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Research Article

A Morphometric Study of Epidermal Appendages in Commonly Existed Angiosperms in Faisalabad, Pakistan

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Abstract: Epidermal appendages are mono-cellular or multicellular, root hairs, and trichomes that grow on an epidermis. Commonly found members of angiospermic families were selected focusing on their presence or absence, position, and types of surface appendages (colliculate, hairy, wart and papil). Trichomes types (simple hairs, tubercle-based hair minerals) concrete, secretory hairs, vesicular hairs, moniliform hairs, and dendritic hairs were also considered. The epidermal appendages were studied in thirty species of angiospermic families collected from Botanical Garden University of Agriculture Faisalabad, Pakistan, by using light microscope. The data was then subjected to ANOVA and cluster analysis to investigate relationship among different angiosperm species. The Maximum cell length/width on abaxial epidermis is recorded in *Strelitzia reginae Regel & Koch* and *Terminalia bellirica* (Gaertn.) Roxb, which is closely related to the *Alpinia allughas* Retz., and *Crinum asiaticum L.*. Minimum cell length on upper epidermis is recorded in *Ficus lyrata* which is closely related to the *Colocasia esculentum.* Maximum number of stomata is observed in *Kigela africana.* Minimum number of stomata is recorded in *Terminalia bellirica* and *Ficus lyrata*. Maximum number of stomata on adaxial epidermis is recorded in *Pentas lanceolata.* Minimum number of stomata is recorded in *Ravenala medaghas cariensis*. On adaxial surface, the maximum number of trichomes is recorded in *Terminallia bellirica* and *Kigela Africana*, and the minimum number is recorded in *Campsis rodicum.* The present study emphasizes the distinctive and similar features of those epidermal appendages. These observations will provide as a basis for subsequent research on the physiology and ethnobotany of the chosen species.

Keywords: Plants, Families, Leaves, Root Hairs, Stomata, Trichome.

1. INTRODUCTION

Flowering plants, also called angiosperms, are recognized members of numerous plant families worldwide (total 400-500) with around 4 million species. These plants, constituting at least 95% of all vascular plant species, significantly impact the human world and its survival. Taxonomy plays a crucial role in categorizing and distinguishing plant species by organizing them according to morphological, epidermal, and phytochemical traits [1]. Within plant taxonomy, diverse plant species are designated and described based on genus and species. Various taxonomists utilize epidermal features to recognize plants belonging to specific genera and families. In the field of plant taxonomy, attributes like trichome, stoma characteristics, and anticlinal cell wall patterns in epidermal cells are employed for identification. [2-5]. Epidermal traits encompass epidermal cells, stomata, and epidermal inserts. These attributes play a crucial role in the classification of taxa within different plant families

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[6]. Epidermal analysis mainly involves the classification of stoma type and trichome variation [7]. Trichomes and hydathodes represent additional characteristics that can serve as effective taxonomic instruments in the epidermis and other plant organs. Numerous systematic approaches have employed them for both systematic and classification objectives [8-12].

A specific trichome species frequently serves as a distinguishing feature for species, genera, and entire plant families. Trichome morphology is a key aspect in characterizing the epidermal properties within the taxonomy of the Combretaceae family. Trichomes, as a taxonomic trait, hold significance across various plant groups, ranging from specific to familial levels. Different feather types are specified to highlight distinct sub-genera and sections within the *Jatropha* L. genus [13]. The characteristics of the leaf epidermis are becoming more and more important in solving the current taxonomic disturbances [14, 15].

In the realm of leaf epidermis, anatomical features like stomata, trichomes, and other markers have demonstrated their potential taxonomic significance as valuable anatomical tools [16, 17]. In leaves of dicotyledonous plants, stomata are dispersed, while in monocotyledonous leaves, they are arranged in parallel rows. The quantity of stomata on leaf surfaces varies considerably among distinct plant species, but typically, the lower epidermis of the leaf tends to possess a greater number of stomata compared to the upper surface [18]. Plant stoma patterns and distribution were found to be very useful as diagnostic tools in plant taxonomy and systematic [19]. Trichomes are found on the surfaces of plant organs, serving as outer extensions of living cells that interface initially with the external environment surrounding the plant.

The scientific literature proposes various functions of trichomes, including safeguarding against herbivores and small chewing insects, minimizing transpirational water loss, aiding gaseous exchange in humid conditions, and attracting pollinators [20-22]. The aim of this study was to explore the diversity, taxonomic traits, types, positioning, patterns, and significance of epidermal appendages observed in different families of angiosperm plants.

2. MATERIALS AND METHODS

2.1. Collection and Preservation of Leave Samples

Frequent visits of botanical gardens were carried out in university of agriculture, Faisalabad, to collect leaves specimens of some angiosperm families. In total 30 species of angiosperm plants were considered (Table 1). The leave samples were preserved by labeling them. The leave samples were preserved in anatomical jars, dipping them in 70% methanol solution. The observations and measurements were taken many times in order to ensure accuracy.

2.2. Anatomical Studies

The preserved samples as well as fresh leaves were used for anatomical features studies. For the study of different anatomical features light microscope was used. Magnifier 10X was used for observation of epidermal peels of leave specimens. The epidermis of the leaves specimens was peeled-off with the help of scalpel razor blade as its method has proved to be effective and accurate for peeling-off the large number of specimens. The debris on leave was removed by brush. To peel off the abaxial surface the leaf must be placed on the tile with its adaxial surface facing upwards. The adaxial surface was scrapped until the abaxial surface appeared. The epidermal peels were dipped in water for few seconds so as to clear the surface from chloroplasts (traces of greenish material). And then peels were washed with water. The abaxial and adaxial epidermis was removed along with the mesophyll cells by using scalpel blade, until only the epidermis of the leaf remained on the tile. The epidermal peels of scrapped surfaces were placed on the clean glass slides also putting few drops of water and cover the peels with cover slips. The following anatomical Characteristics of both the abaxial and adaxial epidermis were studied during investigation:

- Epidermal cell Length and width
- Stomatal number
- Trichome number & length

2.2.1. Cell length

To measure the length of cells in the abaxial and adaxial epidermis of leaves, fresh leaf samples are collected and the epidermal layers are carefully

Table 1. Trichome analysis from the study of epidermal appendages in some angiosperm families.

Sr. No.	Species	Common name	Family	Trichome type abaxial/adaxial
1	*Hemarocallis fulva* L.	Orange day-lilly	Asphodelaceae	Absent/absent
2	*Pentas lanceolata* (Forssk.) Defiers	Egyptian star cluster	Rubiaceae	Absent/Absent
3	*Chlorophytum comosum* (Thunb.) Jaques.	Spider plant	Asparagaceae	Absent/Present
4	*Alpinia allughas* Retzius.	Black galangal	Zingiberaceae	Absent/Absent
5	*Ravenala medaghas* Sonn.	Traveler tree, Traveler palm	Strelitzaceae	Absent/Absent
6	*Strelitzia reginae* Banks.	Crane flower, Bird of Paradise	Strelitzaceae	Absent/Present
7	*Gardenia floribunda* L.	Cape jasmine	Rubiaceae	Present/T-shaped
8	*Rhondelatia adorata* Jacq.	Cleveland	Rubiaceae	Absent/ Unicellular
9	*Campsis rodicum* (L.) Seem. ex-Bureau.	Trumpet vine	Egnoniaceae	Absent/ Hair clusters
10	*Campsis grandiflora* (Thunb.) K. Schum.	Chinensis trumpet vine	Egnoniaceae	Absent/Absent
11	*Cana indica* L.	Wild cana lilly	Cannaceae	Absent/Absent
12	*Colocasia esculentum* (L) Schott.	Elephant ears	Araceae	Absent/Present
13	*Cricum asiaticum* Linn.	Giant Cricum asiaticum	Amaryllidaceae	Present/Present
14	*Barlaria cristata* L.	Philippine violet	Acanthaceae	Absent/Absent
15	*Terminallia bellirica* (Gaertn.) Roxb.	Bastard myrobalan	Combretaceae	Unicellular/Unicellular
16	*Morus leavigata* Wall.	Mulberries	Moraceae	Absent/Absent
17	*Schizinthus terebunthifolus* Raddi.	Brazilian pepper tree	Anacardaceae	Present /T-shaped
18	*Litchi chinensis* Sonn.	Lychees	Sapindaceae	Absent/Absent
19	*Kigela Africana* (Lam.) Benth.	Sausage	Bignoniaceae	Absent/Unicellular
20	*Tamarindis indica* L.	Tamarind	Fabaceae	Short branched/present
21	*Artocarpous lakucha* Roxb.	Monkey fruit	Moraceae	Present/present
22	*Erthrina herbaca* L.	Cherokee bean	Phylanthaceae	Short-branched/ dendritic
23	*Hematoxylon campechianum* L.	Logwood tree	Fabaceae	Absent/Absent
24	*Diospyrose embryopteris* (Desr.) Kostel.	Butter fruit	Enabaceae	absent /Hair-clusters
25	*Annona squamosal* L.	Sugar apple	Annonaceae	Absent/ Absent
26	*Lawsonia innermis* L.	Henna	Lythraceae	Absent/Absent
27	*Ficus netalensis*Hochst.	Banyan	Moraceae	Absent/Absent
28	*Ficus lyrata* Warb.	Fiddle leaf fig	Moraceae	Absent/Short branches
29	*Ficus bengalensis* L.	Natal fig matuba	Moraceae	Absent/Absent
30	*Ficus elastic* (Roxb.) Hornem.	Rubber fig	Moraceae	Absent/Absent

peeled off. The epidermal strips are cleared using a 10% NaOH solution or a commercial clearing agent, then stained with 0.1% toluidine blue to enhance cell visibility. The stained samples are mounted on glass slides and observed under a light microscope at 40x magnification. Digital images are captured and analyzed using image analysis which is calibrated with a micrometer scale for accurate measurements. A statistically significant number of cells are measured from multiple fields of view on both the abaxial and adaxial surfaces. The average cell length and variability are then determined through statistical analysis [23, 24].

2.2.2. Stomatal number and length

To identify and calculate the number of stomata, fresh leaf samples are collected and cleared using a 10% NaOH solution, then neutralized in 5% acetic acid. The cleared samples are stained with 0.1% toluidine blue to highlight the stomatal structures. The stained leaf samples are mounted on glass slides and observed under a light microscope at 40x or higher magnification. Images are captured using a digital camera attached to the microscope. Stomatal density is calculated by counting the number of stomata in a defined area (e.g., 0.1 mm^2) and averaging the counts from multiple fields of view, expressed as stomata per square millimeter. Stomatal size is measured using the software's measurement tool, and statistical analysis is performed to determine the average size [25].

2.2.3. Trichome number and length

Fresh leaf samples were collected carefully. Using a stereomicroscope or light microscope at 10x to 40x magnification, trichomes were observed and counted in a defined area (e.g., 1 cm^2) on both the abaxial and adaxial surfaces. The length of individual trichomes was measured using image analysis software or a calibrated eyepiece. Recent studies have utilized similar methods for accurate trichome analysis [26, 27].

2.3. Statistical Analysis

The data on stomatal, trichome number and length were analyzed using ANOVA to identify significant differences among angiosperm species. The cluster analysis was performed to explore relationships and groupings based on trichome characteristics. The data was then subjected to multivariate (PCA and cluster) analysis to investigate the relationship between different angiosperm species samples collected from new and old botanical gardens of the university of agriculture, Faisalabad, Pakistan.

3. RESULTS

3.1. Comparative Leaf Epidermal Studies

3.1.1. Abaxial epidermal cell length/ width

Maximum cell length/width on abaxial epidermis is recorded in *Strelitzia reginae* and *Terminalia bellirica* which is closely related to the *Alpinia allughas, Crinum asiaticum*. Minimum cell length on upper epidermis is recorded in *Ficus lyrata* which is closely related to the *Colocasia esculentum* (L). Schott (Figure 1(a)).

3.1.2. Adaxial epidermal cell length/width

Maximum cell width is recorded in *Lawsonia innermis* and *Ficus netalensis* L., and while other shows little variations in cell width. Minimum cell width on abaxial epidermis is recorded in *Gardenia floribunda* L. and *Ficus lyrata* Warb (Figure 1(b)).

3.1.3. Abaxial epidermal stomatal length

Maximum stomatal length is recorded in *Gardenia floribunda, Campsis rodicum.* Minimum stomatal length is recorded in *Terminelia bellirica* and *Ficus lyrata*.

3.1.4. Adaxial epidermal stomata length

Maximum stomatal length is recorded in *Ravenala medaghas cariensis* and *Litchi chinensis.* Minimum stomatal length is recorded in *Diospyros embryopteris* and *Rhondolatia adorata.*

3.1.5. Abaxial number of stomata

Maximum number of stomata is observed in *Kigela africana.* Minimum number of stomata is recorded in *Terminalia bellirica* and *Ficus lyrata* (Figure 2(a)).

3.1.6. Adaxial number of stomata

Maximum number of stomata on adaxial epidermis is recorded in *Pentas lanceolata.* Minimum number of stomata is recorded in *Ravenala medaghas cariensis* (Figure 2(b)).

3.1.7. Abaxial trichome length

Maximum length of trichome on abaxial epidermis is recorded in *Tamarindis indica* and *Terminallia bellirica*. Minimum length is observed in *Gardenia floribunda* (Figure 3(a)).

3.1.8. Adaxial trichome length

Maximum trichome length is recorded in *Tamarindis indica* and *Colocasia esculentum*. Minimum trichome length is recorded in *Chlorophytum comosum* and *Gardenia floribunda* (Figure 3(b)).

3.1.9. Abaxial number of trichomes

Maximum number of trichomes is recorded in *Terminallia bellirica and Schizinthus terebunthifolus* and while minimum number is recorded in *Gardenia floribunda* (Figure 4(a)).

3.1.10. Adaxial number of trichomes

Maximum number of trichomes is recorded *Terminallia bellirica and Kigela africana.* Minimum number is recorded in *Campsis rodicum* (Figure 4(b)).

Dendogram is clustered into two main groups first group contains 15 species and second group contains 15 species. Both groups are further subclustered in two more groups. In first group *Tamarindis indica and Erthrina herbaca shows more similarity as they are close to cluster.* In second group *Hemamatoxylon campechianum and Ficus netalensis* more similarity as they are close to each other.

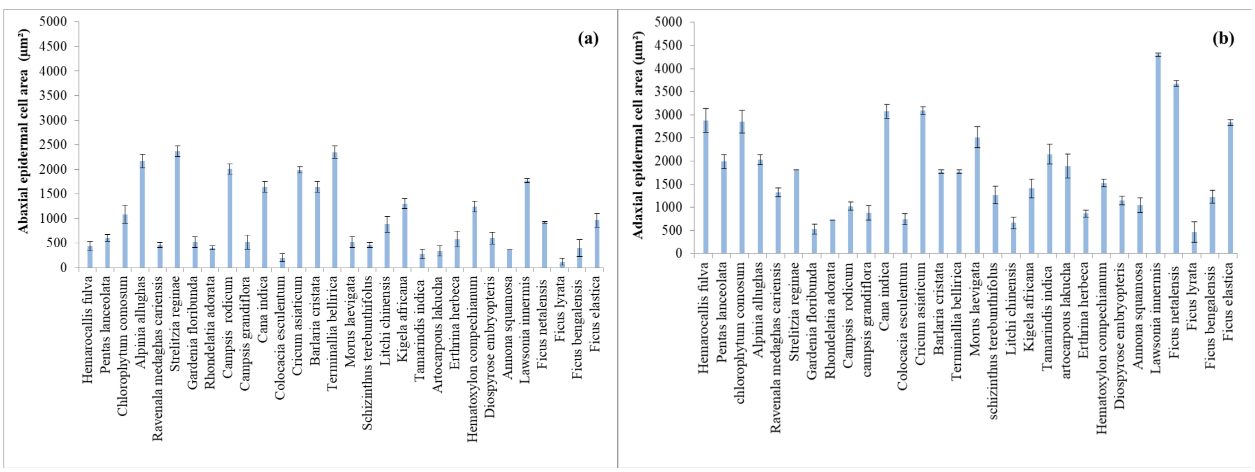

Fig. 1. (a) Abaxial Epidermal cell areas (µm²) from the study of epidermal appendages in some angiosperm families and **(b)** Adaxial epidermal cell areas (µm²) from the study of epidermal appendages in some angiosperm families.

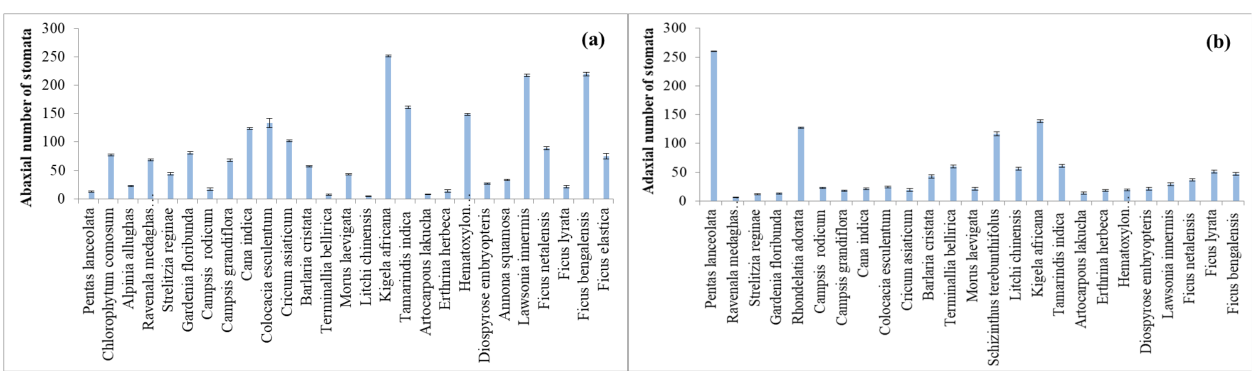

Fig. 2. (a) Abaxial stomata number from the study of epidermal appendages in some angiosperm families and **(b)** Adaxial stomata number from the study of epidermal appendages in some angiosperm families.

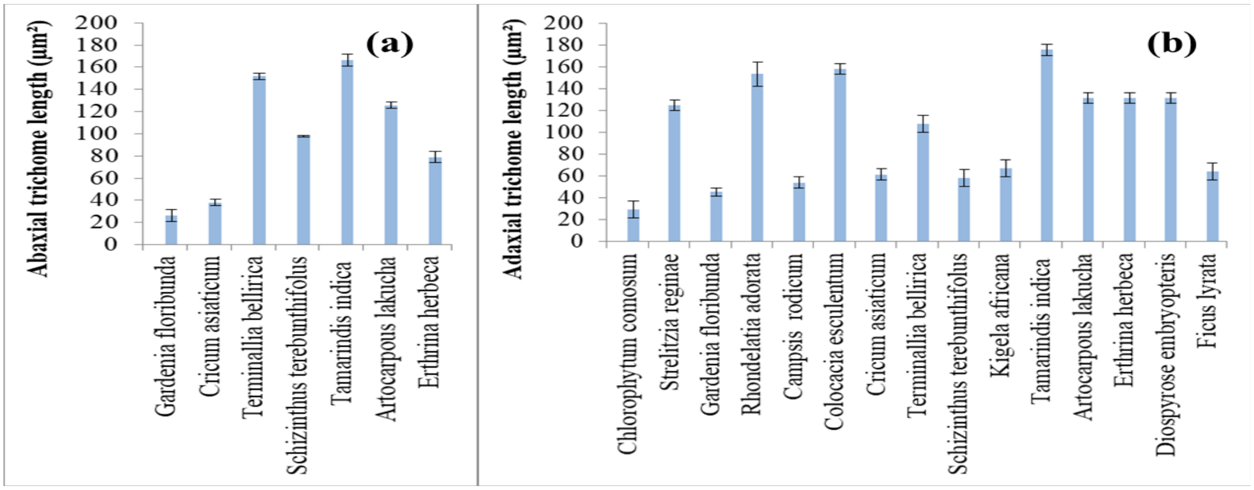

Fig. 3. (a) Abaxial trichome lengths (µm²) from the study of epidermal appendages in some angiosperm families and **(b)** Adaxial trichome lengths (µm²) from the study of epidermal appendages in some angiosperm families.

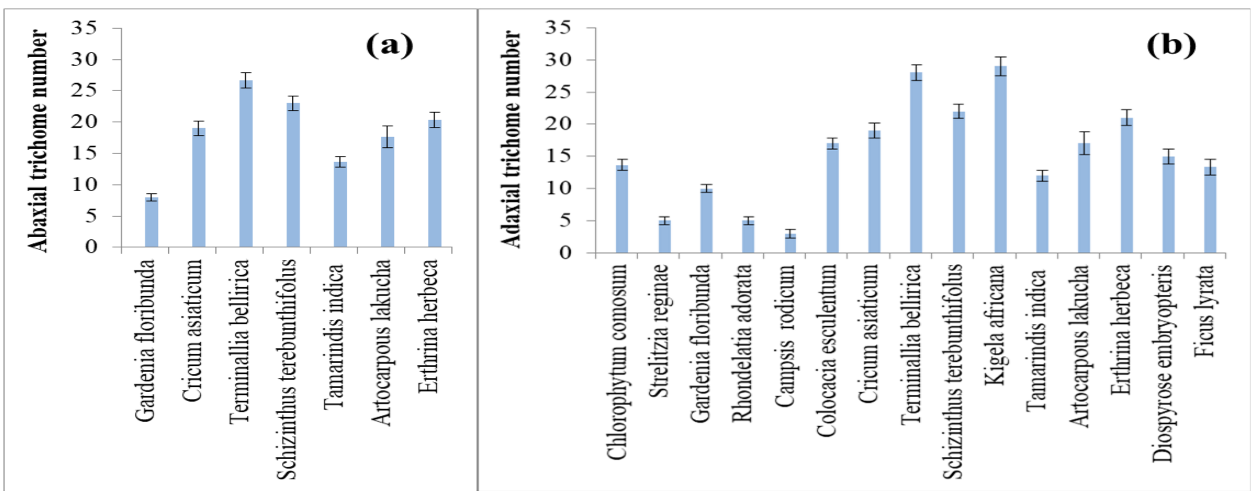

Fig. 4. (a) Abaxial trichome number from the study of epidermal appendages in some angiosperm families and **(b)** Adaxial trichome number from the study of epidermal appendages in some angiosperm families.

4. DISCUSSION

The ANOVA results in Table 2, demonstrated significant differences in trichome characteristics across the studied parameters. For abaxial trichome length (µm²), the species factor was highly significant (F = 11.71), with a mean square (MS) of 5451.60, indicating substantial interspecies variation. The standard error (SE = 12.46) reflects reliable measurements. Similarly, for adaxial trichome length (µm²), the species effect was pronounced (F = 21.85, MS = 8327.08, SE = 11.27), highlighting its dependence. For abaxial trichome number, the species factor was non-significant (F = 0.0003), suggesting uniformity across species. In contrast, adaxial trichome number showed significant variation among species (F = 4.56), indicating a potential role in species differentiation or adaptive mechanisms. The larger SE values associated with trichome numbers may reflect higher variability in environmental influence or sampling differences. These findings suggest that trichome length is a more consistent trait compared to trichome number, which may be influenced by external factors. Studies on the epidermal surfaces revealed a number of important micro-morphological characters and revealed interesting specific differences that are important for the identification of these characters. There are many changes in the smooth and glabrata species. For example, the highest and lowest stomatal length/width (L/W) ratios were found among species. Taxonomic studies of epidermal appendages of angiosperm families' species revealed interesting

Table 2. Analysis of variance (ANOVA) abaxial and adaxial trichome length and number respectively.

Sr. no.	ANOVA	SOV	DF	SS	MS	F-ratio	SE
1.	Abaxial trichome length (μm²)	Species	29	158096.5088	5451.603752	11.71024378	12.45714574
		Error	60	27932.4864	465.54144	--	--
		Total	89	186028.9952	--	--	--
2.	Adaxial trichome length (μm²)	Species	29	241485.5534	8327.08805	21.84841472	11.27135129
		Error	60	22867.8048	381.13008	--	--
		Total	89	264353.3582	--	--	--
3.	Abaxial trichome number	Species	29	1868.377778	0.626682667	0.000289327	722
		Error	60	2226	2166	--	--
		Total	89	357.6222222	--	--	--
4.	Adaxial trichome number	Species	29	8016.233333	1.292920007	4.55692	9457.555556
		Error	60	28432.66667	28372.66667	--	--
		Total	89	36448.9	--	--	--

SOV: Source of variable, **DF:** Degree of freedom**, SS:** Sum of square, **MS:** Means of square**, F-ratio:** F- ratio, **SE:** Standard error

significant results on observing the epidermis of the leaves and comparative leaf studies by comparing the characters of leaf surfaces of the angiosperm plant species (see Table 1). Epidermis of leaf cells having different length/width ratios most of the specie's epidermis lack stomata or less density of stomata and trichomes. Herewith micro-hairs were often present but most probably on the leaf surface not on observing epidermis of the leaf. Thirty species were observed here with from *Hemarocallus fulva* to *Ficus elastica*. Epidermal glands are important in relative research in angiosperms arising from epidermal cells [28]. Glandular characters were observed on studying the leaf surfaces of angiosperm plant species. Clearly found glands on the surfaces of leaf *Chlorophytum comosum* showed clear glands on the adaxial surface of leaf but on adaxial and abaxial surface clear glands were observed in *Gardenia floribunda.* Trichomes can vary widely in families and smaller plant groups and even in the same plant.

On the other hand, trichomes within a group of plants sometimes have significant uniformity. Plant hair types have been used successfully in the classification of genera and even in some families and in the identification of specific hybrids [29]. Observational and comparative studies of leaf surfaces of angiosperm families were observed and trichomes were present on the leaf surface. Unicellular, multicellular, hair-clusters, y-shaped, t-shaped, dendrites, with short branches, and gland-headed leaf epidermal features are sometimes influenced by environmental conditions [30, 31]. There is substantial evidence for their overall genetic control [32-34]. As such, they have been employed for plant species discrimination at various taxonomic ranks [35]. *Ficus lyrata* sword is bifacial and hypostomatic. The upper epidermal (adaxial epidermis layer) cells are iso-diametric to the lower rectangle according to the shape and are covered with a visible soft flat cuticle. This is followed by a sub epidermal cell layer - hypodermis - followed by lithocysts (12 m length and 12 m width) formed by large cells found here and giant epidermal cells protruding into the mesophyll. The function of these facialized cells is unknown. Inside the lithocysts, long solitary systolites (which accumulate calcium carbonate) are found suspended from a left arm (attached to the top of the lithocysts) [36]. On the other hand, *Ficus lyrata* is fiddle leaf fig belongs to Moraceae family. The present study gives the Abaxial and adaxial surfaces with epidermal appendages that unicellular, short branched trichomes appears on the adaxial surface. The comparative study showed the distinguished characters of leaf of *Ficus lyrata*. The leaf blade has elliptical shape, which means distribution or regulation of a vascular system, such as in the wing or leaf of an insect. Vascular patterns in insect wings are often used to identify and distinguish species. The venation pattern is observed as secondary veins branched towards margins. The leaf margins are lobed. Apex of the leaf is emarginated and base is obtuse. On the abaxial surface there are young and permanent old trichome bases consisting of one or three surrounding cells with one or less circular rings in the middle and *M. oppositifolius* var. pubescens; however, trichome bases were recorded only on the adaxial surface of the genus. The hair base as a character has been shown to be taxonomically useful in distinguishing these characters from *Mallotus* [37]. On both the surfaces of leaf epidermis and leaf area trichomes were observed *Terminallia bellirica* and *Tamarindis indica* have the maximum number of trichomes and the minimum number is recorded in *Crinum asiaticum*. On adaxial surface the maximum number of trichome is recorded. *Terminallia bellirica* and *Schizinthus terebunthifolus, Kigela africana*. The minimum number is recorded in *Campsis rodicum*.

5. CONCLUSIONS

The present study provides detailed insights into the epidermal characteristics crucial for taxonomy and physiological understanding. Analysis revealed significant variations in epidermal traits such as cell length, stomatal density, and types of epidermal appendages like trichomes. Species-specific distinctions were observed, such as *Pentas lanceolata* showing maximum abaxial cell length and *Ficus elastica* exhibiting minimum upper epidermal cell length. *Strelitzia reginae* displayed the lowest stomatal count on the abaxial surface, contrasting with species like *Crinum asiaticum* and *Morus leavigata*, which showed higher stomatal densities. ANOVA and cluster analysis provided statistical validation of these distinctions, highlighting relationships among species based on their epidermal features. These findings contribute foundational knowledge for future studies in plant taxonomy, physiology, and ethnobotany, emphasizing the importance of epidermal characteristics in plant classification and ecological adaptation.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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