Taxonomic Implications of Leaf Anatomical Patterns in Riparian Apocynaceae Species

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Abstract

Aim of study: This study described micro magnified anatomical variable patterns to understand the complex relationship between the macroscopic and microscopic features of leaves within the riparian Apocynaceae species.

Area of study: The biomagnification process provides crucial insights into the structural adaptations and ecological roles from riparian zone of Punjab.

Material and method: Comparative foliar anatomical features of 25 Apocynaceous taxa were examined using light microscopy and scanning electron microscopy (SEM) to document epidermal characteristics and trichome diversity.

Main results: Both surfaces of the leaves exhibited differences in their leaf anatomical traits including type of stomata, epidermis, anticlinal wall, lobes and diversity of trichomes. The epidermal cells shape reported as irregular, rectangular, polygonal, undulated, isodiametric and uniseriate. The largest epidermal cells were examined in *Vinca major* (59.6 μm) and (77.6 μm) on adaxial and abaxial surfaces respectively. Largest stomatal complex was recorded for *Beaumontia grandiflora* (48.6 μm) while smallest for *Cryptolepis dubia* (13.2 μm). The maximum stomatal index (SI) was calculated (37.2%) in *Asclepias curassavica*. Multicellular non-glandular trichomes were observed on both surfaces of *Beaumontia grandiflora* while uniseriate trichomes located in inter-coastal zone in *Trachelospermum jasminoides* especially on the abaxial surface. The longest trichome length was measured for *Pergularia tomentosa* (135 μm) along adaxial side, while along the abaxial surface, maximum length was calculated for *Asclepias curassavica* (262 μm).

Research highlights: The study using LM and SEM, key taxonomically relevant features including trichome types, stomatal complex arrangements, and epidermal cell patterns were identified. These micromorphological markers proved valuable in distinguishing closely related species and contributed to the development of diagnostic taxonomic keys.

Keywords: Taxonomic Markers, Micro-histology, Epidermis, Trichome Structure, Riparian Zone

Kıyısal Apocynaceae Türlerinde Yaprak Anatomik Desenlerinin Taksonomik Sonuçları

Öz

Çalışmanın amacı: Bu çalışmada, kıyısal Apocynaceae türlerindeki yaprakların makroskobik ve mikroskobik özellikleri arasındaki karmaşık ilişkiyi anlamak için mikro büyütmeli anatomik değişken desenleri tanımlanmıştır.

Çalışma alanı: Biyobüyütme süreci, Pencap kıyı bölgesindeki yapısal adaptasyonlar ve ekolojik roller hakkında önemli bilgiler sağlamaktadır.

Materyal ve yöntem: Epidermal özellikleri ve trikom çeşitliliğini belgelemek amacıyla 25 Apocynaceous taksonunun karşılaştırmalı yaprak anatomik özellikleri ışık mikroskobu ve taramalı elektron mikroskobu (SEM) kullanılarak incelenmiştir.

Temel sonuçlar: Yaprakların her iki yüzeyi de stoma tipi, epidermis, antiklinal duvar, loblar ve trikom çeşitliliği dahil olmak üzere yaprak anatomik özelliklerinde farklılıklar göstermiştir. Epidermal hücre şekli düzensiz, dikdörtgen, poligonal, dalgalı, izodiametrik ve uniseriat olarak bildirilmiştir. En büyük epidermal hücreler Vinca major'da (59.6 µm) ve adaksiyal ve abaksiyal olmayan yüzeylerde (77.6 µm) incelenmiştir. En büyük stoma kompleksi Beaumontia grandiflora için (48.6 µm) kaydedilirken en küçüğü Cryptolepis dubia için (13.2 µm) kaydedildi. Maksimum stoma indeksi (SI) Asclepias curassavica'da hesaplanmıştır. (%37.2). Beaumontia grandiflora'nın her iki yüzeyinde çok hücreli glandüler olmayan trikomlar gözlemlenirken, Trachelospermum jasminoides'te özellikle abaksiyal yüzeyde kıyılar arası bölgede bulunan uniseriat trikomlar gözlenmiştir. En uzun trikom uzunluğu Pergularia tomentosa için abaksiyal tarafta ölçüldü (135 µm), abaksiyal yüzey boyunca ise maksimum uzunluk Asclepias curassavica için hesaplanmıştır (262 µm).

Araştırma vurguları: Çalışma, Pakistan'ın Pencap eyaletinin kıyı bölgelerinden toplanan 25 Apocynaceous taksonundaki yaprak mikromorfolojik özelliklerinin kapsamlı bir karşılaştırmalı analizini sunmaktadır. Işık mikroskobu ve taramalı elektron mikroskobu kullanılarak, trikom tipleri, stoma kompleksi düzenlemeleri ve epidermal hücre desenleri gibi taksonomik olarak önemli özellikler belirlenmiştir. Bu mikromorfolojik belirteçler, yakın ilişkili türleri ayırt etmede değerli olduğunu kanıtlamış ve tanısal taksonomik anahtarların geliştirilmesine katkıda bulunmuştur. Bulgular, temel anatomik veriler sağlıyor ve Apocynaceae'deki yaprak epidermal yapılarının ekolojik uyarlanabilirliğini ve sistematik önemini vurgulamaktadır..

Anahtar Kelimeler: Taksonomik Belirteçler, Mikro-histoloji, Epidermis, Trikom Yapısı, Kıyı Bölgesi

Introduction

The family Apocynaceae, first formally described by Antoine Laurent de Jussieu in 1789 as "Apocineae," is a well-established group within the order Gentianales, known for its taxonomic complexity, wide ecological distribution, and considerable ethnobotanical value (Endress & Bruyns, 2000). Globally, the family comprises approximately 215 genera and over 1900 species. In the context of Pakistan, the *Flora of Pakistan* reports six native genera and species, while an additional 13 genera and 20 species are cultivated, bringing the total to 19 genera and 26 species across the country (Khan & Shaukat, 2006; Khan et al., 2023). This representation underscores the ecological amplitude and adaptive strategies of Apocynaceae taxa in diverse habitats, particularly within the riparian zones of Punjab, where environmental conditions such as high soil moisture, alluvial substrates, and transitional microclimates support an impressive diversity of both native and introduced members of the family (Saqib et al., 2024; Liu et al., 2025; Saqib et al., 2025). Apocynaceous plants are perennial or, in rare cases, annual herbs, shrubs, or trees. Simple, alternate, opposite, or whorled leaves petiolate or sessile are found. The leaves are ovate, obovate, oblong, linear, lanceolate, or elliptic in shape, with an entire or undulate border and an acute apex (El-Fiki et al., 2019). In bio systematics, numerical taxonomy is a classification system that deals with plant classification. Rather than employing a subjective assessment of plant specimens, the numerical approach to taxonomic traits is based on their unique phytography (Ullah et al., 2018). The method of using numerical algorithms, such as cluster analysis and dendrogram-based phylogenetic approach, is utilized for analysis to develop

statistical numerical taxonomic authentication (Majeed et al., 2022).

The riparian zones of Punjab, Pakistan, represent ecologically dynamic interfaces between terrestrial and aquatic ecosystems, characterized by periodic hydrological regimes, high soil moisture, and rich alluvial deposits conditions that foster a high degree of floristic diversity and ecological specialization. Within this unique environmental setting, members of the family Apocynaceae exhibit remarkable structural and adaptive diversity, making them ideal candidates for micromorphological and taxonomic investigations. The present study was conducted across selected riparian habitats of Punjab, which support a notable assemblage of twenty-five Apocynaceous taxa, including *Alstonia scholaris*, *Asclepias curassavica*, *Beaumontia grandiflora*, *Calotropis gigantea*, *Calotropis procera*, *Carissa carandas*, *Carissa macrocarpa*, *Carissa spinarum*, *Cascabela thevetia*, *Catharanthus roseus*, *Cryptolepis dubia*, *Cynanchum acutum*, *Nerium oleander*, *Oxystelma esculentum*, *Pergularia daemia*, *Pergularia tomentosa*, *Plumeria rubra*, *Rhazya stricta*, *Tabernaemontana divaricata*, *Trachelospermum jasminoides*, *Vinca major*, *Vincetoxicum arnottianum*, *Vincetoxicum hirsutum*, *Vincetoxicum spirale*, and *Wattakaka volubilis*. These taxa, comprising both native and cultivated species, reflect a broad ecological amplitude and phenotypic plasticity in response to riparian conditions. The selection of Punjab's riparian corridor as the study area is thus justified due to its high representational value of Apocynaceae diversity, as well as its suitability for examining species anatomical responses to fluctuating environmental gradients, which are central to understanding taxonomic differentiation within this family.

Plants exhibit anatomical traits and adaptive mechanisms that allow them to function and survive in a wide range of habitats. The commercialized material is scraped and difficult to discover, anatomical traits serve an important role in identifying species of pharmaceutical value. Furthermore, these features can provide important insights to recognizing the diverse mechanisms and adaptations seen in plants of varying growth forms (Tripathi et al., 2023). Anatomy studies cover the ground vascular system and epidermal cells, as well as their functions, cell kinds, and tissues. Plant morpho-anatomical structure and arrangement are used to classify and differentiate angiosperms. Stomatal complex number and density are crucial diagnostic aspects of the anatomy of the leaf epidermis (Bashir et al., 2020). The stomatal number varies with the age of the leaf, but due to fluctuations in the external environment, the stomatal index remains relatively constant. It has been used to distinguish between closely related species of the same genus in dry and moist vegetational flora (Guilfoyle et al., 2015). One of the prominent methods used for identifying and to compare the plants at the subfamily and species level using morpho-structural anatomy (Uma et al., 2022). In the Apocynaceae, it is basic to use anatomical micromorphology to classify species and address taxonomical complexities (Simões et al., 2007). The anatomy of the vegetative parts has proven a useful aid in taxonomic authentication at various taxonomic levels to adapt the plants in diverse ecological habitats (Pirolla-Souza et al., 2019).

Microanatomical characterization refers to internal structures which are not visible with the naked eye and can only be seen with an electron microscope or any other powerful light microscope (Abbas et al., 2022; Majeed et al., 2022). The study of internal plant tissues and their organization at the microscopic level is referred to as plant anatomy (Zahra et al., 2025). Using Scanning Electron Microscopy (SEM), the micromorphological investigation revealed key taxonomic traits and offered a clearer insight into plant structural details through detailed bio-imaging visualization (Majeed et al., 2024; Khan et al., 2025; Samatova et al., 2025). The examination of various micromorphology in context of their systematic significance could be useful to delimiting species (Baxtiyorovna et al., 2025). The taxonomic assemblages to specific invasive vegetation types with a new spectrum with specific morphological-anatomical parameters, provide a solid foundation to trace the evolutionary adaptation in ecological habitats (Qayyum et al., 2025).

The surface of the leaf is an important taxonomic character, and the invention of scanning microscopy has added revolutionized dimension contributed to microanatomy and its systematic relevance (Gul et al., 2019; Shah et al., 2019). Scanning microscopic imaging methods were used to explore the trichome diversity and address their taxonomic significance (Ullah et al., 2021; Jamal et al., 2024). The SEM analysis revealed a variety of trichome types distributed across the leaf surface, and the structural features of the stomatal complex were found to possess considerable significance for systematic classification (Beilstein et al., 2006). The foliar epidermal anatomy provides sufficient relevant data to differentiate between angiosperms families. Many epidermal features, such as the length and shape of epidermal cells, stomata, and hairs, have become important identification tools to classify species among angiosperm families (Hussain et al., 2019).

Various authors have studied the anatomy of the family Apocynaceae globally (Guidoti et al., 2015; Ugwu, 2018; Seenu et al., 2019; Adeniran et al., 2022). Bashir et al. (2020) researched the foliar leaf epidermis and systematics of Apocynaceae taxa from the Peshawar. His findings show that quantitative aspects of leaf epidermis provide insight into micromorphological structure. Nisa et al. (2019) identified stomatal novelties in *Vincetoxicum arnottianum* observed the non-contiguous stomatal cluster of abnormal stomatal pattern in six populations. Comparative morpho-anatomical standardization of foliar epidermal anatomy of 25 Apocynaceous taxa clustering revealed epidermal cells anticlinal wall pattern, stomatal shape, size, and also distribution, the shape of guard cells by Singh et al. (2012) from India. Recently Beckers et al. (2022) examined woody structures Secamonoideae and Asclepiadoideae subfamilies reconstruct the phylogenetic informative wood anatomical origin.

The present study aims to investigate structural variations in the foliar epidermis and the ultrastructural diversity of trichomes among selected taxa of the Apocynaceae family, utilizing polarized light and scanning electron microscopy. The developed taxonomic keys are intended to effectively highlight the systematic relevance of epidermal traits for accurate species identification.

Material and Methods
Apocynaceous Plant Sampling

The various field trips were conducted from August 2024 to February 2025, during the monsoon and spring seasons to explore the diversity of Apocynaceous taxa. The flat and mountainous phytogeographic areas of moist and dry subtropical areas were visited to collect dicotyledonous samples of the Apocynaceae family (Figure 1). According to regional floristic surveys and herbarium records, the province of Punjab, Pakistan, supports a substantial representation of Apocynaceae, with approximately 30 species documented across various habitats, including cultivated, naturalized, and native taxa. These species exhibit diverse ecological behaviours and morphological traits, particularly in riparian environments where edaphic and hydrological conditions favour their establishment. For the present study, 25 representative taxa were selected based on their ecological occurrence, accessibility, and morphological distinctiveness within riparian zones. The selection prioritized taxa that are either widespread, ecologically dominant, or exhibit notable micromorphological variability, which are critical for comparative anatomical investigations. This targeted sampling approach was designed to ensure a comprehensive yet manageable dataset, enabling in-depth analysis of foliar epidermal structures and their taxonomic relevance, while maintaining focus on species that reflect the structural and ecological diversity of Apocynaceae in the region.
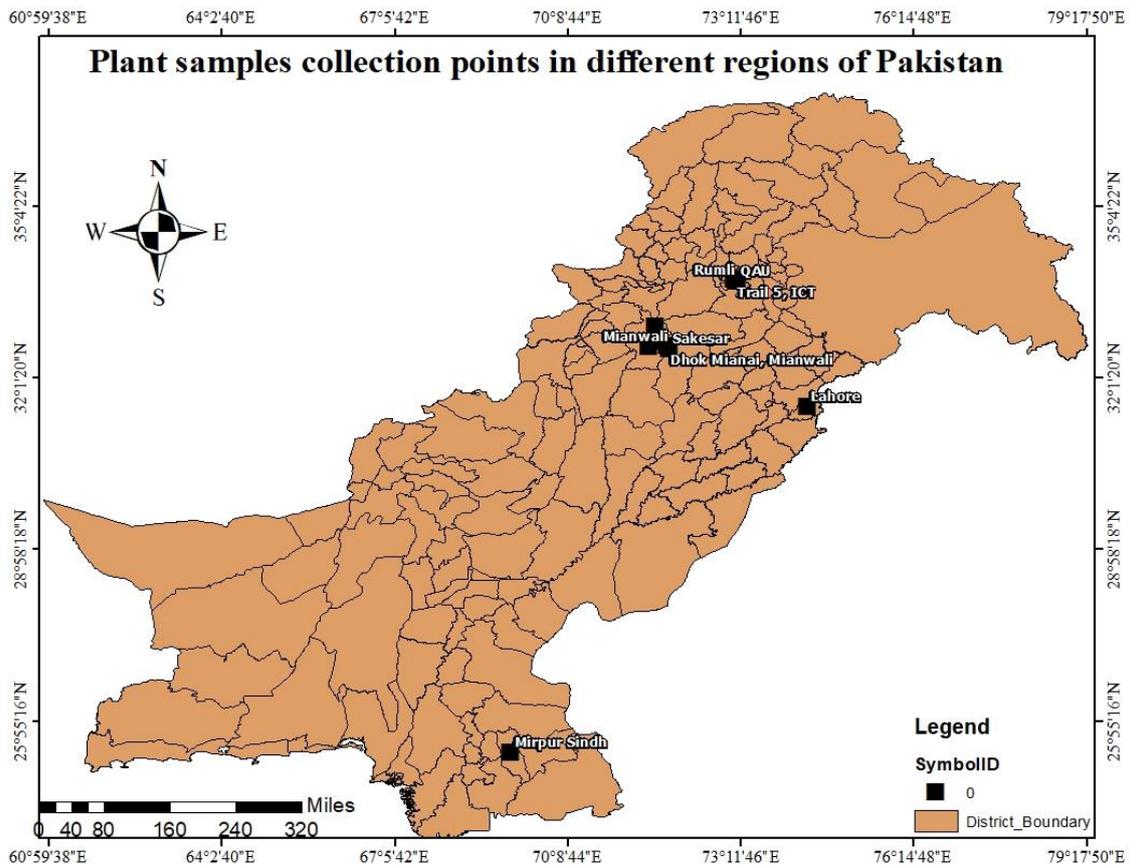
Figure 1. Map showing the sampling localities of Apocynaceous taxa

Each Apocynaceous taxa was field photographed as shown in Figure 2, 3, 4, 5 and 6 and field notes were recorded as shown in (Table 1). The collected specimens were processed following standard herbarium protocols as outlined by Bridson and Forman (1999). Specifically, freshly collected Apocynaceous plant samples were pressed in newspaper sheets using a plant press with consistent pressure and regularly changed blotters to prevent fungal growth and preserve morphological integrity. Specimens were air-dried in a well-ventilated area for 7–10 days, depending on the moisture content of the samples. Once fully dried, the specimens were mounted on standard herbarium sheets (11.5 × 16.5 inches), labeled with detailed field data including species name, collection date, GPS coordinates, habitat, habit, collector name, and accession number. The identification was carried out with the help of expert taxonomists and compared with the flora of Pakistan (Url-1).

The authenticated plant name was confirmed from the WFO plant list URL-2). These mounted specimens were then deposited in the ISL Herbarium, Islamabad, for reference and future taxonomic verification. This documentation ensured the preservation of key morphological traits critical for accurate identification and systematic comparisons.

Figure 2. Field pictorial view: (a) *Alstonia scholaris* (b) *Beaumontia grandiflora* (c) *Calotropis procera,* (d) *Calotropis gigantea*

Figure 3. Field pictorial view: (e) *Carissa carandas,* (f) *Carissa macrocarpa,* (g) *Carissa spinarum,* (h) *Cascabela thevetia*

Figure 4. Field pictorial view: (i) *Catharanthus roseus,* (j) *Cryptolepis dubia,* (k) *Nerium oleander,* (l) *Plumeria rubra*

Figure 5. Field pictorial view: (m) *Pergularia daemia,* (n) *Rhazya stricta,* (o) *Tabernaemontana divaricata,* (p) *Trachelospermum jasminoides*
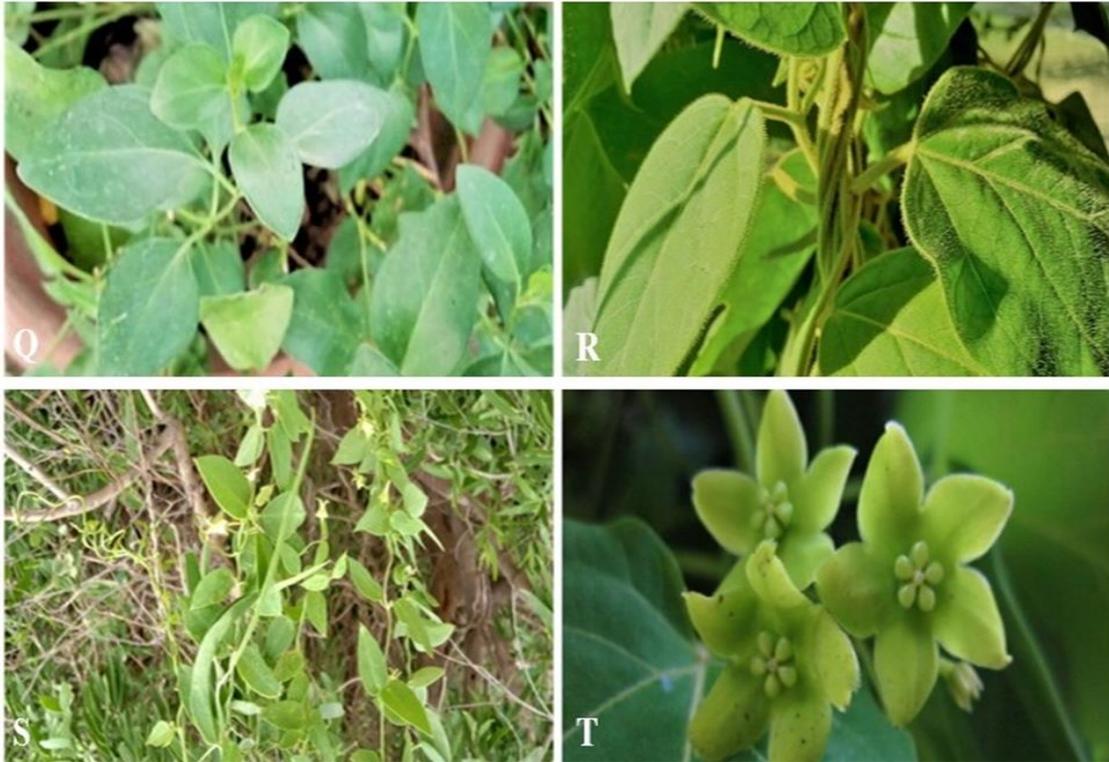
Figure 6. Field pictorial view: (q) *Vinca major,* (r)*Vincetoxicum hirsutum,* (s) *Vincetoxicum spirale,* (t) *Wattakaka volubilis*

Table 1. Checklist of Apocynaceous plants sampling

Sr. No.	Taxa	Flowering Period	Voucher no.	Localities	Coordinates	Collector's name	Accession No.	Altitude (feet)	Province
1.	*Alstonia scholaris* (L.) R.Br.	November to January	AS-16	Lahore	31.513099° N, 74.373667° E	M. Rizwan Khan	ISL-133415	712	Punjab
2.	*Asclepias curassavica* L.	June to October	AC-32	Rawalpindi	33.567410° N, 73.085568° E	M. Rizwan Khan	ISL-133428	1680	Punjab
3.	*Beaumontia grandiflora Wall.*	March to April	BG-20	ICT	33.745615° N, 73.137888° E	M. Rizwan Khan	ISL-133494	2031	Islamabad
4.	*Calotropis gigantea (L.) Dryand*	May to August	CG-33	Mirpur	25.514458° N, 69.023728° E	Salman Majeed	ISL-133493	60	Sindh
5.	*Calotropis procera* (Aiton) Dryand.	October to December	CP-06	Mianwali	32.576547 ° N, 71.575377 ° E	M. Rizwan Khan	ISL-133416	688	Punjab
6.	*Carissa carandas Lour*	March to may	CC-22	Mianwali	32.576547 ° N, 71.575377 ° E	M. Rizwan Khan	ISL-133491	688	Punjab
7.	*Carissa macrocarpa* (Eckl.) A.DC.	October to November	CM-15	Chakwal	32.939325° N, 72.864004° E	M. Rizwan Khan	ISL-133422	1965	Punjab
8.	*Carissa spinarum* L.	July to August	CS-10	Rumli	33.752956° N, 73.136338° E	Salman Majeed	ISL-133426	2346	Islamabad
9.	*Cascabela thevetia* (L.) Lippold	June to October	CT-11	ICT	33.745615° N, 73.137888° E	M. Rizwan Khan	ISL-133418	2031	Islamabad
10.	*Catharanthus roseus* (L.) G.Don	July to August	CR-03	QAU	33.745623° N, 73132074° E	M. Rizwan Khan	ISL-133419	2031	Islamabad
11.	*Cryptolepis dubia* (Burm.f.) M.R.Almeida	June to August	CD-13	Sakesar	32.541652° N, 71.934362° E	M. Rizwan Khan	ISL-133430	4960	Punjab
12.	*Cynanchum acutum L.*	June to August	CA-26	Malakand	34.5123452° N, 71.8916382° E	M. Rizwan Khan	ISL-133497	1506	KPK
13.	*Nerium oleander* L.	July to September	NO-12	Talagang	32.913338° N, 72.426670° E	Salman Majeed	ISL-133424	1666	Punjab
14.	*Oxystelma esculentum (L. f.) Sms*	July to January	OE-23	Mirpur	25.504108° N, 69.037589° E	M. Rizwan Khan	ISL-133427	63	Sindh
15.	*Pergularia daemia* (Forssk.) Chiov.	October to November	PD-14	Jehlum	32.951902° N, 73.689400° E	M. Rizwan Khan	ISL-133429	768	Punjab

Lactic Acid Nitric Acid Procedure

The microscopic techniques to conduct foliar micromorphology, we followed the Nazir et al. (2013) procedure with a little modification. To remove the dust particles from both surfaces of the leaf, collected leaves were briefly soaked in water. 4-5 leaves were then heated in a test tube for 2–3 minutes to make the leaves transparent using 30% nitric acid and 70% lactic acid. Adaxial and abaxial leaf surfaces were separated using a camel brush and needle after being put onto Petri dishes. The detached epidermis was put on a clean slide, sealed with a coverslip, and treated with a drop of lactic acid to help it clear. For each plant species, four to six samples of both surfaces were made. All features were examined using a Nikon and Meiji (Japan) light microscope. Photographs were captured using a LIECA-DM-1000 light microscope and a Meiji Affinity DK-5000 camera.

Scanning Microscopic Analysis

For scanning electron microscopic (SEM) studies, mature and healthy leaves were carefully rinsed with distilled water to remove surface debris and then air-dried at room temperature $(25 \pm 2°C)$ in a dust-free environment for 7–10 days. This passive air-drying method was chosen to preserve the foliar surfaces, minimizing structural distortion or shrinkage. Once thoroughly dried, leaf samples were stored in labelled airtight paper envelopes with silica gel desiccants to prevent moisture absorption until further SEM preparation. This method ensured optimal preservation of surface micromorphology for high-resolution imaging and accurate comparative analysis. From each leaf sample, two pieces from both upper and lower sides were taken for mounting on stubs with a two-fold coating of scotch tape. The sputtering of leaf samples was done with gold palladium and examined under a JEOL JSM-5910 scanning electron microscope. Polaroid (P/N 665) film was used to take photographs.

Qualitative and Quantitative Characteristics

In the present study, foliar microanatomical features were comprehensively examined on both adaxial and abaxial leaf surfaces. Qualitative characteristics included stomatal type, trichome presence and type, epidermal cell shape, and the pattern of anticlinal walls. Quantitative traits comprised numerical and dimensional parameters such as the number, length, and width of epidermal cells, stomata, subsidiary cells, guard cells, stomatal pores, and trichomes, along with the stomatal index. Quantitative measurements were performed under a calibrated light microscope using a standard ocular micrometer. For each character, ten independent readings were recorded to ensure statistical reliability. Data were processed using SPSS software (Abbas et al., 2022), and results were expressed as Mean ± Standard Error. These parameters were selected based on their diagnostic value in previous anatomical and taxonomic studies. This approach enabled the robust statistical evaluation of interspecific variation, supporting taxonomic delimitation among the studied taxa.

Statistical Analysis
Stomatal Index (SI)

The formula determines the stomatal index is given Equation 1,

$$S.I = \frac{S}{E + S} \times 100 \qquad (1)$$

Where (S) is the number of stomata per unit area and (E) is the number of epidermal cells per unit area.

Trichome Index (TI)

The formula determines the trichome index is given Equation 2,

$$T.I = \frac{T}{E + T} \times 100 \qquad (2)$$

Where (T) is the number of trichome per unit area and (E) is the number of epidermal cells per unit area.

Cluster Analysis

To assess the interspecific relationships among the Apocynaceous taxa based on foliar micromorphological traits, both hierarchical clustering and Principal Component Analysis (PCA) were conducted. Cluster analysis was performed using the Unweighted Pair Group

Method with Arithmetic Mean (UPGMA), which generated a dendrogram to illustrate phenetic affinities among the species. For dimensionality reduction and to identify the most significant variables contributing to variation among taxa, PCA was applied using the multivariate statistical software PAST (version referenced in Khan et al., 2023). The analysis was based on standardized quantitative data, including parameters such as stomatal dimensions, epidermal cell metrics, guard cell size, and stomatal index. PCA helped visualize the grouping patterns by projecting species onto principal component axes, highlighting major trends in data variability and aiding in species discrimination based on micromorphological traits.

Results

Leaf epidermal micro-morphology of twenty-five riparian Apocynaceous species was investigated using light and scanning microscopy (LM and SEM). The current findings described variations in both qualitative and quantitative foliar anatomical characteristics of amphistomatic and hypostomatic leaves (Table 2 and 3). The leaf epidermal surface visualized under light and scanning micrographs were illustrated in Figures 7, 8,9 and 10.
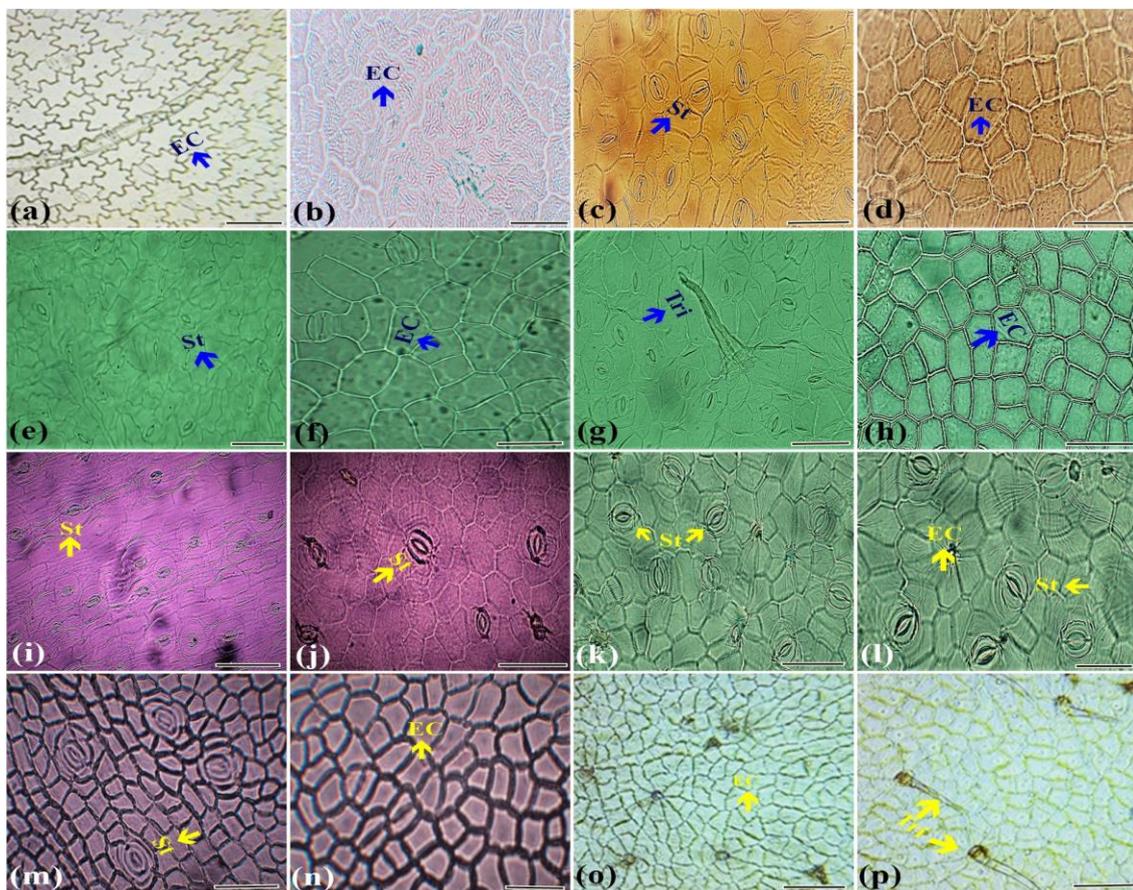

Figure 7. Foliar anatomical micrographs of Epidermal cells (EC) , Stomata (St) and Trichomes (Tri)

(a) Abaxial surface , *P. rubra* showing EC (20x) (b) Adaxial surface, *P. rubra* having rectangular EC (20x) (c) Abaxial surface, *A. scholaris* showing anomocytic St(20x) (d) Adaxial surface, *A. scholaris* with irregular EC (20x) (e) Abaxial surface, *A. curassavica* having anomocytic St (20x) (f) Adaxial surface, *A. curassavica* showing irregular EC(20x) (g) Abaxial surface, *B. grandiflora* with non-glandular tri (10x) (h) Adaxial surface, *B. grandiflora* showing rectangular EC (10x) (i) Abaxial surface, *C. gigantea* with diacytic St (10x) (j) Adaxial surface, *C. gigantea* showing rectangular EC (10x) (k) Abaxial surface, *C. procera* with paracytic St (10x) (l) Adaxial surface , *C. procera* having rectangular EC (10x) (m) Abaxial surface, *C. carandas* showing anomocytic St (40x) (n) Adaxial surface, *C. carandas* with flat rectangular EC (40x) (o)Abaxial surface,*C. macrocarpa* having rectangular EC (20x) (p) Adaxial surface , *C. macrocarpa* showing unicellular tri(20x)

Figure 8. Foliar anatomical micrographs of Epidermal cells (EC) , Stomata (St) and Trichomes (Tri)

(a) Abaxial surface, *C. spinarum* having paracytic St (20x) (b) Adaxial surface, *C. spinarum* showing EC (20x) (c) Abaxial surface, *C. thevetia* with multicellular tri (20x) (d) Adaxial surface, *C. thevetia* having irregular EC (20x) (e) Abaxial surface, *C. roseus* showing anisocytic stomata (40x) (f) Adaxial surface, *C. roseus* with polygonal EC (40x) (i) Abaxial surface, *C. dubia* with paracytic St (40x) (h) Adaxial surface, *C. dubia* showing polygonal EC(40x) (i) Abaxial surface, *C. acutum* having anomocytic St (40x) (j) Adaxial surface, *C. acutum* with irregular EC (40x) (k) Abaxial surface, *N. oleander* with tr (20x) (l) Adaxial surface, *N. oleander* having rectangular EC (20x) (m) Abaxial surface, *O. esculentum* showing anomocytic St (40x) (n) Abaxial surface, *O. esculentum* with glandular tri (40x) (o) Adaxial surface, *P. daemia* having regular EC (40x) (p) Abaxial surface, *P. daemia* with unicellular tri (40x)
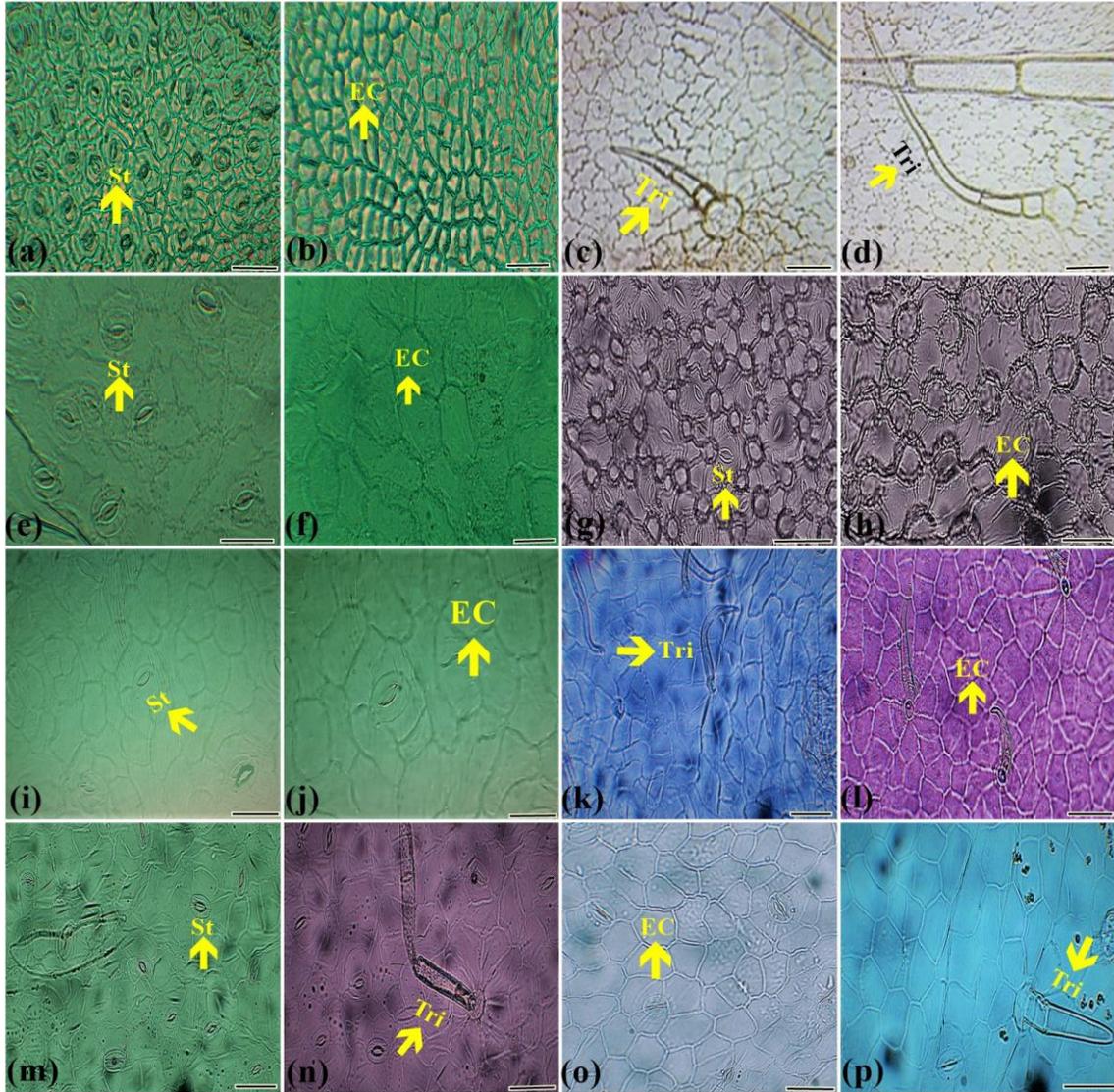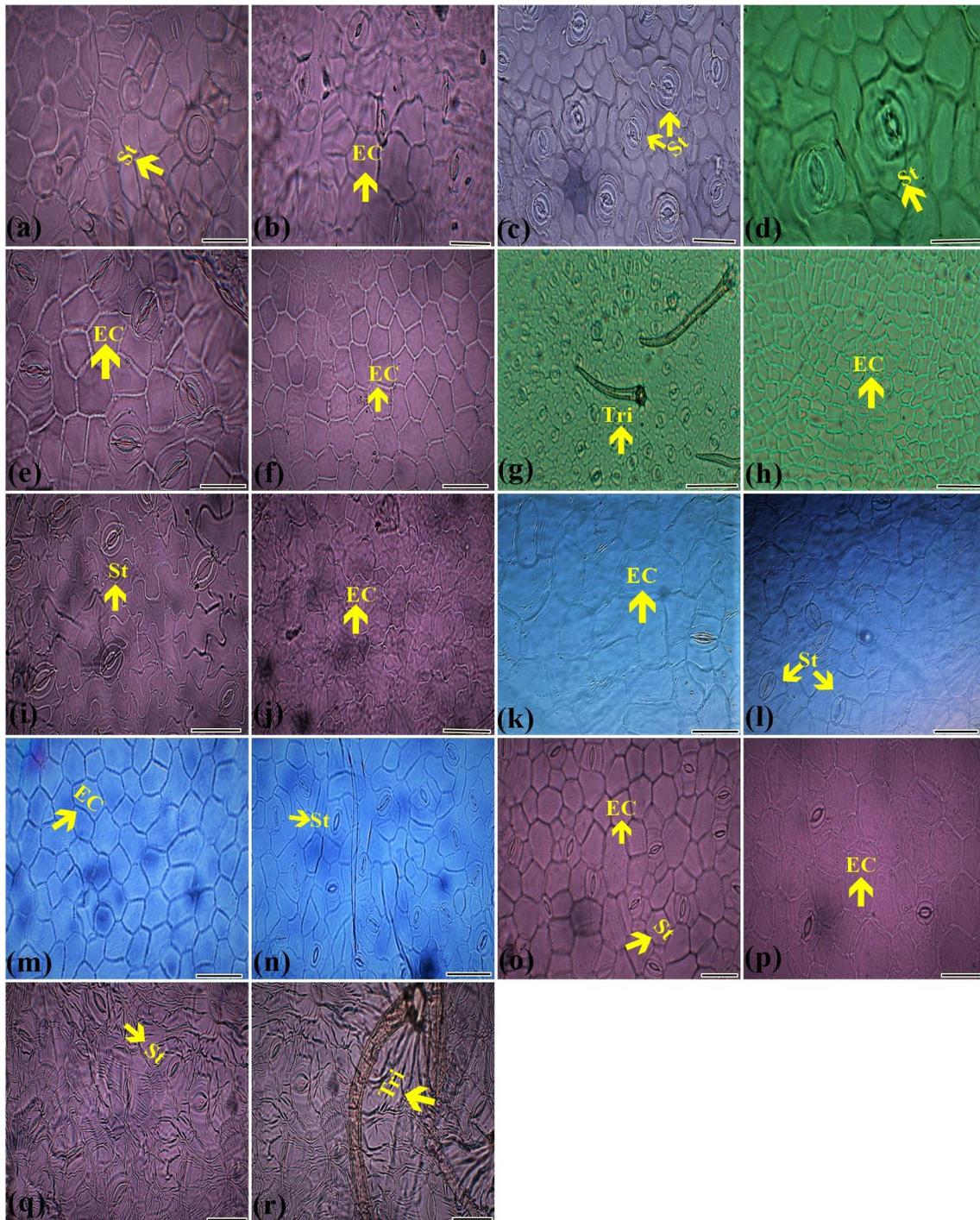
Figure 9. Foliar anatomical micrographs of Epidermal cells (EC) , Stomata (St) and Trichomes (Tri)

(a) Abaxial surface, *P. tomentosa* showing paracytic St (20x) (b) Adaxial surface, *P. tomentosa* with regular EC (20x) (c) Abaxial surface, *R. stricta* showing paracytic St(20x) (d) Adaxial surface, *R. stricta* having paracytic *St* (20x) (e) Abaxial surface, *T. divaricata* having anomocytic stomata St (40x) (f) Adaxial surface, *T. divaricata* with rectangular EC (40x) (g) Abaxial surface, *T. jasminoifes* having non-glandular tri (20x) (h) Adaxial surface, *T. jasminoides* with rectangular EC (20x) (i) Abaxial surface, *V.major* showing paracytic St (40x) (j) Adaxial surface, *V. major* having irregular EC (40x) (k) Abaxial surface, *V. arnottianum* with irregular EC (40x) (l) Adaxial surface, *V. arnottianum* having anomocytic St (40x) (m) Abaxial surface, *V. hirsutum* showing irregular EC (40x) (n) Adaxial surface, *V. hirsutum* with anomocytic St (40x) (o) Abaxial surface, *V. spirale* showing anomocytic St (40x) (p) Adaxial surface, *V. spirale* with rectangular EC (40x) (q) Abaxial surface, *W. volubilis* having anomocytic St (20x) (r) Adaxial surface, *W. volubilis* showing non-glandular trichome (20x).
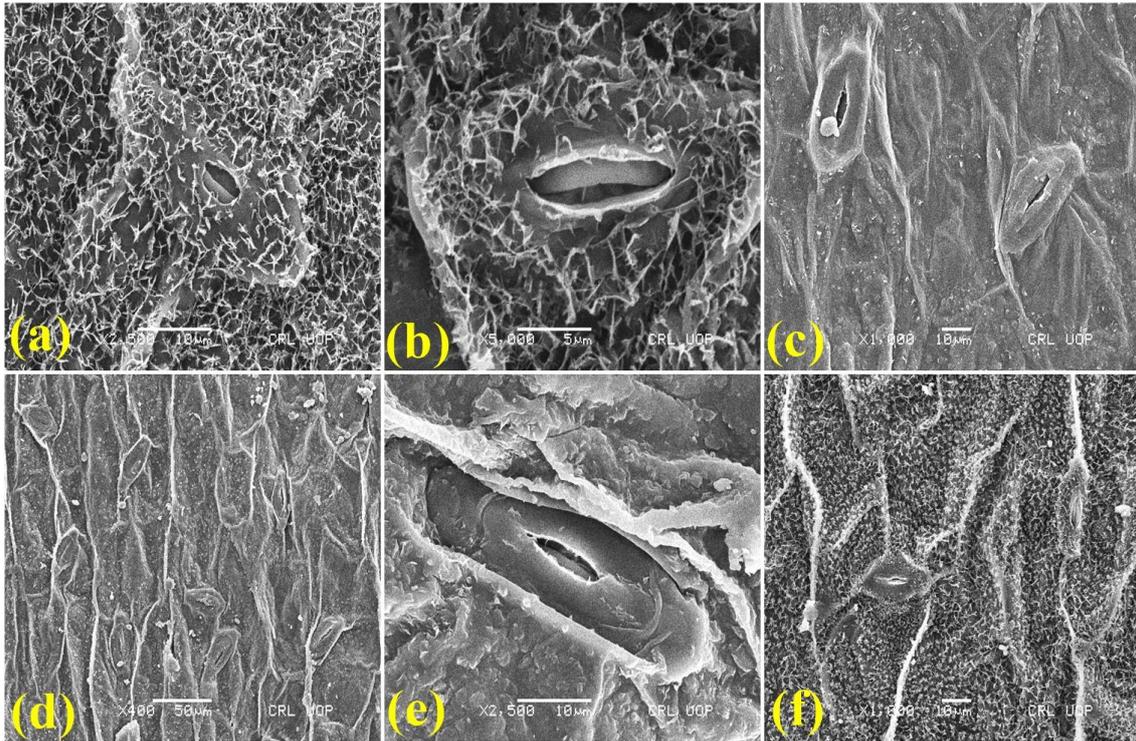
Figure 10. SEM micrographs of Apocynaceous taxa

(a) Abaxial surface, *C. procera* having stoma (10μm) (b) Adaxial surface, *C. procera* showing stoma (5μm) (c) Abaxial surface, *R. stricta* having stomata (10 μm) (d) Adaxial surface, *R. stricta* showing stomata (50 μm) (e) Adaxial surface, *C. acutum* with stoma (10 μm) (f) Abaxial surface, *C. acutum* with stomata(10 μm)

Table 2. Qualitative Analysis of Adaxial and Abaxial surfaces among Apocynaceous taxa

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	St (P/A)	ST	SS	GCS	SCS	Tri (P/A)	Trichome	DT
Alstonia scholaris (L.) *R. Br.*	Hypostomatic	Ad	Irregular	Straight curved	A	A	A	A	A	A	A	A
		Ab	Irregular	Straight curved	P	Anomocytic	Elliptic	Kidney Shape	Elongate, Irregularly shaped	A	A	A
Asclepias curassavica L.	Amphistomatic	Ad	Irregular	Undulate	P	Anomocytic	Elongate elliptic	Kidney shape	Undulate margin, enclosing GC	A	A	A
		Ab	Irregular	Undulate	P	Anomocytic	Elongate elliptic	Kidney shape	Undulate margins enclosing GC	A	A	A
Beaumontia grandiflora Wall	Hypostomatic	Ad	Rectangular	Straight	A	A	A	A	A	P	Muticellular and non-glandular	Intercostal zone
		Ab	Rectangular	Straight	P	Anomocytic	Elliptic	Kidney shape	Elongated, Irregularly shaped enclosing GC	P	Muticellular and non-grandular	Intercostal zone
Calotropis gigantea (L.) Dryand	Amphistomatic	Ad	Rectangular	Smooth	P	Diacytic	Elongate elliptic	Kidney shape	Rectangularly shaped Enclosing Partly GC	A	A	A
		Ab	Rectangular	Smooth	P	Diacytic	Elongate elliptic	Kidney shape	Rectangularly shaped Enclosing Partly GC	A	A	A
Calotropis procera (Aiton) Dryand.	Amphistomatic	Ad	Rectangular	Straight	P	Paracytic	Elongate elliptic	Kidney shape	Oblong shaped Enclosing Partly GC	A	A	A
		Ab	Rectangular	Straight	P	Paracytic	Elongate elliptic	Kidney shape	Rectangularly shaped Enclosing Partly GC	A	A	A
Carissa carandas Lour.	Hypostomatic	Ad	Flat Rectangular	Undulated	A	A	A	A	A	A	A	A
		Ab	Flat Rectangular	Undulated	P	Anomocytic	Elliptic	Kidney shape	Undulate margin, enclosing GC	A	A	A
Carissa macrocarpa (Eckl.) A.DC.	Hypostomatic	Ad	Rectangular	Straight	A	A	A	A	A	A	A	A
		Ab	Rectangular	Straight	P	Anomocytic	Elliptic	Kidney shape	Rectangularly shaped, Enclosing GC	A	A	A
Carissa spinarum L.	Hypostomatic	Ad	Regular	Straight curved	A	A	A	A	A	A	A	A
		Ab	Regular	Straight curved	P	Anomocytic	Elliptic	Kidney shape	Curved, wavy, enclosing GC	A	A	A
Cascabela thevetia (L.) Lippold	Hypostomatic	Ad	Regular	Sinuated	A	A	A	A	A	A	A	A
		Ab	Regular	Sinuated	P	Anisocytic	Elliptic	Kidney shape	Sinuated margins, wavy, enclosing GC	A	A	A
Catharanthus roseus (L.) G.Don	Amphistomatic	Ad	Polygonal	Undulate	P	Anisocytic	Broad Elliptic	Broad kidney shape	Undulate margins, enclosing GC	A	A	A
		Ab	Polygonal	Undulate	P	Anisocytic	Broad Elliptic	Broad Kidney shape	Undulate margins, enclosing GC	A	A	A
Cryptolepis dubia (Burm.f.) M.R.Almeida	Hypostomatic	Ad	Polygonal	Sinuated to wavy	A	A	A	Broad kidney Shaped	Margin sinuous, enclosing GC	A	A	A
		Ab	Polygonal	Sinuated to wavy	P	Paracytic	Elongate Elliptic	Broad kidney Shaped	Margin sinuous, enclosing GC	A	A	A

Table 2. (Continued)

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	St (P/A)	ST	SS	GCS	SCS	Tri (P/A)	Trichome	DT
Cynanchum acutum L.	Amphistomatic	Ad	Irregular	Perpendicular	P	Anomocytic	Elongate Elliptic	Broad kidney shape	Lobed margins, wavy, enclosing GC	A	A	A
		Ab	Irregular	Perpendicular	P	Anomocytic	Elongate Elliptic	Broad kidney shape	Lobed margins, wavy, enclosing GC	A	A	A
Nerium oleander L.	Hypostomatic	Ad	Regular	Rectangular	A	Crypts	Crypts	A	A	A	A	A
		Ab	Regular	Rectangular	P	Crypts	Crypts	A	Oblong shaped	P	Multicellular and non-grandular	Intercoastal zone
Oxystelma esculentum (L. f.) Sms	Hypostomatic	Ad	Irregular	Rectangular	A	A	A	A	A	A	A	A
		Ab	Irregular	Rectangular	P	Anomocytic	Elliptic	Kidney shape	Oblong shaped, enclosing GC	P	Multicelluar and glandular	Intercoastal zone
Pergularia daemia (Forssk.) Chiov	Hypostomatic	Ad	Regular	Rectangular	A	A	A	A	A	A	A	A
		Ab	Regular	Rectangular	P	Anomocytic	Elliptic	Kidney shape	Oblong shape, enclosing GC	P	Unicellular	Coastal zone
Pergularia tomentosa L.	Hypostomatic	Ad	Regular	Undulate	A	A	A	A	A	A	A	A
		Ab	Regular	Undulate	P	Paracytic	Broad Elliptic	Broad kidney shape	Undulate margins, enclosing GC	A	A	A
Plumeria rubra L.	Hypostomatic	Ad	Rectangular	Straight	A	A	A	A	A	A	A	A
		Ab	Rectangular	Straight	P	Anisocytic	Elliptic	Kidney shape	Oblong shape, enclosing GC	P	Multicellular and glandular	Coastal zone
Rhazya stricta Decne.	Amphistomatic	Ad	Regular	Straight	P	Paracytic	Broad Elliptic	Broad kidney Shape	Lobed margins, enclosing GC	A	A	A
		Ab	Regular	Straight	P	Paracytic	Broad Elliptic	Broad kidney Shape	Lobed margins, enclosing GC	A	A	A
Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.	Amphistomatic	Ad	Rectangular	Straight	P	Anomocytic	Elongate Elliptic	Kidney shape	Slightly oblong, enclosing GC	A	A	A
		Ab	Rectangular	Straight	P	Anomocytic	Elongate Elliptic	Kidney shape	Slightly oblong, enclosing GC	A	A	A
Trachelospermum jasminoides (Lindl.) Lem.	Hypostomatic	Ad	Rectangular	Sinuated	A	A	A	A	A	A	A	A
		Ab	Rectangular	Sinuated	P	Paracytic	Broad Elliptic	Kidney Shape	Sinuatedmargins, enclosing GC	P	Uniseriate, non-glandular	Intercoastal zone
Vinca major L.	Amphistomatic	Ad	Irregular	Sinuated	P	Paracytic	Elongate Elliptic	Kidney Shape	Sinuatedmargins, enclosing GC	A	A	A
		Ab	Irregular	Sinuated	P	Paracytic	Elongate Elliptic	Kidney shape	Sinuatedmargins, enclosing GC	A	A	A
Vincetoxicum arnottianum (Wight) Wight	Amphistomatic	Ad	Irregular	Straight	P	Anomocytic	Elliptic	Kidney Shape	Slightly elongated, enclosing GC	A	A	A
		Ab	Irregular	Straight	P	Anomocytic	Elliptic	Kidney Shape	Sligtly enlongated, enclosing GC	A	A	A

Table 2. (Continued)

Plant species	Leaves Condition	Ad × Ab	ECS	AWP	St (P/A)	ST	SS	GCS	SCS	Tri (P/A)	Trichome	DT
Vincetoxicum hirsutum (Wall.) Kuntze	Hypostomatic	Ad	Irregular	Straight	A	A	A	A	A	A	A	A
		Ab	Irregular	Straight	P	Anomocytic	Elongate Elliptic	Kidney Shape	Lobed margins, slightly enclosing GC	P	Unicellular, nonglandualr	Intercoastal zone
Vincetoxicum spirale (Forssk.) D.Z.Li	Amphistomatic	Ad	Rectangular	Smooth angular	P	Anomocytic	Elliptic	A	Angualr margins, slightly enclosing GC	A	A	A
		Ab	Rectangular	Smooth angular	P	Anomocytic	Elliptic	Kidney Shape		P	Hairlike, non-glandular	Coastal zone
Wattakaka volubilis Stapf	Hypostomatic	Ad	Irregular	Sinuated	A	A	A	A	A	A	A	A
		Ab	Irregular	Sinuated	P	Anomocytic	Broad Elliptic	Broad Kidney Shape	Sinuated margins, enclosing GC	P	Multicellular and non-glandular trichome	A

Ad:Adaxial, Ab:Abaxial, ECS:Epidermal cell size, AWP:Anticlinal wall pattern, NES:Nature of epidermal cells, LPC:Lobes per cell, St: Stomata ; ST:Stomata type, SS:Stomata shape, GCS:Gaurd cells shape, SSC:Subsidiary cell shape, Tri:Trichome, DT:Distribution of trichomes, A:Absent, P:Present

Table 3. Quantitative Analysis of Adaxial and Abaxial surfaces of epidermal cells, stomata and trichomes of Apocynaceous taxa

Taxa	Ad × Ab	L × W	Avg No. of Epidermal Cell	Epidermal Cell (Min–Max = Mean ± SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean ± SE)	SI (%)	Trichome No. per unit area	Trichome (Min–Max = Mean ± SE)	TI (%)
Alstonia scholaris (L.) R.Br.	Ad	L	29	27.5–85.5 = 55.5 ±1.7	A	A	A	A	A	A
		W		10.5–28.4 = 20.3 ±0.1						
	Ab	L	26	13.5–26.5 = 19.4 ±1.7	7	23.7–29.6 = 26.7 ±0.9	34.09	A	A	A
		W		11.8–21.7 = 16.5 ±1.6		18.5–21 = 19.85 ±0.4				
Asclepias curassavica L.	Ad	L	35	31.2–39.1 = 35.8 ±1.4	14	20.7–23.7 = 21.8 ±0.5	45.14	6	101–172.2 = 128.5 ±1.5	24.58
		W		30.2–37.2 = 33.9 ±0.8		8.5–9.6 = 8.65 ±0.1			15.2 ±0.3	
	Ab	L	42	24.5–33.7 = 27.8 ±1.6	5	19.5–20.75 = 20.15 ±0.2	37.25	8	231.7–301 = 262 ±0.9	28.98
		W		25.3–35.6 = 30.5 ±0.6		17.25–22.89 = 19.5 ±0.7				
Beaumontia grandiflora Wall.	Ad	L	38	22.7–30.2 = 26.5 ±0.4	A	A	A	A	A	A
		W		15.2–24.5 = 19.5 ±0.5						
	Ab	L	29	11.2–25.2 = 17.5 ±2.6	16	40.7–55.7 = 48.6 ±0.6	27.77	A	A	A
		W		10.4–24.4 = 16.5 ±0.8		10.8–12.3 = 11.48 ±0.3				
Calotropis gigantea (L.) Dryand.	Ad	L	43	21.2–30.7 = 24.9 ±1.7	11	24.5–28.2 = 26.4 ±0.9	25.36	A	A	A
		W		14.5–19.5 = 17.3 ±1.2		19.1–21.5 = 20.5 ±0.5				
	Ab	L	55	29.7–35.2 = 33.5 ±1.6	16	22.7–28.7 = 25.8 ±1.6	31.71	A	A	A
		W		11.5–26.4 = 18.2 ±1.9		13.5–16.4 = 15.7 ±0.3				
Calotropis procera (Aiton) Dryand.	Ad	L	49	28.2–32.4 = 30.1 ±0.2	8	2.7–30.7 = 23.9 ±0.7	28.43	A	A	A
		W		18.5–28.5 = 22.9 ±2.6		9.5–14.5 = 11.6 ±1.1				
	Ab	L	41	25.2–35.5 = 30.8 ±1.5	11	25.5–29.5 = 27.2 ±0.6	35.71	A	A	A
Carissa carandas Lour.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
Carissa macrocarpa (Eckl.) A.DC.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
Carissa spinarum L.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
Cascabela thevetia (L.) Lippold	Ad	L	54	17.5–33.5 = 24.2 ±1.2	A	A	A	A	A	A
		W		13.5–16.5 = 15 ±0.4						
	Ab	L	43	41.7–45.5 = 43.8 ±1.8	13	34.2–38.2 = 35.7 ±0.2	27.58	A	A	A
		W		13.3–14.8 = 14.5 ±0.2		16.4–26.5 = 21.44 ±0.5				
Catharanthus roseus (L.) G.Don	Ad	L	76	25.2–42.7 = 35.7 ±0.1	7	32.2–34.5 = 33.4 ±0.4	16.46	A	A	A
		W		10.5–11.3 = 10.36 ±0.5		18.3–18.5 = 18.42 ±0.3				
	Ab	L	36	40.2–43.2 = 41.8 ±0.1	11	24.5–27.7 = 25.6 ±0.5	22.91	A	A	A
		W		18.5–16.7 = 17.6 ±0.3		25.5–38.9 = 3.2 ±0.6				
Cryptolepis dubia (Burm.f.) M.R.Almeida	Ad	L	39	32.8–50.3 = 38.1 ±3.1	A	A	A	A	A	A
		W		24.5–23.8 = 24.1 ±1.3						
	Ab	L	43	25.5–35.5 = 30.8 ±0.1	9	2.1–3.2 = 2.6 ±0.5	32.69	A	A	A
		W		15.3–17.5 = 16.4 ±1.3		7.3–9.6 = 8.4 ±0.9				

Table 3. (Continued)

Taxa	Ad × Ab	L × W	Avg No. of Epidermal Cell	Epidermal Cell (Min–Max = Mean ± SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean ± SE)	SI (%)	Trichome No. per unit area	Trichome (Min–Max = Mean ± SE)	TI (%)
Cynanchum acutum L.	Ad	L	66	38.5–44.5 = 41.9 ±0.8	5	30.2–34.7 = 31.8 ±0.7	9.67	A	A	A
		W		26.2–38.2 = 32 ±1.3		28.7–35.3 = 30.4 ±1.2				
	Ab	L	49	29.5–41.4 = 36 ±0.8	12	32.2–39.1 = 34.4 ±1.2	21.42	A	A	A
		W		15.7–34.7 = 23.9 ±1.2		15.8–17.5 = 16.2 ±0.5				
Nerium oleander L.	Ad	L	55	21.1–25.7 = 23.4 ±0.6	A	A	A	A	A	A
		W		24–29.5 = 26.5 ±0.2						
	Ab	L	28	25.2–40.5 = 36.5 ±0.1	14	25.2–28.1 = 26.9 ±0.1	26.31	3	60.5–176 = 116.2 ±1.4	30.25
		W		22–28 = 25.4 ±0.3		26.1–26.9 = 26.8 ±0.7				
Oxystelma esculentum (L.f.) Sm.	Ad	L	69	12.6–13.2 = 12.9 ±0.1	7	21.4–24.3 = 22.3 ±0.9	A			
		W		28.2–45.7 = 38.3 ±0.4		22.5–38.4 = 30.4 ±1.5	A			
	Ab	L	52	31.2–48.4 = 41.1 ±1.1	25		A			
		W		32.2–49.1 = 42.5 ±1.6			A			
Pergularia daemia (Forssk.) Chiov.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
		W								
Pergularia tomentosa L.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
		W								
Plumeria rubra L.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
		W								
Rhazya stricta Decne.	Ad	L	63	22.2–39.8 = 32.5 ±0.1	8	15.6–19.15 = 17.3 ±0.6	A	A	A	A
		W	18.2–35.7 = 26.9 ±1.2		10.23–14.76 = 12.4 ±1.5	A	A	A	A	
	Ab	L	46	15.5–20.7 = 14.6 ±0.3	6	23.2–27.7 = 25.2 ±1.5	A			
		W	20.2–18.4 = 19.3 ±1.3	18.6–22.8 = 20.7 ±1.8	A					
Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.	Ad	L	31	20.5–32.7 = 26.7 ±0.8	8	17.2–25.5 = 19.8 ±1.5	A			
		W	13.3–27.5 = 18.6 ±0.9							
	Ab	L	24	25.9–29.9 = 27.1 ±1.3	7	17.2–25.5 = 19.8 ±1.5	A			
		W	19.6–25.2 = 23.4 ±0.8	15.2–21.7 = 19.8 ±1.8	A					
Trachelospermum jasminoides (Lindl.) Lem.	Ad	L	58	21.5–26.7 = 24.9 ±0.6	15	25.05	A			
		W	16.2–20.2 = 18.8 ±0.8							
	Ab	L	37	29.2–48.1 = 36.8 ±1.4	15.57	31.37	A			
		W	27.3–37.6 = 32.5 ±0.6							

Table 3. (Continued)

Taxa	Ad × Ab	L × W	Avg No. of Epidermal Cell	Epidermal Cell (Min–Max = Mean ± SE)	No. of Stomata (Avg)	Stomata (Min–Max = Mean ± SE)	SI (%)	Trichome No. per unit area	Trichome (Min–Max = Mean ± SE)	TI (%)
Vinca major L.	Ad	L	A	A	A	A	A	A	A	A
		W								
	Ab	L	A	A	A	A	A	A	A	A
		W								
Vincetoxicum arnottianum (Wight) Wight	Ad	L	86	23.2–37.5 = 33.5 ±1.2	5	27.7–32.7 = 30.4 ±0.8	9.23	A	A	A
		W	18.5–22.5 = 20.5 ±0.9							
	Ab	L	63	35.12–38.5 = 37 ±1.2	14	26.2–28.7 = 27.6 ±1.2	24.54	A	A	A
		W	24.5–30.5 = 26.5 ±1.5							
Vincetoxicum hirsutum (Wall.) Kuntze	Ad	L	71	52.4–24.7 = 43.6 ±2.1	8	18.7–22.2 = 20.6 ±0.5	A			
		W	82.6–34.9 = 23.9 ±1.3	20.7–24.5 = 20.6 ±1.7	A					
	Ab	L	63	42.2–34.1 = 38.9 ±1.2	7	525.7–768 = 116.2 ±1.4	A			
		W	22.5–14.2 = 22.5 ±0.4	18.75–22.2 = 20.6 ±1.5	A					
Vincetoxicum spirale (Forssk.) D.Z.Li	Ad	L	88	40.2–60.8 = 50.5 ±0.2	5	23.1–110.1 = 43.7 ±1.6	A			
		W	35.7–47.5 = 41.5 ±2.2	15.7–35.9 = 25.8 ±0.3						
	Ab	L	70	25.9–35.1 = 29.4 ±1.7	9	25.2–32.1 = 28.7 ±1.4	24.32	A		
		W	25.8–35.3 = 29.3 ±0.9	20.3–27.4 = 23.9 ±1.8	3					
Wattakaka volubilis Stapf	Ad	L	94	26.7–37.6 = 32.6 ±0.8	7	24.51–50.5 = 43.3 ±0.9	A			
		W	29.3–40.2 = 33.3 ±1.9	22.6–29.4 = 26.12 ±1.3	7					
	Ab	L	58	23.5–41.9 = 34.2 ±1.2	25	24.5–50.5 = 43.3 ±0.9	A			
		W	19.3–26.7 = 22.5 ±0.6	18.6–38.2 = 28.1 ±1.3	32.10					

Ad=Adaxial, Ab=Abaxial, L=Length, W=Width, M=Mean, SE=Standard Error, Max=Maximum, Min=Minimum, SI=Stomatal Index, TI=Trichome Index; Avg=Average; A=Absent

Epidermal Cell Morphotypes

Significant differences in foliar epidermis structure were observed on both the adaxial and abaxial sides of Apocynaceous taxa. The epidermis cells differ in size depending on the species being examined. Diverse epidermal cell types were observed; irregular, rectangular, polygonal, undulated and uniseriate (Table 2). Various types of anticlinal wall patterns were observed, i.e., angular, wavy, straight, undulating, slightly straight, rounded, curved, sinuous, and deeply sinuate. The average number of epidermal cells ranged from 24 to 88 on both surfaces. Similarly, the number of lobes per cell differs from 3 to 14 among Apocynaceous taxa.

The largest epidermal cell length and width were measured on the adaxial side of *Vinca major* (L = 59 .6 µm) and (W = 66.4 µm), respectively. Whereas on the abaxial surface, maximum length (L = 77.6 µm) and width (W = 47.5 µm) were also found in *V. major*. The shortest epidermal cell length (L = 26.7 µm) and width (W = 14.1 µm) were measured in *Trachelospermum jasminoides* on the adaxial surface, while on the abaxial side, minimum length (L = 17.5 µm) and width (W = 16.5 µm) were noted in *Beaumontia grandiflora* (Figure 9). The figure has been updated to present high-resolution micrographs with clearly defined scale bars and precise annotations. This allows the recorded dimensions to be accurately visualized and easily interpreted within the morphological context. Subsidiary cells have also been found in all species with considerable variations. Four subsidiary cell arrangement types were examined; margin sinuous, enclosing guard cells in most Apocynaceous taxa, followed by lobed margins, wavy, enclosing guard cells, and lobed margins, wavy and partly enclosing guard cell shapes. On the adaxial side of *Vincetoxicum spirale*, the largest subsidiary cell length (L = 52 µm) and the maximum length (L = 54 µm) on the abaxial side were noted. The width of the largest subsidiary cell was noted in *Asclepias curassavica* (W = 30.6 µm) on the adaxial surface, while in *Beaumontia grandiflora* on the abaxial surface (W = 28.4 µm), as summarized in Table 4.

Table 4. Taxonomic key based foliar epidermal characters of Apocynaceous species.

Character State	Present(+) Absent (-)	Diagnostic Features	Species name
1	-	Anomocytic stomata, straight curved anticlinal wall	*Alstonia scholaris*
	+	Amphistomatic leaves, undulate anticlinal wall, trichomes absent	*Asclepias curassavica*
2	-	Straight anticlinal wall, Multicellular glandular trichomes	*Beaumontia grandiflora*
	+	Diacytic stomata and raised, smooth anticlinal wall	*Calotropis gigantea*
3	-	Straight anticlinal wall, paracytic stomata	*Calotropis procera*
	+	Anomocytic stomata, undulated anticlinal wall	*Carissa carandas*
4	-	Straight anticlinal wall, anomocytic stomata	*Carissa macrocarpa*
	+	Straight curved anticlinal wall, anomocytic stomata	*Carissa spinarum*
5	-	Sinuated anticlinal wall, anisocytic stomata	*Cascabela thevetia*
	+	Anisocytic stomata, undulate anticlinal wall	*Catharanthus roseus*
6	-	Paracytic stomata, sinuate to way anticlinal wall	*Cryptolepis dubia*
	+	Perpendicular anticlinal wall, anomocytic stomata	*Cynanchum acutum*
7	-	Rectangular anticlinal wall, Multicellular non-glandular trichomes	*Nerium oleander*
	+	Rectangular anticlinal wall, Non-glandular intercostal trichomes	*Oxystelma esculentum*
8	-	Anomocytic stomata, unicellular trichomes	*Pergularia daemia*
	+	Paracytic sunken stomata, undulate anticlinal wall	*Pergularia tomentosa*
9	+	Leaves hypostomatic, anisocytic stomata, straight anticlinal wall, multicellular trichomes	*Plumeria rubra*
	-	Straight anticlinal wall, paracytic stomata	*Rhazya stricta*
10	+	Anomocytic stomata, straight anticlinal wall	*Tabernaemontana divaricata*
	-	Sinuate anticlinal wall, uniseriate non-glandular trichomes	*Trachelospermum jasminoides*

Morpho-Structure of Stomatal Complex

In the present study, stomata were observed on leaf surfaces in all Apocynaceous species. Thirteen species have anomocytic types of stomata, followed by anisocytic types (3 species) and paracytic stomata (7 species),

while in *Calotropis gigantea* and *Nerium oleander*, the diacytic type of stomata and crypts on the epidermal surface was examined, respectively. Stomatal shape differences were observed as elliptic, elongate elliptic, and broad elliptic were observed in *Alstonia scholaris*, *Asclepias curassavica*, and *Catharanthus roseus*. The difference in stomatal size varied among species, and on the lower surface, the largest stomatal size was observed than on the upper surface. Lengthwise, the largest stomata were noted in *Vincetoxicum spirale* (L = 43.7 μm) and the maximum width along the adaxial surface (W = 30.4 μm) was measured for *Cynanchum acutum*. Whereas maximum length (L = 48.6 μm) and width (W = 40.4 μm) along abaxial side was calculated in *Beaumontia grandiflora*.

The guard cells were mostly examined with a kidney shape, while some species with broad kidney shaped guard cells were examined. Guard cells mean variations in length ranged from (L = 16.28 μm) in *Alstonia scholaris* to (L = 31.85 μm) in *Vinca major*. Quantitatively stomatal pore showed variations as shown in Table 4, on both sides length was observed maximum in *Vincetoxicum spirale* (L = 22.10 μm) and *Nerium oleander* (L = 18.65 μm) respectively. Whereas minimum length was noted for *Cynanchum acutum* on adaxial side (L = 9.75μm) and of *Cascabela thevetia* along abaxial surface (L = 4.6 μm). On the adaxial side, maximum width was observed in *Catharanthus roseus* (L = 9.75 μm), while on the abaxial in *Trachelospermum jasminoides* (L = 16.10 μm). *Cynanchum acutum* had a minimum width of (W = 4.20 μm) on the adaxial surface and *Vincetoxicum hirsutum* (W = 2.30 μm) on the abaxial surface. For many species, the stomatal index was measured on both the adaxial and abaxial surfaces of the epidermis. Stomatal index was measured maximum and minimum along adaxial and abaxial sides in *Asclepias curassavica* (24.58% and 7.29%), respectively. While stomatal index on abaxial surface was calculated (35.71%) in *Calotropis procera*, whereas minimum along adaxial surface (8.92%) in *Vincetoxicum arnottianum*.

Trichome Appendages

Table 2 presents 12 species out of 25 that have trichome of various forms, i.e. slender, unicellular, uniseriate, multicellular, stellate, capitate, glandular, and non-glandular. Selected light and scanning micrographs of different types of trichomes are shown in different figures. Widely distributed glandular and non-glandular, long and unbranched trichomes were recorded on the epidermis of most of the Apocynaceous species. The longest trichome was found on the adaxial surface in *Pergularia tomentosa* (L = 135.5 μm), while the smallest was examined for *Rhazya stricta* (L = 125.7 μm). On the abaxial surface, maximum length was measured for *Asclepias curassavica* (L = 262 μm), whereas the shortest in *Plumeria rubra* was (L = 38.3 μm). Differences were also observed in the trichome width along the adaxial side, with a maximum in *P. rubra* (W = 37.2 μm) and a minimum in *A. curassavica* (W = 16.50 μm). While the abaxial surface of *P. rubra* has a maximum width of (W = 37.2 μm), whereas the minimum examined in *Asclepias curassavica* is (W = 16.50 μm).

Trichome index was recorded highest (25.05%) on the adaxial surface of *Pergularia tomentosa* and lowest (22.67%) for *Rhazya stricta*. Whereas on abaxial side maximum was measured for *Trachelospermum jasminoides* (42.42%) and minimum (26.41%) for *Rhazya stricta*.

Taxonomic Key Constructed Based on Anatomical Traits

The discussed foliar anatomical features in Apocynaceaea was distinguished further to accurately identified the species constructing the taxonomic key.

UPGMA Cluster Analysis

The dendrogram revealed hierarchical clustering between foliar micro-morphological qualitative and quantitative traits of 25 Apocynaceae species. The Euclidean cluster analysis based on similar traits reveals various levels of phenotypic relationships (Figure 11). The cluster analysis was carried out by the Euclidean distance method, based on 25 foliar micromorphological attributes. The main cluster (C1) of the dendrogram based on

similar characters using qualitative and quantitative data is divided into 2 main groups represented by C1 and C2. Group 1 (C1) has only single species including *Vinca major*, while group 2 (C2) comprises 24 species two further sub-cluster. Furthermore, C2 is divided into 2 sub-groups (C2a1) and (C2a2). In sub-cluster C2a1 *Carissa macrocarpa* and

Cynanchum acutum were closely placed due to similar anomocytic stomatal character. In sub-cluster, C2a2 *Oxystelma esculentum* and *Pergularia daemia* were shown to have the least Euclidean distance shared similar epidermal cell shape and anticlinal wall pattern.
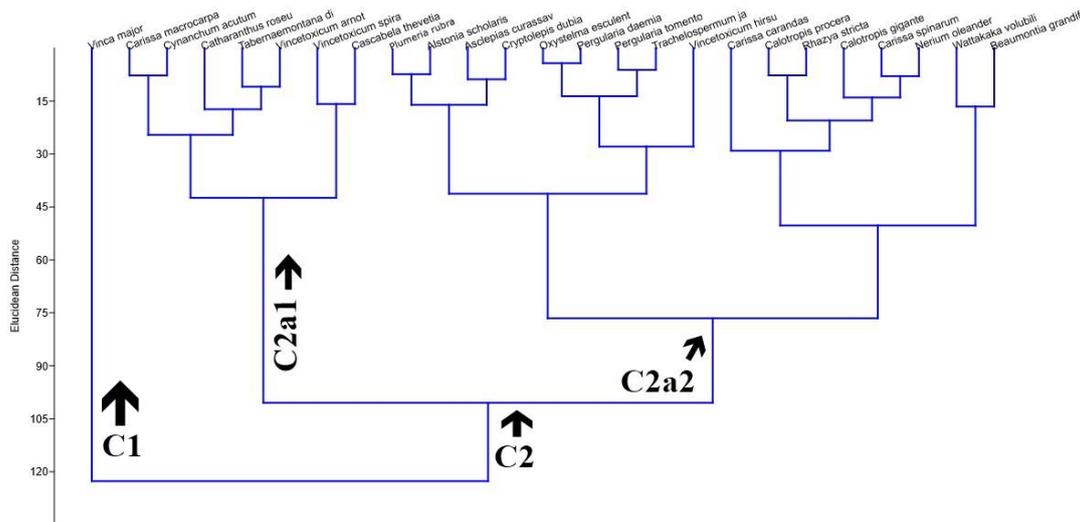
Figure 11. Cluster groupings via dendrogram of Apocynaceous species based on foliar epidermal morphometric features

PCA Cluster Analysis

The resemblance in the attributes in context of correlation variance factor statistical principal component analysis (PCA) was performed using microanatomical traits of 25 Apocynaceaous species (Figure 12, Table 5). Total number of variables in principle component analysis is represented by Eigenvalues, which is frequently used to assess the number of factors to retain. According to metric variables loadings of PCA biplot, a total of (65.55%) variations were observed for the first two principal component. The PC1 represent 41.64% variance show strong positive relation with respect to stomatal length and width whereas trends shows strong negative relation between the abaxial epidermis length and width. In PC2 (23.91%) variance was observed negative relation with respect to width of guard cell, while strong positive relationship with respect to length of guard cells.

Table 5. Eigenvalues, percentage of total variance explained by each axis among Apocynaceous species.

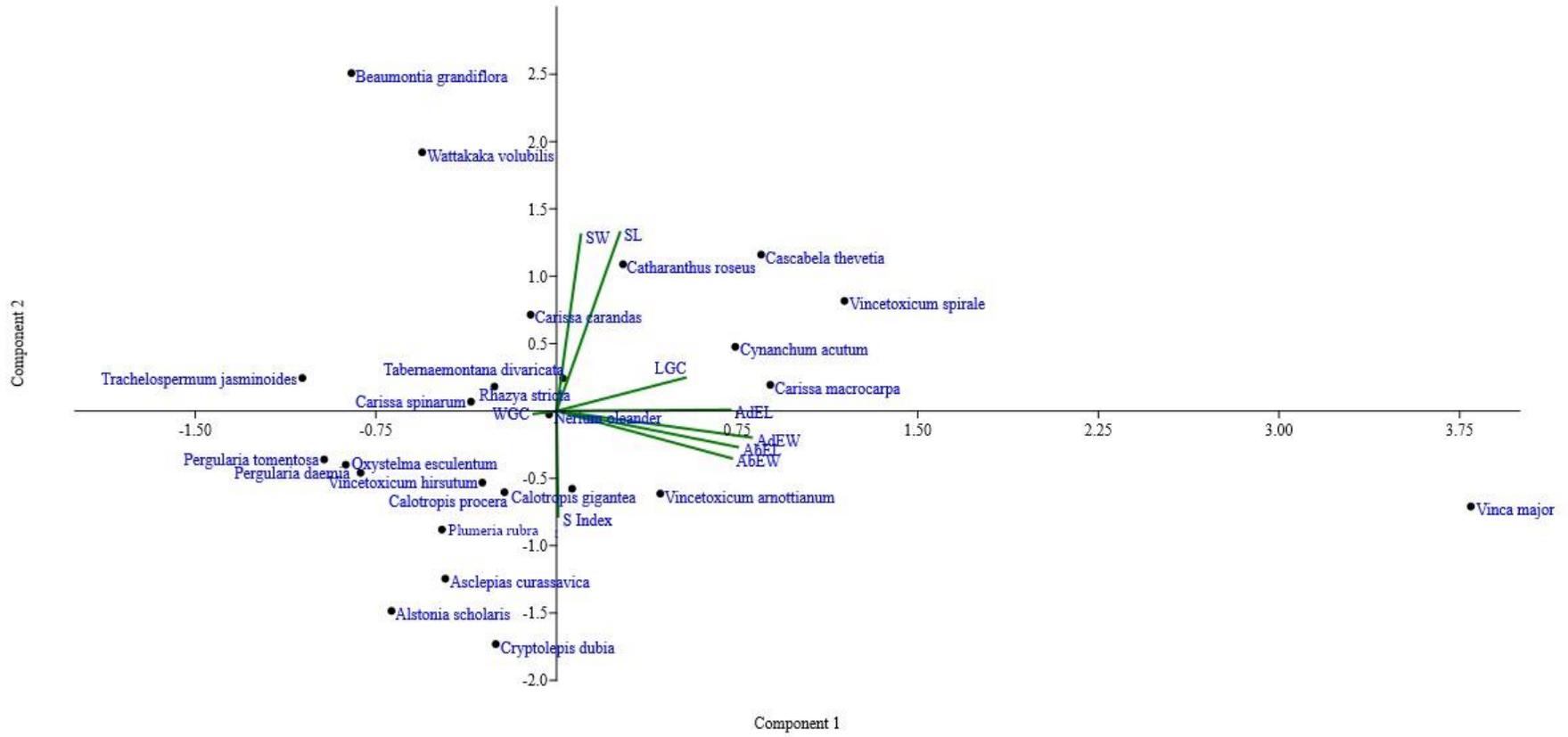| PC	Eigenvalue	% Variance
1	3.33153	41.644
2	1.91312	23.914
3	1.07228	13.403
4	0.719448	8.9931
5	0.525355	6.5669
6	0.274055	3.4257
7	0.115281	1.441
8	0.048934	0.61168

445

Figure 12. Principal component analysis (PCA) biplot of matric variables of Apocynaceous taxa

Discussion

In the present study, the anatomical and surface foliar epidermal characteristics of the leaves belonging to nineteen genera (*Alstonia, Asclepias, Beaumontia, Calotropis*, *Carissa, Cascabela, Catharanthus, Cryptolepis, Cynanchum, Nerium, Oxystelma, Pergularia, Plumeria, Rhazya, Tabernaemontana, Trachelospermum, Vinca, Vincetoxicum* and *Wattakaka*) of Apocynaceous taxa were examined using LM and SEM. Light microscopy (LM) and scanning electron microscopy (SEM) were used in combination to provide a comprehensive assessment of foliar anatomy. LM enabled quantitative evaluation of internal epidermal features, while SEM offered high-resolution imaging of surface micromorphology. This integrative approach enhanced taxonomic resolution by capturing both structural and ultrastructural details essential for species differentiation. The SEM has been shown to be quite useful for observing the surface morphology of plant material due to its better depth of field and high resolution, which are not attainable with the LM (Bahadur et al., 2018). The purpose of the study was to identify diagnostic traits that may be used to distinguish among Apocynaceous genera and species. Based on both quantitative and qualitative anatomical traits, cluster analysis via dendrogram and PCA ordination of analyzed species revealed taxonomic importance. Statistical accuracy was confirmed through PCA explaining 65.55% of total variance, a cophenetic correlation coefficient of 0.85 for cluster analysis, and a KMO value of 0.76 with Bartlett's test showing significance (p < 0.001), supporting the robustness of the analysis. This study provides comprehensive micro-morphological information among Apocynaceous taxa using botanical techniques. The micro-morphological attributes basically coincide slightly with the descriptions of other Apocynaceous species previously studied using an optical microscope (Abdalla et al., 2016; EL-Fiki et al., 2019; Bashir et al., 2020; El-Taher et al., 2020).

Leaf epidermal micro-morphological traits of 25 species belonging to nineteen genera, which were not previously explored using a scanning electron microscopy approach, have

demonstrated significant taxonomic significance for their accurate identification and relationships among them. Earlier, a few studies on the micro-morphological characters of various species of Apocynaceous were carried out by Khan et al. (2014), Nisa et al. (2019) and Bashir et al. (2020). However, no comprehensive study of the leaf microstructure of Apocynaceous species in the dry and moist subtropical region has been conducted. Therefore, the current report was undertaken with the aim of providing an identification guide based upon foliar micro-morphology using scanning microscopy.

The leaf epidermis is the subject of most taxonomic studies, and the foliar epidermal structure is one of the most significant taxonomic aspects in biosystematics. Epidermal cells, stomata, trichome sizes, lengths, distribution, orientation, and frequency, as well as their distribution, orientation and frequency are all significant phylogenetic indicators (Voigt et al. 2021; Majeed et al., 2024). However, according to Akinsulire et al. (2020), the leaf possesses a range of anatomical traits that are taxonomically significant. Certain epidermal characteristics, such as the morphology of subsidiary cells of the stomata, microhairs, trichomes, and prickles, are of systematic relevance, according to Metcalfe (1968). The size, shape, and orientation of stomata, guard cells and subsidiary cells, structural peculiarities of epidermal cells, and frequency of stomata are just a few of the significant diagnostic features found in the epidermis that can aid in identification. Akinsulire et al. (2018) also stated that data recorded in leaf micro-morphological studies were taxonomically useful and the characters elaborated include the stomatal distribution, which is largely hypostomatous, though sometimes amphistomatous as documented.

Plant species that have anticlinal walls of epidermal cells that are curved or straight are features of plant species that flourish in dry environments (Munir et al., 2011). According to Fajuke et al. (2018), as both stomatal index and guard cell area exhibited significant interspecific variation among the 25 Apocynaceous taxa examined. These parameters demonstrated taxonomic

relevance by contributing substantially to the variance explained in PCA (stomatal index and guard cell length/width strongly loading on PC1 and PC2, respectively). Their consistent differentiation among genera and species supports their utility as reliable diagnostic traits for taxon delimitation within the family.

Foliar epidermal features are often regarded as a useful technique for establishing taxonomic relationships. In the case of taxonomic resolutions in the Apocynaceae, SEM is preferable to other traditional microscopic methods because it reveals different characteristics of significant microstructural features (Shaheen et al., 2010). Many researchers have used plant anatomical methods to resolve identification problems among groups of plants from diverse phyto-geographic regions of Pakistan. Such studies include the works of Gul et al. (2019) on Lamiaceous species., Rashid et al. (2020) on some species of tribe *Trifolieae*, Shah et al. (2019) on selected species of ferns from family Pteridaceae and Dryopteridaceae, Ullah et al. (2018) on the Caryophyllaceae species., Raza et al. (2022) on selected *Acanthus* species, Ashfaq et al. (2019) on Convolvulaceous taxa and Khan et al. (2019) on identification of Gymnosperms.

Agustiar et al. (2020) and Bashir et al. (2020) showed in *Plumeria rubra* the paracytic type of stomata, glandular trichomes which are close to current findings where only elliptic shape, multicellular glandular trichomes are present on the abaxial surface. According to EL-Fiki et al. (2019) and Bashir et al. (2020) trichomes are absent but two types of stomata, anisocytic and anomocytic, were found in different varieties of *Alstonia scholaris* whereas the stomatal index may also varies. Similarly, these findings also supported our findings of absence of trichomes, anisocytic type and elliptic stomata. In the specie *Asclepias curassavica*, irregularly arranged layers of epidermal cells, trichome absent, anomocytic stomata were observed on the both surfaces of the leaf. El-Taher et al. (2020) reported previously the acute apex that are bent and rounded, polygonal and hexagonal shaped epidermal cells, stomata were flat with a slit on the abaxial and adaxial surface, these findings

were differ from our findings. We found rectangular shaped epidermal cells, anomocytic and multicellular non-glandular trichomes in *Beaumontia grandiflora*, which is similar to the findings of EL-Fiki et al. (2019). Hence, anatomical attribute sensitivity in *Calotropis gigantea* could change to adapt to the water regime reflected in stoma density on leaf surfaces (Tao et al., 2009; Ullah et al., 2025). While our findings explain rectangularly shaped epidermal cells having smooth anticlinal walls epidermis and diacytic stomata, trichomes were absent in *C. gigantea.* While xeromorphic plant *C. gigantea* have amphistomatic leaves, anomocytic and elliptical stomata (Abeysinghe, 2022) these results differ from our findings.

The anatomical features of *Calotropis procera* having paracytic stomata, absence of trichomes, rectangular epidermal cells, elongate elliptic shaped stomata, these finding were not similar to Abeysinghe (2022), having amphistomatic, anomocytic stomata and diacytic (Ahmad et al., 2009), guard cells were surrounded by four to five irregularly shaped subsidiary cells. The current findings in *Carissa carandas, Carissa spinarum*, *Carissa macrocarpa* shows hypostomatic leaves, regular to flat epidermal cells, the absence of trichomes on both sides, Paracytic to anomoscytic stomata, undulated, straight to straightly curved anticlinal walls pattern respectively. Our findings differ from EL-Fiki et al. (2019), Bashir et al. (2020), El-Taher et al. (2020) and Alsudani & Altameme (2021) having anisocytic, cyclocytic and stephanocytic type of stomata, epidermal cell are oblong, square shape, polygonal to irregular. According to our observations *Cascabela thevetia* have anisocytic stomata, sinuated anticlinal wall pattern, absence of trichomes and *Catharanthus roseus* have polygonal epidermal cells, anisocytic stomata, absence of trichome, undulate anticlinal wall pattern. These observations relate with the findings of Khan et al. (2014), Abdalla et al. (2016) and Bashir et al. (2020).

According to Joubert (2013), EL-Fiki et al. (2019) *Cryptolepis dubia* and *Cynanchum acutum* comprises of tabular periclinal wall, striated cuticle, absence of trichomes, uniseriate epidermis, have been identified in

the research work. These findings mainly varies with our observations including polygonal to irregular epidermal cells, sinuated to perpendicular anticlinal wall pattern, paracytic to anomocytic stomatal type and absence of trichomes. The non-glandular trichomes, regular to polygonal epidermal cells , stomata are in the form of crypts to anisocytic in *Nerium oleander* confirms our findings from those of Abdalla et al. (2016), According to research work of Poornima et al. (2009) in *Oxystelma esculentum*, stomata are amphistomatic to anomocytic type, stomatal index is more at the abaxial surface , these findings confirms our observations of anomocytic stomata, abaxial surface with more stomatal index and multicellular glandular trichome. The present findings show that the *Pergularia daemia* and *Pergularia tomentosa* has mostly anomocytic types of stomata, unicellular trichomes, rectangular anticlinal wall pattern and paracytic type, undulate anticlinal wall, absence of trichomes, which is similar to the earlier work of (Patil & Malphatak, 2016) and (Bhoyar and Biradar, 2016) who reported paracytic and anomocytic types of stomata, trichome is present in *P.daemia* while absent in *P.tomentosa* respectively. According to the recent work of Bukhari et al. (2017), *Rhazya stricta* had anomocytic stomata, it varies with our findings, the species has paracytic stomata and absence of trichomes.

El-Taher et al. (2020) described trichome and stomatal diversity in *Tabernaemontana divaricata* with an anomocytic stomatal type, verrucose ornamentation of epidermal cells, while our research shows similarities having rectangular epidermal cells, anomocytic, and absence of trichomes. In foliar epidermal anatomy in *Trachelospermum jasminoides* and *Vinca major* revealed a paracytic type of stomata, uniseriate non-glandular trichomes and sinuated anticlinal wall pattern, absence of trichomes, respectively. But our observations varies with Ciorita et al. (2021) having a arrow head stomata, non-glandular trichomes were only present in the midvein.

In *Vincetoxicum arnottianum* and *Vincetoxicum hirsutum have* anomocytic type of stomata, straight anticlinal wall pattern and unicellular non-glandular trichomes, respectively. These results confirms from the findings of Nisa et al. (2019) and El-Taher et al. (2020) having anomocytic to paracytic stomatal type. According to Ilcim et al. (2010) and Shivani et al. (2023), *Vincetoxicum spirale* and *Wattakaka volubilis* have simple trichomes, hypostomatic and anomocytic type which relates with our observations as non-glandular trichomes and anomocytic stomatal type.

Conclusion

This study is based on the hypothesis that foliar micromorphological and anatomical traits such as stomatal configuration, trichome morphology, and epidermal cell patterns can serve as stable, species-specific markers for the taxonomic identification of Apocynaceae taxa in riparian zones of Punjab, Pakistan. The current findings reveal that foliar anatomical features, including the diversity of epidermal cells, trichome morphology, and variation of stoma types, are potentially useful to delimit the taxa at a specific level. Apocynaceous species can easily be differentiated on account of their epidermal cell shape. The size of epidermal cells, their shape, and the stomata type of these species were considerably variable and different from each other.

The diversity in the foliar trichomes at the species level also served as a useful taxonomic tool. Each trait has its own systematic importance in the delimitation of taxa. Given the limitations of macromorphological features due to ecological plasticity and morphological overlap among species, a comparative analysis using light microscopy (LM) and scanning electron microscopy (SEM) was conducted on 25 representative taxa. The study is justified by the need for reliable anatomical datasets to resolve taxonomic ambiguities and to understand structural adaptations of these species to riparian environments, thereby contributing to more accurate classification and systematic documentation of the family in the region. Future research should include a wider range of Apocynaceous species to improve comparative analyses and taxonomic resolution. Integrating micromorphological data with molecular tools like DNA barcoding could help clarify species boundaries. Advanced imaging techniques such as SEM

are also recommended for more precise trait analysis. Additionally, considering ecological influences on foliar traits and creating a centralized micromorphological database would support accurate identification, pharmacognostic use, and biodiversity conservation.

Ethics Committee Approval
N/A

Peer-review
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Author Contributions
Conceptualization, M.R.K., M.Z., and S.M.; methodology, T.M., N.N., and S.I.; data curation, M.I., A.S., and M.O.B.; sample collection and identification, L.B. and N.E.; writing—original draft, M.R.K., M.Z., and S.M.; writing—review & editing, N.I. and S.I.; supervision, S.M.; project administration, S.M.; funding acquisition, S.M. All authors have read and approved the final manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

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