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Comparative Foliar Epidermal Micromorphology of Four Medicinal Plant Species from Rivers State University Campus, Port Harcourt, Nigeria

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Abstract

This study investigated the foliar epidermal micromorphology of four medicinal plant species (*Ocimum gratissimum, Mentha piperita, Aspilia africana, and Vernonia amygdalina*) collected from the Rivers State University campus in Port Harcourt, Nigeria. Fresh leaf samples were processed using standard epidermal peeling techniques, stained with safranin and methyl blue, and examined under a compound light microscope. Quantitative parameters including trichome density and dimensions, epidermal cell characteristics, and stomatal features were measured and analyzed using ANOVA. Results revealed considerable interspecific and surface-specific variation in epidermal traits. Aspilia africana exhibited the highest abaxial trichome density (9.33 ± 1.53 trichomes per field) and stomatal density (40.33 ± 6.03 stomata per field), while *Menthapiperita* displayed the largest guard cells on the abaxial surface (71.19 ± 10.20 µm). *Ocimum gratissimum* showed the highest adaxial epidermal cell density (92.00 ± 31.19 cells per field) and the longest adaxial trichomes (138.93 ± 88.79 µm). Significant differences ($p < 0.05$) were observed among species for several parameters including abaxial trichome width, abaxial epidermal cell number, adaxial epidermal cell length, adaxial epidermal cell width, abaxial stomatal number, abaxial guard cell length and width, adaxial stomatal pore length, and adaxial stomatal pore width. These micromorphological variations reflect adaptive strategies and provide taxonomic evidence useful for species identification and authentication.

Keywords: Epidermal micromorphology, stomata, trichomes, medicinal plants, *Ocimum gratissimum, Mentha piperita, Aspilia africana, Vernonia amygdalina*

Introduction

The leaf epidermis serves as the primary interface between plants and their environment, performing critical functions in gas exchange, transpiration, and defense against biotic and abiotic stressors (Agbede *et al*., 2023; Kolawole *et al*., 2023). The micromorphological features of the epidermis, including epidermal cell shape and size, stomatal density and dimensions, and trichome characteristics, exhibit considerable variation across plant taxa and are influenced by both genetic factors and environmental conditions. These features have proven valuable in plant taxonomy, particularly when morphological similarities among related species complicate identification based on macroscopic characters alone (Agbede *et al*., 2023; Adeniran *et al*., 2026).

Medicinal plants constitute a significant component of healthcare systems in Nigeria and across Africa, with many species harvested from wild populations for traditional therapeutic applications. Accurate identification and authentication of these plants are essential for ensuring efficacy and safety in ethnopharmacological use. Epidermal micromorphology offers a relatively rapid and accessible approach for species verification, particularly when fresh material is available (Agbede *et al*., 2023; Ugbabe *et al*., 2023).

The four species examined in this study, *Ocimum gratissimum* (African basil), *Mentha piperita* (peppermint), *Aspilia africana* (wild sunflower), and *Vernonia amygdalina* (bitter leaf), represent important medicinal taxa from the Lamiaceae and Asteraceae families. *Ocimum gratissimum* and *Mentha piperita* are aromatic herbs widely used for their essential oils and antimicrobial properties (Ugbabe *et al*., 2023; Fernandes *et al*., 2024). *Aspilia africana* and *Vernonia amygdalina* are prominent in traditional medicine for wound healing, anti-inflammatory, and antimalarial applications (Agbede *et al*., 2023; Kemka-Evans *et al*., 2014). Despite their ethnopharmacological importance, comparative data on their foliar epidermal characteristics from the Niger Delta region remain limited.

This study aimed to characterize and compare the epidermal micromorphology of these four medicinal species, documenting variation in trichome, epidermal cell, and stomatal features across adaxial and abaxial leaf surfaces. The findings contribute to the growing body of taxonomic evidence for these species and provide baseline data for authentication purposes.

Materials and Methods

Study Area

The study was conducted at the Rivers State University (RSU) campus, located within Port Harcourt metropolis in Rivers State, Nigeria. Port Harcourt is an industrialized cosmopolitan city situated in the Niger Delta region. The RSU campus lies within Latitudes 4°31'–4°40'N and Longitudes 7°0'–7°10'E, with an elevation of approximately 10–15 m above sea level (Ubong *et al*., 2015).

Collection of Plant Materials

Fresh, healthy, and mature leaves of *Ocimum gratissimum* (Lamiaceae), *Mentha piperita* (Lamiaceae), *Aspilia africana* (Asteraceae), and *Vernonia amygdalina* (Asteraceae) were collected from Port Harcourt. Samples were collected during early morning hours (7:00–9:00 AM) to minimize water stress effects and ensure turgid epidermal cells (Agbede *et al*., 2023). Collected samples were placed in labeled polythene bags, transported to the laboratory, and

identified and authenticated by Prof. M. G. Ajuru, a Plant Taxonomist in the Department of Plant Science and Biotechnology.

Preparation of Epidermal Peels

Epidermal peels were prepared using standard peeling techniques (Agbede *et al*., 2023; Omolokun, 2020). Leaf samples were cleaned with distilled water, and small pieces (approximately 1 cm × 1 cm) were cut from the middle portion of each leaf, avoiding the midrib and major veins. The epidermis was carefully peeled using fine forceps. For thick leaves, mesophyll tissue was scraped away using a sharp razor blade. For leaves that were difficult to peel, samples were treated with weak sodium hydroxide solution (2–4 hours at room temperature) to facilitate epidermal separation. At least five peels were prepared for each leaf surface of each species.

Staining and Mounting

Epidermal peels were stained with 1% safranin solution (in 70% ethanol) for 2–3 minutes, followed by brief washing with distilled water. Methyl blue was applied to enhance contrast and differentiation of epidermal structures. Stained specimens were mounted in 50% glycerine on clean glass slides, covered with cover slips, and labeled appropriately.

Microscopic Examination

Prepared slides were examined using a compound light microscope (Olympus CX23) equipped with an ocular micrometer and calibrated stage micrometer. Observations were conducted at magnifications ranging from ×40 to ×400, depending on the structure under examination.

Data Collection

Quantitative parameters recorded for each species, organ, and surface included: epidermal cell length (ECL, µm), epidermal cell width (ECW, µm), number of epidermal cells per field of view (×400 magnification), stomatal pore length (SPL, µm), stomatal pore width (SPW, µm), guard cell length (GCL, µm), guard cell width (GCW, µm), number of stomata per field of view, trichome length (µm), trichome width (µm), and number of trichomes per field of view.

Photomicrography

Photomicrographs were captured using a digital camera attached to the light microscope. Representative images of stomata, trichomes, and epidermal cells from each species and surface were documented at various magnifications.

Statistical Analysis

Descriptive statistics (mean and standard deviation) were calculated for all quantitative parameters. Data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics version 27. Means were separated using Fisher's Least Significant Difference (LSD) test at $p < 0.05$.

Results

Trichome Characteristics

The trichome characteristics on both leaf surfaces of the four species are presented in Table 1 and Plates 1 and 2. Trichome density varied considerably among species. *Ocimum gratissimum* exhibited the lowest trichome density on the adaxial surface (1.67 ± 1.15 trichomes per field), while *Mentha piperita* showed the highest adaxial density (6.33 ± 3.06). On the abaxial surface, *Aspilia africana* demonstrated the highest trichome density (9.33 ± 1.53), which was notably higher than its adaxial surface (3.33 ± 2.31).

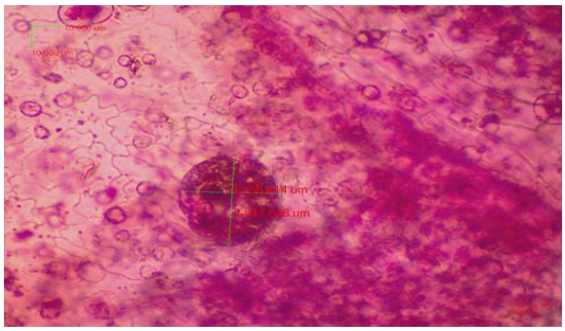
Trichome length showed marked interspecific variation. *Ocimum gratissimum* exhibited the longest trichomes on the adaxial surface (138.93 ± 88.79 µm), whereas *Vernonia amygdalina* showed the shortest adaxial trichomes (31.44 ± 6.49 µm). On the abaxial surface, A. africana displayed the longest trichomes (139.70 ± 71.89 µm).

Trichome width also varied considerably with*Mentha piperita* exhibiting the widest adaxial trichomes (47.31 ± 19.79 µm), while *O. gratissimum* displayed the widest abaxial trichomes (29.96 ± 5.54 µm). ANOVA revealed significant interspecific differences for abaxial trichome width (p = 0.002).

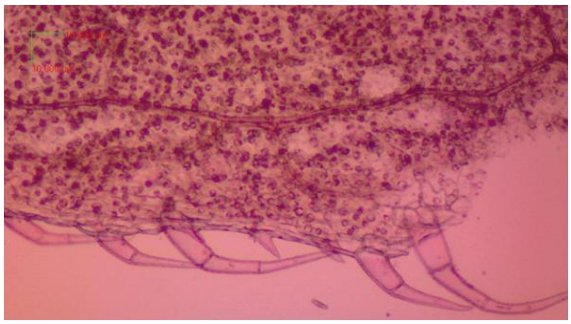
Table 1: Trichome Characteristics on Leaf Surfaces of the Four Medicinal Plant Species

Plant Species	**Number of Trichomes per Field**		**Trichome Length**(µm)		**Trichome Width**(µm)	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Ocimum gratissimum	1.67 ± 1.15	6.67 ± 1.53	138.93 ± 88.79	72.69 ± 28.71	21.36 ± 8.15	29.96 ± 5.54
Mentha piperita	6.33 ± 3.06	5.67 ± 4.16	118.85 ± 56.79	48.91 ± 7.50	47.31 ± 19.79	13.48 ± 2.82
Aspilia africana	3.33 ± 2.31	9.33 ± 1.53	96.88 ± 88.03	139.70 ± 71.89	24.23 ± 17.91	16.21 ± 2.44
Vernonia amygdalina	3.67 ± 2.08	5.67 ± 4.16	31.44 ± 6.49	48.91 ± 7.50	16.74 ± 2.60	13.48 ± 2.82
P Value	0.165	0.506	0.316	0.080	0.106	0.002
LSD	4.25	5.83	129.42	74.74	26.39	6.63

Values are presented as Mean ± Standard Deviation (SD)



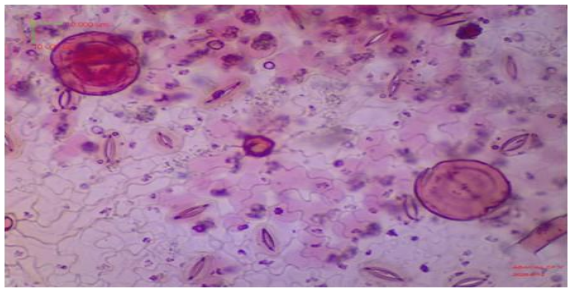
A (100X)



B (40X)

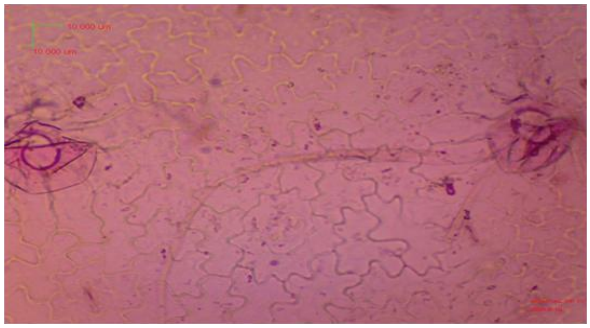


C (40X)

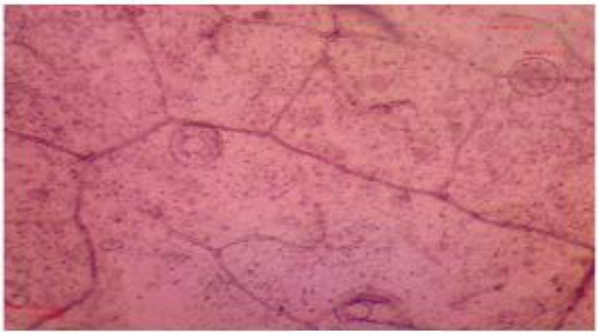


D (100X)

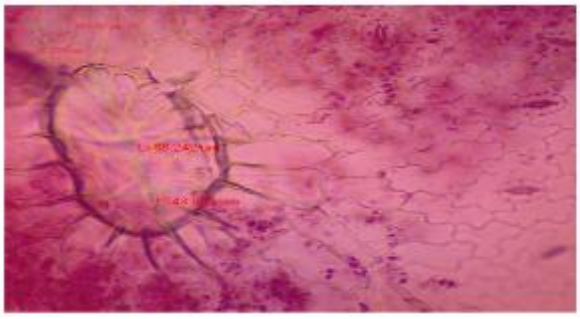
Plate 1: Abaxial Surface Trichomes of: A = *O. gratissimum*; B = *M. piperita;* C = *A. africana;* D = *V. amygdalina*



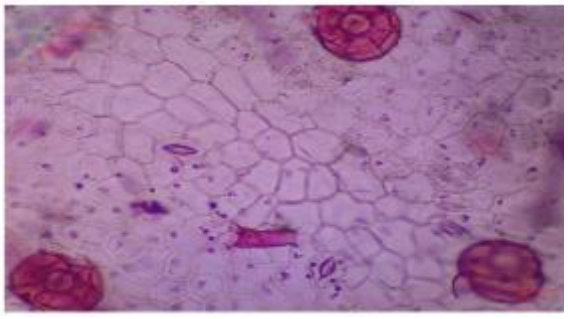
A (100X)



B (40X)



C (100X)



D (100X)

Plate 2: Adaxial Surface Trichomes of: A = *O. gratissimum*; B = *M. piperita;* C = *A. africana;* D = *V. amygdalina*

Epidermal Cell Characteristics

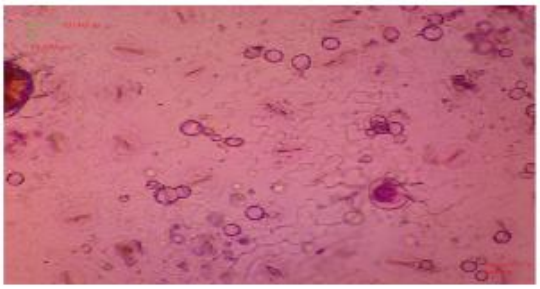
Epidermal cell characteristics are summarized in Table 2 and Plates 3 and 4. The number of epidermal cells per field of view varied considerably. *Ocimum gratissimum* exhibited the highest adaxial epidermal cell density (92.00 ± 31.19 cells per field), while *Mentha piperita* showed the lowest abaxial density (8.00 ± 3.00 cells per field). *Aspilia africana* demonstrated relatively consistent densities on both surfaces (56.33 ± 3.06 adaxial; 62.33 ± 5.03 abaxial), while *V. amygdalina* showed moderate densities (78.33 ± 6.81 adaxial; 64.67 ± 20.60 abaxial).

Epidermal cell length varied significantly among species. *Ocimum gratissimum* exhibited the longest adaxial epidermal cells (37.82 ± 4.67 µm), while *V. amygdalina* showed the shortest (23.40 ± 3.54 µm). On the abaxial surface, *M. piperita* (35.84 ± 11.49 µm) and *V. amygdalina* (34.60 ± 1.40 µm) displayed relatively longer cells. Epidermal cell width followed similar patterns, with *O. gratissimum* exhibiting the widest adaxial cells (25.46 ± 8.43 µm) and M. piperita the narrowest (17.01 ± 3.10 µm). Notably, *O. gratissimum* displayed considerably larger epidermal cells on the adaxial surface compared to the abaxial surface (13.28 ± 2.90 µm). Significant interspecific differences were observed for abaxial epidermal cell number ($p < 0.001$), adaxial epidermal cell length ($p = 0.003$), and adaxial epidermal cell width ($p = 0.034$).

Table 2: Epidermal Cell Characteristics of the Four Medicinal Plant Species

Plant Species	**Number of Epidermal cells per view**		**Epidermal cell length** (µm)		**Epidermal cell width** (µm)	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Ocimum gratissimum	92.00 ± 31.19	57.33 ± 5.13	37.82 ± 4.67	29.82 ± 11.92	25.46 ± 8.43	13.28 ± 2.90
Mentha piperita	85.33 ± 15.95	8.00 ± 3.00	35.00 ± 2.73	35.84 ± 11.49	17.01 ± 3.10	16.30 ± 5.51
Aspilia africana	56.33 ± 3.06	62.33 ± 5.03	28.59 ± 1.80	27.41 ± 5.15	17.67 ± 2.14	14.29 ± 1.85
Vernonia amygdalina	78.33 ± 6.81	64.67 ± 20.60	23.40 ± 3.54	34.60 ± 1.40	11.17 ± 0.86	13.97 ± 6.57
P Value	0.161	0.000	0.003	0.619	0.034	0.869
LSD	33.72	20.73	6.32	16.38	8.73	8.70

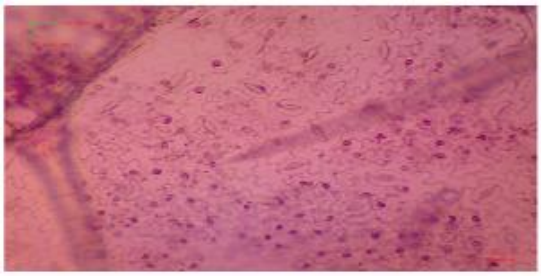
Values are presented as Mean ± Standard Deviation (SD)



A (40X)



B (400X)

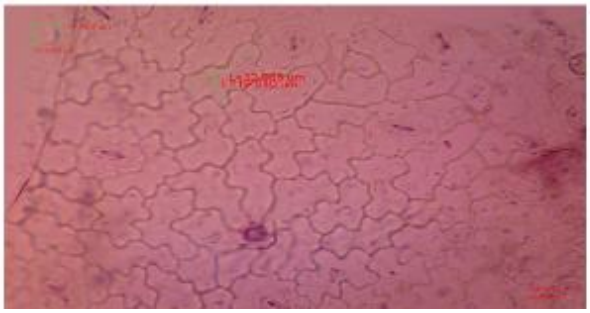


C (40X)

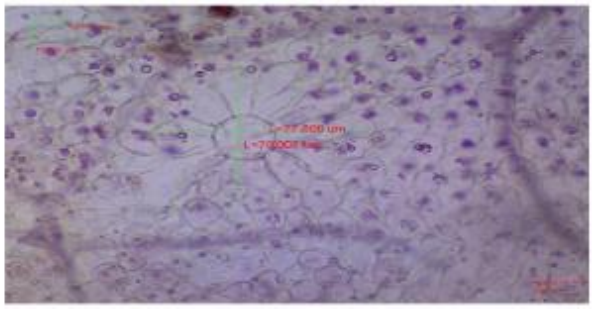


D (100X)

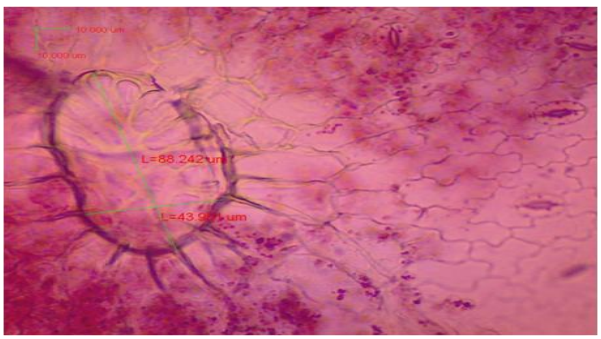
Plate 3: Abaxial Surface Epidermal Cells of: A = *O. gratissimum*; B = *M. piperita;* C = *A. africana;* D = *V. amygdalina*



A (100X)



B (40X)



C (100X)



D (100X)

Plate 4.4: Adaxial Surface Epidermal Cells of: A = *O. gratissimum*; B = *M. piperita;* C = *A. africana;* D = *V. amygdalina*

Stomatal Characteristics

Stomatal characteristics are presented in Tables 3 and 4 and Plates 5 and 6. Stomatal density varied markedly among species and between surfaces. *Aspilia africana* exhibited the highest abaxial stomatal density (40.33 ± 6.03 stomata per field), while *Mentha piperita* showed the lowest adaxial density (2.67 ± 1.15). *Ocimum gratissimum* displayed approximately five times more stomata on the abaxial surface (16.67 ± 2.08) than on the adaxial surface (3.33 ± 3.21). *Vernonia amygdalina* demonstrated relatively moderate densities on both surfaces (8.00 ± 1.73 adaxial; 9.33 ± 7.57 abaxial). Significant differences were observed for abaxial stomatal number ($p < 0.001$).

Guard cell dimensions varied considerably. *Mentha piperita* exhibited the largest guard cells on the abaxial surface (71.19 ± 10.20 µm), while *V. amygdalina* showed the smallest adaxial guard cells (34.15 ± 30.94 µm). *Ocimum gratissimum* exhibited larger guard cells on the adaxial surface (49.98 ± 9.10 µm) compared to the abaxial surface (17.14 ± 3.36 µm), an unusual pattern among the studied species. Significant differences were observed for abaxial guard cell length ($p < 0.001$) and abaxial guard cell width ($p = 0.002$).

Stomatal pore length was relatively consistent across most species and surfaces, ranging from 30.34 ± 16.73 µm (*V. amygdalina*, abaxial) to 43.80 ± 6.09 µm (*V. amygdalina*, adaxial). *Mentha piperita* displayed the longest stomatal pores on the abaxial surface (43.22 ± 18.37 µm). Stomatal pore width showed greater variation, with *V. amygdalina* abaxial exhibiting the widest pores (18.20 ± 14.99 µm) and *M. piperita* abaxial showing the narrowest (6.20 ± 1.81 µm). Significant differences were observed for adaxial stomatal pore length ($p = 0.045$) and adaxial stomatal pore width ($p = 0.001$).

Table 4.3: Stomatal Characteristics of the Four Medicinal Plant Species

Plant Species	Number of stomata per field view		Guard Cell Length (µm)		Guard Cell Width (µm)	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Ocimum gratissimum	3.33 ± 3.21	16.67 ± 2.08	49.98 ± 9.10	17.14 ± 3.36	39.71 ± 9.72	13.12 ± 3.66
Mentha piperita	2.67 ± 1.15	3.33 ± 1.53	44.32 ± 6.73	71.19 ± 10.20	21.29 ± 3.45	52.87 ± 10.95
Aspilia africana	3.33 ± 2.31	40.33 ± 6.03	53.48 ± 3.17	54.00 ± 6.15	20.90 ± 0.96	52.87 ± 10.95
Vernonia amygdalina	8.00 ± 1.73	9.33 ± 7.57	34.15 ± 30.94	76.15 ± 7.33	26.11 ± 25.55	30.48 ± 9.77
P Value	0.064	0.000	0.537	0.000	0.363	0.002
LSD	4.21	9.43	31.16	15.53	25.96	14.71

Values are presented as Mean ± Standard Deviation (SD)

Table 4.4: Stomatal Pore Characteristics of the Four Medicinal Plant Species

Plant Species	Stomatal Pore Length (µm)		Stomatal Pore Width (µm)	
	Adaxial	Abaxial	Adaxial	Abaxial
Ocimum gratissimum	40.38 ± 7.80	32.12 ± 4.62	10.89 ± 1.45	12.31 ± 4.48
Mentha piperita	32.35 ± 2.98	43.22 ± 18.37	7.50 ± 0.85	6.2 ± 1.81
Aspilia africana	30.43 ± 2.90	35.31 ± 10.08	7.02 ± 1.72	13.54 ± 1.59
Vernonia amygdalina	43.80 ± 6.09	30.34 ± 16.73	13.63 ± 0.83	18.20 ± 14.99
P Value	0.045	0.676	0.001	0.379
LSD	10.10	25.62	2.40	14.90

Values are presented as Mean ± Standard Deviation (SD)

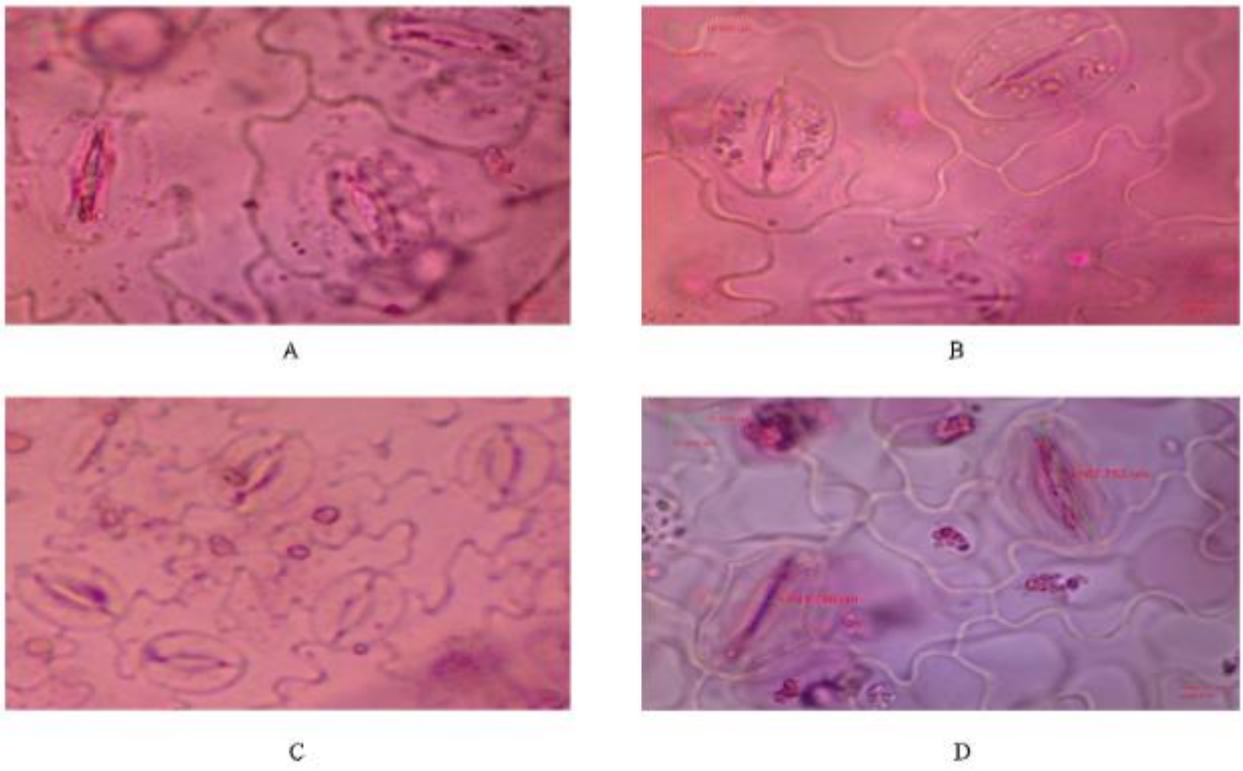


Plate 5: Abaxial Surface Stomata of: A = *O. gratissimum*; B = *M. piperita*; C = *A. africana*; D = *V. amygdalina* (400X)

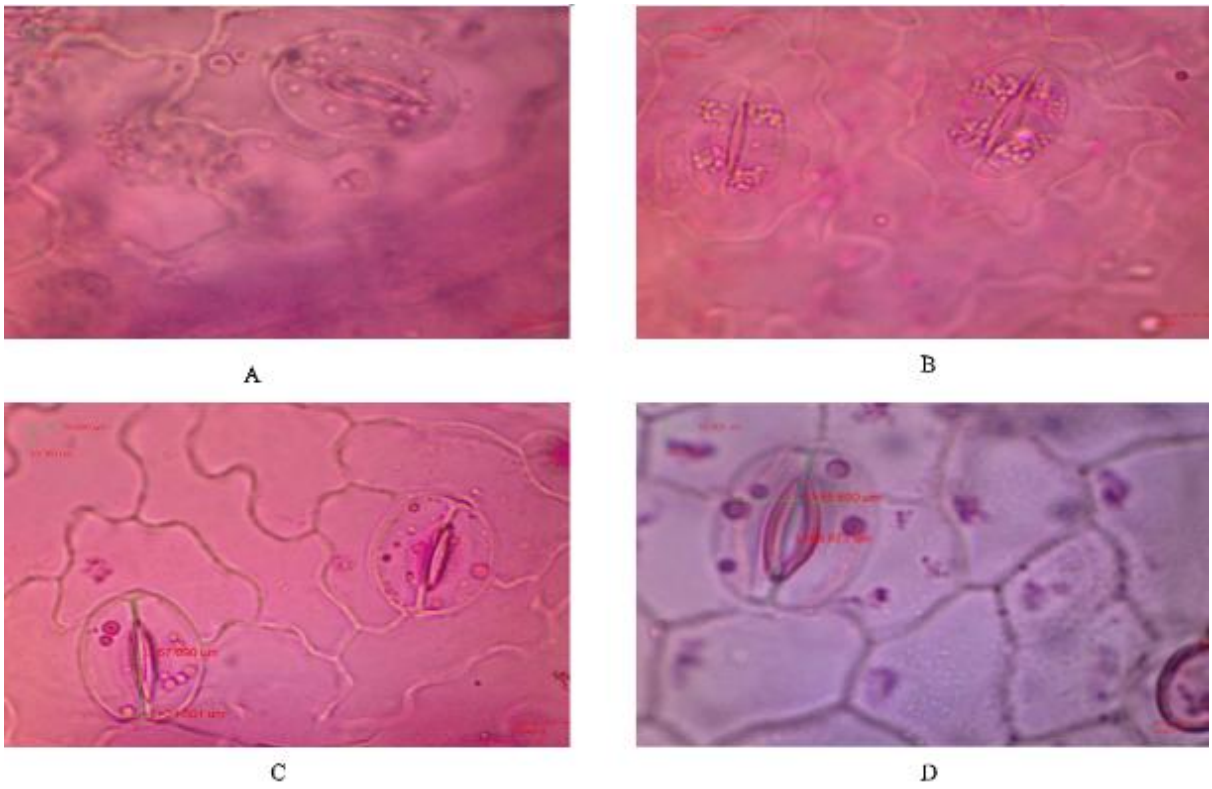


Plate 6: Adaxial Surface Stomata of: A = *O. gratissimum*; B = *M. piperita;* C = *A. africana;* D = *V. amygdalina* (400X)

Discussion

The present study revealed considerable interspecific and surface-specific variation in foliar epidermal micromorphology among the four medicinal plant species examined. These variations reflect both inherent taxonomic differences and adaptive responses to environmental conditions.Trichome density and dimensions are important taxonomic characters in Lamiaceae and Asteraceae. The finding that *Aspilia africana* exhibited the highest abaxial trichome density (9.33 per field) aligns with previous reports describing this species as densely pubescent. Agbede *et al*. (2023) documented that *A. africana* possesses multicellular trichomes on both leaf surfaces, with increased density under dry conditions as an adaptation to reduce water loss and deter herbivores. The relatively long trichomes observed on *O. gratissimum* (138.93 µm adaxial) are consistent with descriptions of this species as having dense trichomes, particularly on the adaxial surface and vascular regions (Ugbabe *et al*., 2023). Trichomes in Ocimum species are known to include both glandular and non-glandular types, with glandular trichomes playing important roles in essential oil secretion (Fernandes *et al*., 2024; Ugbabe *et al*., 2023). The presence of glandular trichomes in *Mentha piperita* has been extensively documented, with studies demonstrating that oil glands are more numerous in this species compared to other Mentha taxa (Yulianti *et al*., 2023). The observation that *M. piperita* exhibited the widest adaxial trichomes (47.31 µm) is consistent with the known morphology of peppermint glandular trichomes, which possess a one- to two-celled stalk and a glandular head of eight radiating cells (USP, 2007).

The significant variation in epidermal cell dimensions among species reflects differences in cell expansion patterns and leaf anatomy. *Ocimum gratissimum* exhibited the largest adaxial epidermal cells, which may relate to its mesophytic leaf structure. Fernandes *et al*. (2024) demonstrated that light intensity influences leaf anatomy in *O. gratissimum*, with increased light leading to greater parenchyma thickness but not affecting epidermal thickness. The observation that *O. gratissimum* displayed considerably larger epidermal cells on the adaxial compared to the abaxial surface is notable, as such surface-related differences in cell size can influence leaf mechanical properties and gas exchange characteristics. Agbede *et al*. (2023) noted that water stress had limited effects on epidermal cell anticlinal wall morphology in Asteraceae species, suggesting that cell size variation may be more strongly genetically determined than environmentally plastic. In *Vernonia amygdalina*, Kemka-Evans *et al*. (2014) described sinuous anticlinal walls on the abaxial surface of the non-bitter variety, with irregular T-shaped trichomes on the bitter leaf variety, characters that have taxonomic value for delimiting taxa within the genus.

The marked differences in stomatal distribution between leaf surfaces have important physiological implications. Amphistomatous leaves (with stomata on both surfaces) typically have higher maximum conductance than hypostomatous leaves. The high abaxial stomatal density in *A. africana* (40.33 per field) is consistent with the amphistomatous nature of this species. Agbede *et al*. (2023) reported that *A. africana* and *V. amygdalina* showed increased stomatal densities on adaxial surfaces during wet seasons, suggesting greater CO_2 uptake capacity when water is abundant. The five-fold difference in stomatal density between surfaces in *O. gratissimum* contrasts with the more even distribution in *V. amygdalina*, reflecting species-specific patterns of stomatal development. Ugbabe *et al*. (2023) documented anomocytic stomata occurring more abundantly on the lower surface of *O. gratissimum*, with glandular trichomes also more abundant on the abaxial surface, a pattern consistent with the present findings. In *Vernonia amygdalina*, Kemka-Evans *et al*. (2014) reported hypoamphistomatic stomatal distribution with anomocytic stomata on all surfaces studied.

The unusually large adaxial guard cells observed in *O. gratissimum* (49.98 µm) compared to its abaxial guard cells (17.14 µm) represents an interesting deviation from typical patterns. In most species, guard cell dimensions are relatively consistent across surfaces, though some variation is common. This pattern may relate to differences in stomatal development or function between surfaces. The large guard cells observed in *M. piperita* abaxial (71.19 µm) are notable, as guard cell size is generally negatively correlated with stomatal density across species (Agbede *et al*., 2023). The pharmacognostic description of peppermint notes that stomata are enclosed by a pair of subsidiary cells whose common wall is at right angles to the guard cells, a diacytic arrangement typical of Lamiaceae (USP, 2007).

The significant differences observed for several parameters, including abaxial trichome width, abaxial epidermal cell number, adaxial epidermal cell length and width, abaxial stomatal number, abaxial guard cell length and width, adaxial stomatal pore length, and adaxial stomatal pore width, indicate that these traits have taxonomic value for distinguishing among these species. The non-significant differences for adaxial trichome number, abaxial trichome length, and abaxial epidermal cell length suggest that some traits are more conserved or exhibit greater phenotypic plasticity. Similar findings have been reported for other medicinal plant groups, where epidermal micromorphology has proven effective for species delimitation. Adeniran *et al*. (2026) demonstrated that micromorphological markers distinguished closely allied species within Crassocephalum, providing reliable diagnostic traits for taxa with overlapping morphological features. In Apocynaceae, epidermal markers

including anticlinal wall patterns and stomatal crypts have been used to discriminate medicinal tree species (Olowokudejo & Nyananyo, 2023).

These findings have practical implications for the authentication of medicinal plant materials. Epidermal micromorphology provides a relatively rapid and cost-effective means of verifying species identity, particularly when macroscopic features are ambiguous or when plant materials are fragmented or processed. The distinctive trichome and stomatal characteristics documented here can serve as reference data for quality control in ethnopharmacological applications. As noted by Ugbabe *et al*. (2023), leaf epidermal microscopy combined with chemo-microscopy can be used for the standardization of medicinal plants, ensuring correct identification and reducing the risk of substitution or adulteration.

Conclusion

This study documented significant interspecific and surface-specific variation in the foliar epidermal micromorphology of four medicinal plant species from the Niger Delta region. The observed differences in trichome density and dimensions, epidermal cell characteristics, and stomatal features reflect both taxonomic distinctiveness and adaptive strategies. These micromorphological data contribute to the taxonomic characterization of these species and provide a basis for their authentication in medicinal plant research and quality control.

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