075151			

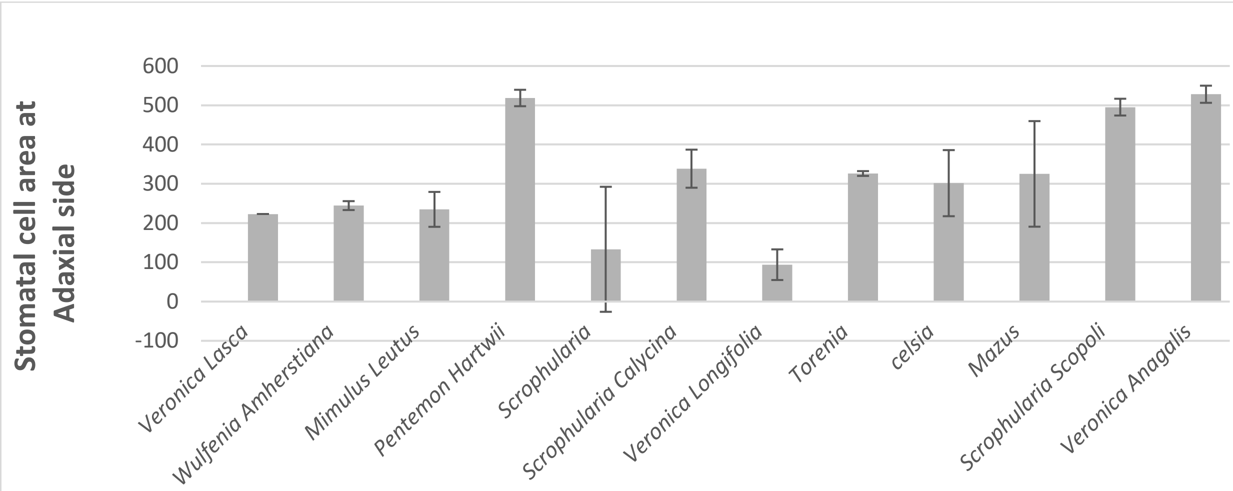
Note; Significant at P<0.05 level



Graphical representation of number of stomata of abaxial side of different plant specimens from UAF

Analysis of Variance of stomatal cell area at adaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	673755	48125	2.87	0.014
Error	21	351662	16746		
Total	35	1025417			

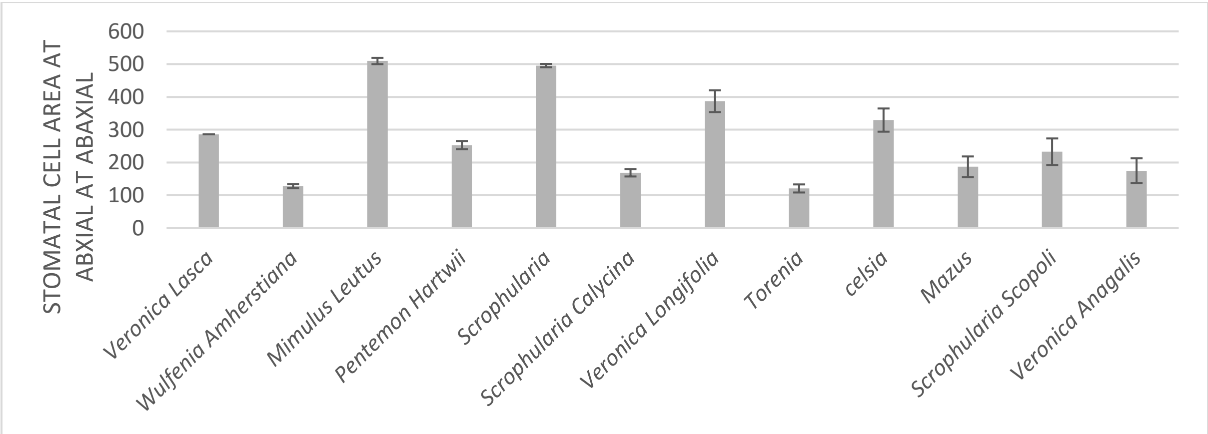


Graphical representation of stomatal cell area at adaxial side of different plant specimens from UAF

Analysis of Variance of stomatal cell area at Abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	595074	42505	20.79	0.000***
Error	21	42935	2045		
Total	35	638009			

Significant at P<0.05 level



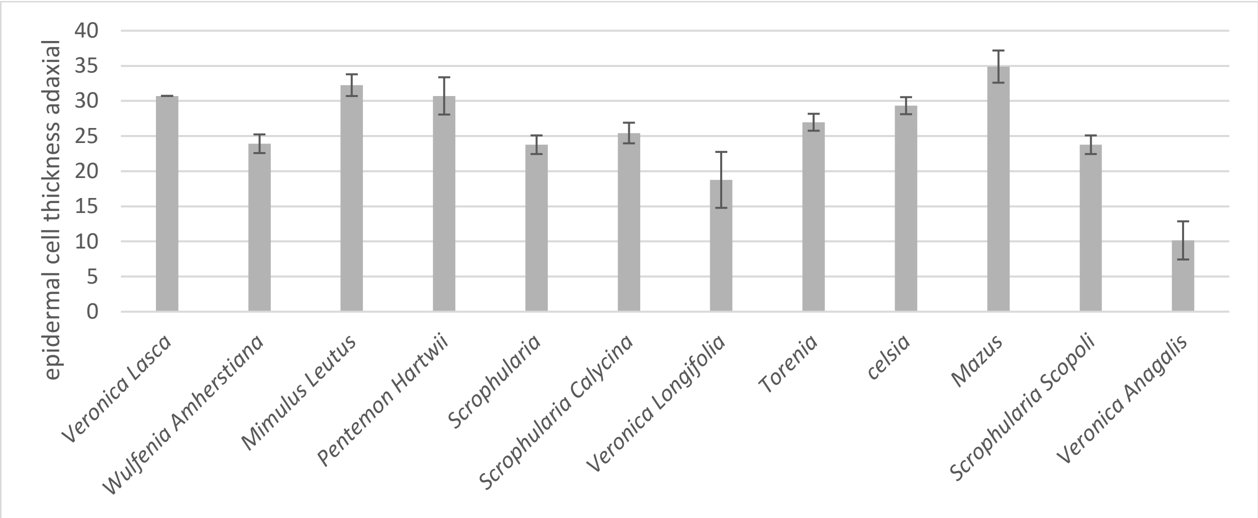
Graphical representation of stomatal cell area at abaxial side of different plant specimens from UAF

Analysis of Variance of epidermal cell thickness at adaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Species	14	1503.3	107.38	8.07	0.000***
Error	21	279.5	13.31		
Total	35	1782.7			

Significant at P<0.05 level

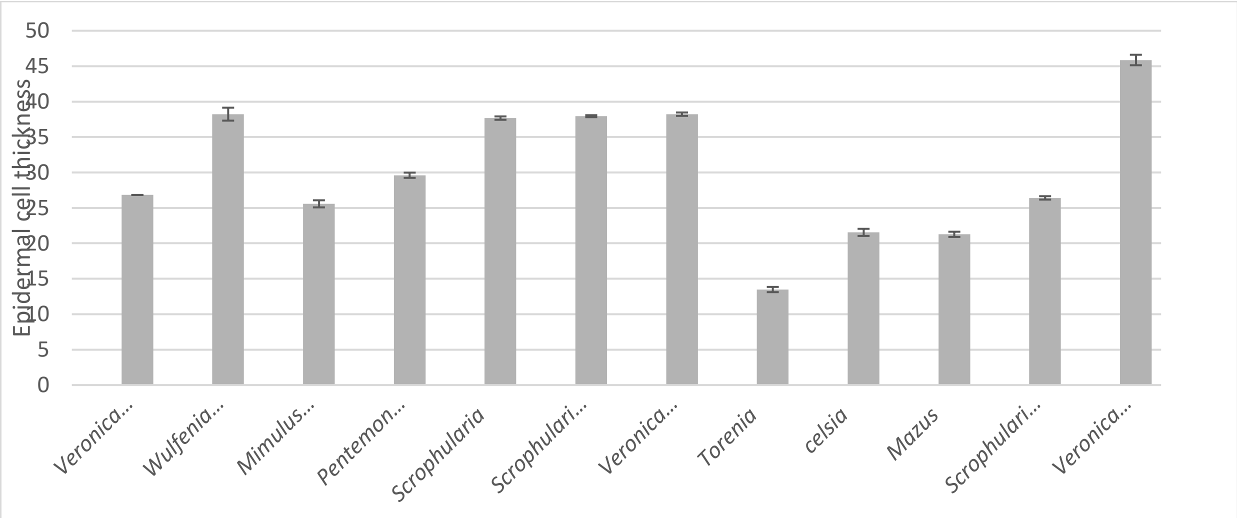


Graphical representation of epidermal cell thickness at adaxial side of different plant specimens from UAF

Analysis of Variance of epidermal cell thickness at abaxial side

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Species	14	2941.59	210.113	188.66	0.000***
Error	21	23.39	1.114		
Total	35	2964.98			

Significant at P<0.05 level



Graphical representation of epidermal cell thickness at abaxial side of different plant specimens from UAF

Multivariate analysis

Principle component analysis (PCA) was constructed among the studied parameters and selected species of family Scrophulariaceae to determine the correlation. AdNS showed strong relation with *Celsia*, wherease, EdCTA showed correlation with *Mazus*. AbECA showed close relationship with *Scrophularia scopoli*, wherease, AbNT showed close relation with *Torenia*. AbSCA showed strong relation with *Scrophularia* and *Mimulus Leutus*, whereas AdECT showed correlation with *Penstemon Hartwegii* and *Veronica lasca*. AbTCA showed strong relation with *Veronica anagalis* while AbNS showed correlation with *Scrophularia calycina*. AdECA showed strong correlation with *Wulfenia Amherstiana*, whereas AbECT showed relation with *Veronica longifolia*.

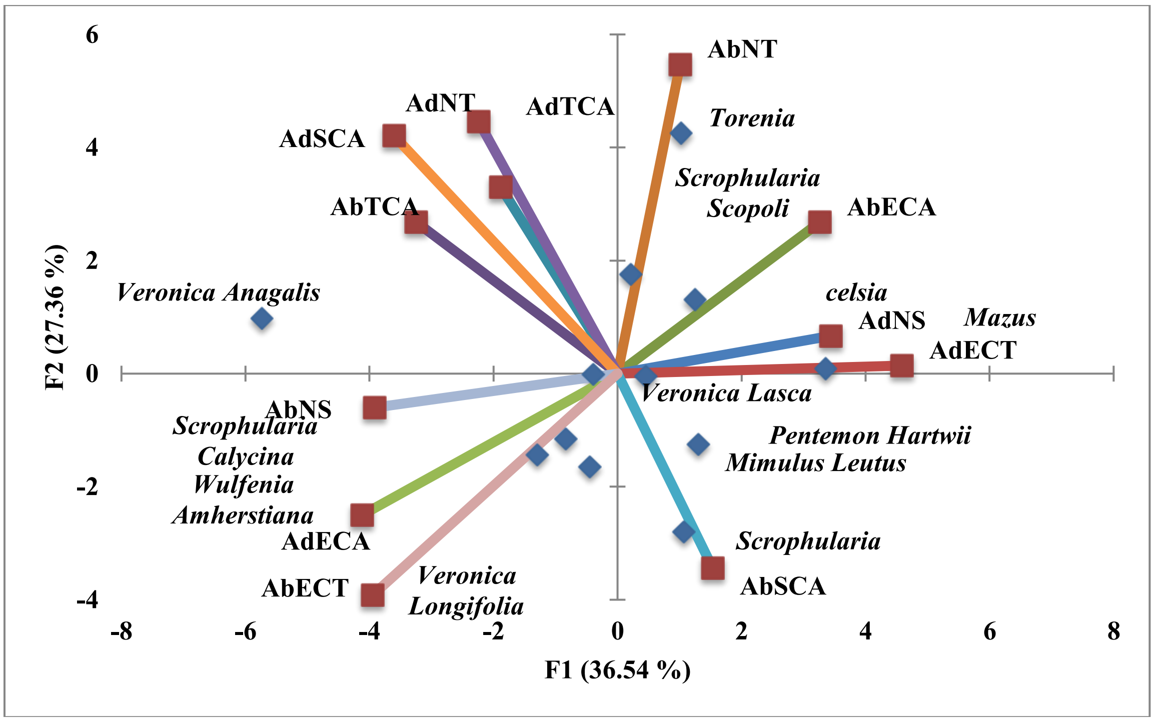


Fig. 4.30. Principle component analysis (PCA) for correlation among studied traits and selected species of family Scrophulariaceae Figure legends: AbSN-abgdhffj, abssh-bdndnd,

Discussion

The family Scrophulariaceae comprises species adapted to a wide range of ecological conditions, and leaf anatomical traits are central to understanding their adaptive capacity and taxonomic delimitation. Investigations of morphological and anatomical structure consistently show that leaf characteristics are among the most informative organs for determining a species' ecological strategy and systematic position (Grodzinskiy, 2013; Shvets, 2013). Species-specific functions such as leaf micromorphology, foliar distribution, photosynthetic efficiency, and air-flow dynamics are influenced by epidermal architecture (Grote et al., 2016). In the present study, the foliar epidermal characters of twelve Scrophulariaceae species from Pakistan were examined, revealing considerable variation in both qualitative and quantitative traits that can be linked to taxonomic identity and environmental adaptation.

Environmental pressures drive the development of specialized anatomical features. A multilayered epidermis, increased sclerification, and particular stomatal arrangements on both leaf surfaces significantly enhance plant tolerance to harsh conditions, including drought and high irradiance (Naz et al., 2009; Hameed et al., 2009). Xeromorphic adaptations such as elevated trichome density and reduced stomatal numbers enable plants to survive under limited water availability (Ali et al., 2009). Our observations support these trends: species exhibiting higher trichome densities tended to display lower stomatal densities on the adaxial surface, a combination that likely reduces transpiration while maintaining protective functions. Conversely, mesophytic taxa such as *Veronica anagallis* showed relatively thin cuticles and fewer trichomes, consistent with their preference for moist habitats.

Leaf micromorphological parameters, including stomatal density, cuticular ornamentation, epidermal cell number, and trichome abundance, are also reliable indicators of a plant's particle-capturing ability and overall physiological performance (Blanusa et al., 2015). In this context, the variation we recorded in epidermal cell area and stomatal dimensions among the studied species suggests differential capacities for gas exchange and pollutant uptake. For instance, the large epidermal cells observed in *Penstemon hartwegii* may be associated with a higher proportion of pavement cells relative to stomata, while the smaller cells in *Veronica anagallis* could accommodate a greater stomatal density per unit area. Recent work on the order Lamiales, which includes Scrophulariaceae, has confirmed that epidermal micromorphological traits remain highly stable within species while varying sufficiently among taxa to serve as reliable systematic markers and may also contribute to antimicrobial resilience through structural features (Aljowaie et al., 2024). Moreover, foliar micromorphology studies in related families such as Lamiaceae have demonstrated the taxonomic utility of these traits when examined with scanning electron microscopy, reinforcing the broader applicability of this approach (Zaman et al., 2022).

Scrophularia species are rarely found in hot deserts; instead, they thrive in coastal to alpine habitats, and leaf morphological characters—including venation pattern, petal length, corolla tube shape, and life form—are traditionally used as diagnostic features (Scheunert et al., 2014). Within the leaf epidermis, the arrangement of guard cells, subsidiary cells, and various trichome types provides taxonomically valuable information in the Scrophulariaceae (Munir et al., 2011). Our qualitative observations confirmed that all studied species possess anomocytic stomata, except *Anticharis glandulosa* (as documented by Bayat & Attar, 2016), which displays diacytic stomata. This consistency in stomatal type across the majority of the studied taxa aligns with earlier reports and supports the use of stomatal architecture as a stable systematic character (Perveen et al., 2007; Ullah et al., 2021). Where diacytic stomata occur, as in *A. glandulosa*, they represent a derived state that helps separate that taxon from the rest of the examined species. Additionally, recent anatomical investigations on Turkish *Scrophularia* species, such as *Scrophularia lepidota*, have confirmed the diagnostic value of foliar epidermal characters within the genus, noting that leaf anatomical traits are often more informative than gross morphology alone ("Anatomical investigations on Turkish vulnerable Scrophularia species," 2025).

Trichomes are among the most polymorphic epidermal structures and serve multiple functions ranging from physical defense to secondary metabolite secretion (Huchelmann et al., 2017; Tissier et al., 2017). Glandular trichomes are particularly important because they produce and accumulate a wide array of primary and secondary metabolites that contribute to plant defense against herbivores, pathogens, UV-B radiation, and excessive heat. Our study found that trichome presence, type, and density varied markedly among the twelve species. Notably, trichomes were entirely absent in *Mazus pumilus* and in all three *Scrophularia* species examined (*Scrophularia* sp., *S. calycina*, *S. scopolii*). In contrast, *Mimulus luteus* and *Penstemon hartwegii* possessed the lowest trichome densities, while other taxa exhibited moderate to abundant indumentum. The absence of trichomes in *Scrophularia* is particularly interesting from a taxonomic standpoint, as previous investigations have often emphasized the diagnostic value of trichome types within this genus (Makbul et al., 2006). The lack of trichomes in these samples may reflect either a species-specific trait or environmental canalization, and further examination of fresh material from natural populations is warranted. Comparative anatomical studies on *Verbascum* taxa, a closely related genus, have also highlighted the importance of trichome micromorphology and epidermal cell characteristics in distinguishing species, with dense multicellular hairs and cuticular ornamentation being particularly valuable characters (Kılıç & Kılıç, 2025; Özerkan et al., 2024).

Quantitative variation was observed in epidermal cell area, stomatal dimensions, and trichome metrics on both leaf surfaces. Among the studied taxa, *Penstemon hartwegii* exhibited the largest adaxial epidermal cell area, whereas *Veronica anagallis* had the smallest. Such differences in cell size may influence the overall mechanical strength of the leaf and its susceptibility to water loss. Furthermore, *Veronica anagallis* displayed the greatest abaxial epidermal thickness, while *Torenia* sp. showed the lowest. The remaining species presented intermediate values with no statistically significant differences among them. These patterns suggest that even within a single family, leaf epidermal traits can be highly plastic, reflecting a combination of phylogenetic constraints and microhabitat specialization.

Stomatal function is critical for regulating the balance between carbon assimilation and water loss. The maximum stomatal conductance of a leaf determines its potential for carbon gain and transpiration, and stomatal aperture is modulated by both passive and active responses of the guard cells to environmental cues. The structure and density of stomata, together with the characteristics of the surrounding subsidiary cells, play a decisive role in preventing desiccation while optimizing the trade-off between CO_2 influx and water vapour efflux. In the present study, stomatal density was generally higher on the abaxial surface (hypostomatic to amphistomatic distribution), a common pattern in mesic-adapted species that reduces direct exposure to solar radiation and lowers transpiration rates.

The taxonomic value of anatomical features in *Scrophularia* has been well established, with characters such as the distribution of idioblasts, stomatal type, and trichome complement serving as systematic microcharacters (Makbul et al., 2006). Our results corroborate the notion that leaf epidermal anatomy, when quantified and combined with qualitative traits, can successfully distinguish species and even identify groups that are morphologically similar. The observed variation in trichome presence/absence, stomatal type, and epidermal cell dimensions aligns with the findings of Ullah et al. (2021) for northern Pakistani Scrophulariaceae and with the broader Lamiales epidermal structural analysis conducted by Aljowaie et al. (2024), further validating the use of foliar epidermal characters in the taxonomy of the family. The recent description of anatomical features in *Verbascum stenostachyum* and other Turkish *Verbascum* taxa also reinforces the value of combining micromorphological, anatomical, and palynological data for species-level identification (Özerkan et al., 2024; Kılıç & Kılıç, 2025).

In summary, the present anatomical investigation demonstrates that foliar epidermal traits, including stomatal complex morphology, trichome diversity, and epidermal cell dimensions, vary significantly among the studied Scrophulariaceae species. These traits not only reflect the ecological adaptations of each species but also provide reliable characters for species delimitation and identification. The absence of trichomes in *Mazus* and the *Scrophularia* taxa, the maximum epidermal cell area in *Penstemon hartwegii*, the smallest cell area in *Veronica anagallis*, and the highest abaxial epidermal thickness in *V. anagallis* are among the most distinctive features that can be incorporated into a diagnostic key. Future studies integrating these anatomical data with molecular phylogenetics and ecophysiological measurements will further clarify the evolutionary and functional significance of leaf epidermal architecture in the family Scrophulariaceae.

CONCLUSIONS

This study confirmed that foliar epidermal anatomical characters are taxonomically significant in Scrophulariaceae. All twelve examined species possessed anomocytic stomata, with stomatal density consistently higher on the abaxial leaf surface. Trichome presence varied dramatically: *Mazus pumilus* and all *Scrophularia* species completely lacked trichomes, while *Torenia* sp. and *Veronica anagallis* showed the greatest trichome densities. *Penstemon hartwegii* and *Veronica anagallis* displayed the extremes in epidermal cell area, and PCA successfully separated the species based on epidermal cell dimensions, stomatal area, and trichome attributes. The findings demonstrate that these micromorphological characters can reliably distinguish even closely related taxa and are valuable for both taxonomic resolution and the authentication of Scrophulariaceae species.

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