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LEAF EPIDERMAL STUDIES OF THREE APOCYNACEAE SPECIES (*Allamanda cathartica Linn., Rauvolfia vomitoria Afzel. and Catharanthus roseus Linn.)*

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ABSTRACT

Apocynaceae is a family of flowering plants that comprises 200 genera and about 2000 species and is widely distributed in tropics and subtropics. In West Africa, it is made up of 37 genera and 138 species. This study focuses on enhancing the taxonomic classification of the *Apocynaceae* plant family in Nigeria by investigating leaf epidermal characteristics of three species. (*Allamanda cathartica Linn*., *Rauvolfia vomitoria Afze*l. and *Catharanthus roseus Linn*) at the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Identification of species within this family is challenging due to the absence of easily distinguishable flowers and fruits. Leaf epidermal peels were obtained following standard procedures. Data obtained were subjected to descriptive analysis and one-way Analysis of variance. Common elliptic stomata shape was observed, except in *A. cathartica*, which exhibited additional circular stomata on the adaxial surface. *C. roseus* was identified as amphistomatic, distinguishing it from the hypostomatic nature of the other two species. Anisocytic stomata type was consistent across all species but *A. cathartica* and *R. vomitoria* displayed extra tetracytic stomata type, while *C. roseus* showcased distinct stomata types like diacytic, and paracytic. The studied species exhibited similarities in anticlinal wall patterns, non-glandular trichomes, and polygonal to irregular epidermal cell shapes. *A. cathartica* had additional and different epidermal cell shapes which ranged from cylindrical, rectangular and triangular. The study emphasizes the taxonomic significance of epidermal characters like leaf surface, stomata type, anticlinal wall patterns, presence of non-glandular trichomes, distribution of stomata on leaf surfaces in delineating boundaries among the *Apocynaceae* species studied.

Keywords: Apocynaceae, Epidermal, Stomata, Taxonomy and Trichomes.

INTRODUCTION

Apocynaceae (*Juss*.) is one of the important families that are present all over the world mainly because of their medicinal importance. The inclusion of species of `*Asclepiadaceae* under the unified classification for *Apocynaceae*, has led to the family enlargement from two to five sub-families (Endress and Bruyns, 2000). According to Li *et al*., (1995), the sub-families are *Apocynoideae*, *Asclepiadoideae*, *Periplocoideae*, *Rauvolfioideae* and *Secamonoideae*. The expanded family now comprises more than 150 genera and 2000 species (Endress and Bruyns, 2000). The family consists of tropical trees, shrubs and vines. The characteristic features of this family are that almost all the species produce milky sap; the leaves are usually simple, op-

posite or whorled; the flowers are large, colourful and slightly fragrant with five contorted lobes; and fruits are in pairs. (Wiart, 2006; Ng, 2006). Various works have been published on the epidermal characterization of many taxa of the family *Apocynaceae*. Kannabiran and Ramassamy, (1988) carried out foliar epidermal study of ten Indian species containing nine genera and reported that *Wriohtia tinctoria*, *Ervatamia divaricata*, and *Catharanthus roseus* were delimited based on leaf type from six other apocynaceous species. El-fiki *et al.* (2019), investigated seven species representing seven genera and recommended merging of *Apocynaceae* and *Asclepiadaceae* to one big family amidst all other studies. However, despite these scientific documentations, the taxonomic relationship among the members of *Apocynaceae* remains unsettled and incomplete. These *Apocynaceae* species are among the trees commonly used for traditional medicine in Nigeria (Wiart, 2006). The decoction of root and bark of *Rauvolfia vomitoria* in tandem with some species of *Meliaceae* was reported by Fabeku, (2006) to be efficacious in the treatment of coated tongue disease. Some of the species with cytotoxic activity include those of Allamanda (Schmidt *et al*., 2006) and *Catharanthus* (Wong *et al*., 2011). However, with the high recognition of the family *Apocynaceae* medicinal importance; their identification can be challenging. Flowers and fruits are not always present or easy to distinguish and sterile plants cannot provide adequate information for identification. This study on leaf epidermis was conducted to provide important taxonomic characters for species identification that could be used as an alternative to flowers and fruits, and could have practical implications for field identification and medicinal use.

MATERIALS AND METHODS

Plant Collection

Fresh leaf samples were collected from Obafemi Awolowo University, Ile Ife, Osun State, Nigeria and University of Ibadan, Ibadan, Oyo State, The GPS coordinates of Obafemi Awolowo University, Nigeria is 7° 31' 14.7612" N and 4° 31' 49.1340" E. It has relatively flat topography and mean altitude of 280 m above sea level. The GPS coordinates of University of Ibadan (UI), Oyo State, Nigeria is 7°23'28.19" N 3°54'59.99" E and a mean altitude of 227 m above sea level. Climatic condition of the two Universities are tropically dominated by rainfall pattern from 1300 – 1500 mm, the average temperature is about 26^0C. The average relative humidity of both areas is about 65%. They both experience two distinct seasons of a dry season, usually from November to March and a raining season, from April to October.

Identification and authentication of the samples were carried out in the herbarium of Obafemi Awolowo University, Ile - Ife, Osun State, Nigeria. The research was carried out in the laboratory of the Department of Pure and Applied Botany, College of Biosciences in Federal University of Agriculture Abeokuta, Ogun State, Nigeria.

Preparation of Epidermal Peels

According to (Oyedapo *et al*., 2018), leaf samples were first preserved in 50 % ethanol before subjection to epidermal characterization. Matured leaves were randomly selected, cut into sizeable sections, and soaked in concentrated nitric acid, ranging from 8 to 24 h depending on the leaf texture (Ibrahim and Ayodele, 2013).

Swollen leaf surfaces with the appearance of

air bubbles are an indication of the readiness of the epidermal layers for separation. Samples with swollen surfaces and air bubbles were then transferred into clean glass Petri-dishes containing water, while the adaxial and abaxial layers were separated using dissecting needle and forceps. The peels were cleaned using a camel-air-brush in water and preserved in storage bottles containing 50 % ethanol (Oyedapo *et al*., 2018, Ibrahim and Ayodele, 2013).

Slide Preparations and Assessment of Epidermal Characters

Epidermal peels were first washed in water before staining with appreciable drops of Safranin O (Oyedapo *et al*., 2018). For clear visibility, peels were counterstained using toluidine blue and the excess stains were removed by washing with water three times. The samples were subjected to a series of ethanol concentrations of 50, 60, 70, 80, 90 and 100 % for approximately 3 min to dehydrate. To completely remove all traces of stains, water and ethanol, the peels were treated using absolute xylene. Each epidermal peel was thereafter mounted on a slide, using 25 % glycerol for the feasibility of the internal structure. Important anatomical features of the peels were observed and recorded using Olympus microscope with attached Amscope digital camera. All measurements were taken using ocular micrometer. Photomicrographs of the slides were taken at both x10 and x40 objectives. Qualitative variables studied include epidermal cell shape, anticlinal wall pattern, stomata type and shape, presence of egarstic substances and trichome types. Quantitative variables such as length and breadth of the epidermal cell, stomata and trichomes, were measured using an ocular micrometre. Guard cell area (GCA) and Stomata index (SI) were estimated as proposed by Metcalfe (1960, 1989).

Data Analysis

The experiment was replicated five times and all the data collected were analyzed statistically using one-way analysis of variance (ANOVA). Means were separated by Duncan's Multiple Range Test (DMRT) at 5% probability level using Statistical Package for Social Sciences (SPSS), version 20 software.

RESULTS

Elliptic stomata shape was common among the three *Apocynaceae* species studied except in *A. cathartica,* which exhibited additional circular stomata on the adaxial surface (Table 1). *C. roseus* was identified as amphistomatic, distinguishing it from the hypostomatic nature of the other two species. *Anisocytic stomata* type was consistent across all species but *A. cathartica* and *R. vomitoria* displayed extra tetracytic stomata type, while *C. roseus* showcased distinct stomata types like diacytic, and paracytic. The species exhibited similarities in anticlinal wall patterns by having curved, straight to undulating anticlinal walls, presence of unicellular, non-glandular trichomes, and polygonal to irregular epidermal cell shapes. *A. cathartica* had additional and different epidermal cell shapes like cylindrical, rectangular, and triangular epidermal cell shapes (Table 1). *R. vomitoria* was unique by having the presence of stylloid and prismatic crystals on the abaxial surface and starch grannules on the adaxial surface respectively as this character separates it distinctly from the other species studied (Figures 1, J and L).

Table 1: Qualitative epidermal characters of three Apocynaceae species

Species		Cell shape	Anticlinal wall	Leaf surface	Stomata type	Stomata shape	Trichome Non - glandular	Egarstic substances
Allamanda Cathartica	Adaxial	Cylindrical/ Polygonal	Curve/ Straight	Hypostomatic	None	Elliptic	Unicellular	Absent
	Abaxial	Cylindrical/ Rectangular/ Triangular/ Polygonal/ Irregular	Curve/ Straight/ Undulating		Anisocytic/ Tetracytic	Elliptic	Unicellular	Absent
Catharanthus roseus	Adaxial	Polygonal	Curve/ Straight	Amphistomatic	Anisocytic	Elliptic/ Circular	Unicellular	Absent
	Abaxial	Polygonal/ Irregular	Curve/ Straight/ Undulating		Anisocytic/ Diacytic/ Paracytic	Elliptic	Unicellular	Absent
Rauvolfia vomitoria	Adaxial	Polygonal	Curve/ Straight	Hypostomatic	None	Elliptic	Absent	Starch granules
	Abaxial	Polygonal/ Irregular	Curve/ Straight/ Undulating		Anisocytic/ Tetracytic	Elliptic	Absent	Styloid and Prismatic crystals

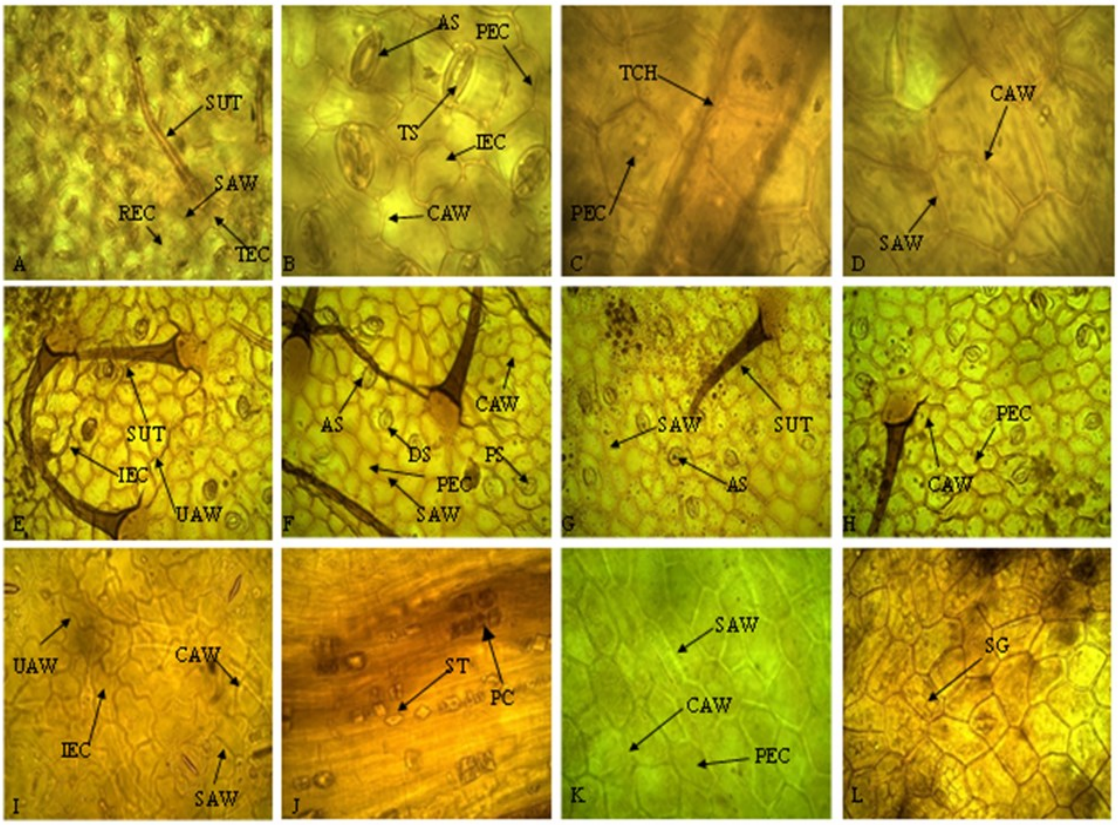

Figure 1: *Allamanda cathartica* surfaces for Abaxial (A-B) and Adaxial (C-D), *Catharanthus roseus* surfaces for Abaxial (E-F) and Adaxial (G-H), *Rauvolfia vomitoria* surfaces for Abaxial (I-J) and Adaxial (K-L)

SAC- Straight anticlinal wall; REC- rectangular epidermal cell; TEC- triangular epidermal cell; SUC- Simple unicellular trichome; IEC- Irregular epidermal cell, PEC- polygonal epidermal cell, CAW- Curve anticlinal wall, TC- Tetracytic stomata and AS- *Anisocytic stomata*, TCH- Trichome, SAW- Straight anticlinal wall E- SUT- Simple unicellular trichome, UAW- Undulating anticlinal wall, DS- Diacytic stomata, PS- Paracytic stomata, ST- Stylloid crystal, PC- Prismatic crystal, SG- Starch granules

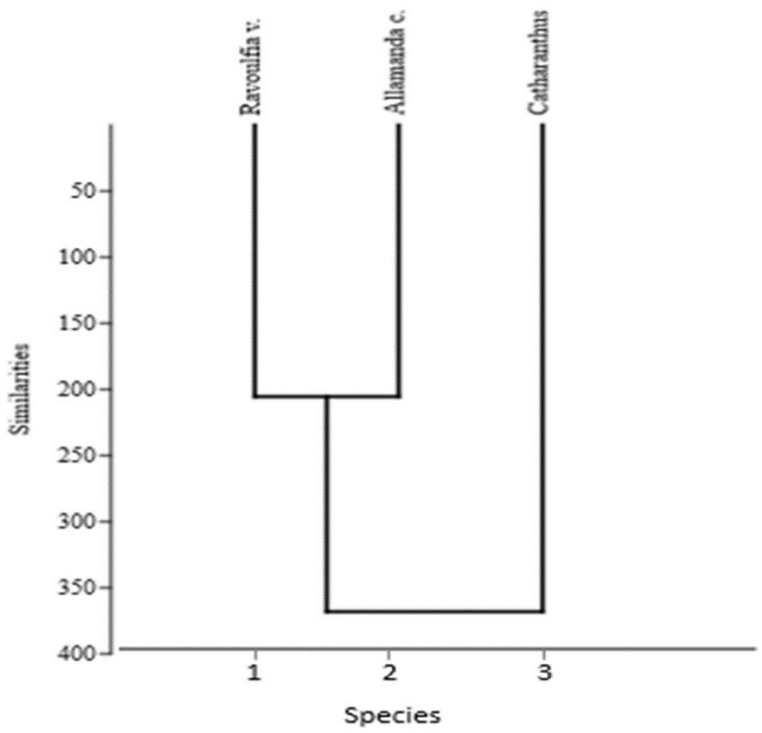

Figure 2: Cluster analysis of three species of *Apocynaceae* based on anatomical characters.

The cluster analysis shows that the three species originated from the same ancestor, *Rauvolfia vomitoria* and *Allamanda cathartica* are closely related at a similarity level of 200 than *Catharanthus roseus*.

The results of the quantitative characters on the epidermal surfaces showed that the highest stomata size was recorded on the abaxial surface of *Catharanthus roseus* (648.00 μm2), while the highest stomata index of 23.28 % was also found on the abaxial surface of *Catharanthus roseus* (Table 2). The highest guard cell area of 331.88 μm2 was recorded in *Catharanthus roseus* on the abaxial surface and the highest epidermal cell area of (2986.88 μm2) found in *Allamanda cathartica* on the abaxial surface (Table 2). *Rauvolfia vomitoria* and *Allamanda cathartica* had the lowest values of 0.00 μm2 in stomata size, stomata index and guard cell area because they both lack stomata on their adaxial surfaces while the lowest value of 2599.88 μm2 for the epidermal cell area was found on the adaxial surface of *Rauvolfia vomitoria* (Table 2), hence they don't have guard cell and the lowest epidermal cell area was found on the adaxial surface of *Rauvolfia vomitoria* (2599.88 μm2).

Table 2: Guard cell and Epidermal cell area of the Abaxial and Adaxial surfaces on the epidermis of the *Apocynaceae* species studied.

Species	Guard cell area (μm²)			Epidermal cell area (μm²)		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Catharanthus roseus (Adaxial)	162.50	270.38	425.00	812.50	2604.88	4687.50
Catharanthus roseus (Abaxial)	187.50	331.88	562.50	937.50	2839.63	5437.50
Rauvolfia vomitoria (Abaxial)	187.50	274.88	450.00	1031.25	2750.75	5568.75
Rauvolfia vomitoria (Adaxial)	0.00	0.00	0.00	812.50	2599.88	5000.00
Allamanda cathartica (Abaxial)	162.50	312.75	475.00	1487.50	2986.50	5437.50
Allamanda cathartica (Adaxial)	0.00	0.00	0.00	1462.50	2758.88	5250.00

Table 3: Stomata size and Stomata range of the Abaxial and Adaxial surfaces of the epidermis of the *Apocynaceae* species studied.

Species	Stomata size (μm²)			Stomata index (%)		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Catharanthus roseus (Adaxial)	243.75	541.63	850.00	10.00	17.60	33.33
Catharanthus roseus (Abaxial)	406.25	648.00	1012.50	16.67	23.28	33.33
Rauvolfia vomitoria (Abaxial)	312.50	507.63	787.50	8.33	19.90	42.86
Rauvolfia vomitoria (Adaxial)	0.00	0.00	0.00	0.00	0.00	0.00
Allamanda cathartica (Abaxial)	325.00	615.50	950.00	11.11	22.65	42.86
Allamanda cathartica (Adaxial)	0.00	0.00	0.00	0.00		

DISCUSSION

There has been some documentations in which epidermal markers were used to discriminate plant taxa (Ibrahim and Ayodele, 2013; Ogunkunle, 2013; Fayose and Freke, 2016; Haruna and Ashir, 2017; Shokefun *et al.*, 2017). Results obtained from this study have demonstrated that epidermal markers could provide a reasonable value for discriminating the selected medicinal tree species of *Apocynaceae*. For instance, only *Catharanthus roseus* out of the three medicinal species con-

sidered was amphistomatic Fig.1 (E - H). This marker could be used as a veritable means of discriminating it from other taxa. The result agrees with (Omino, (1996), in which amphistomatic distribution of stomata was used as a marker to set the boundary between the species of East African *Apocynaceae*.

Lack of stomata in *Allamanda cathartica* and *Rauvolfia vomitoria* may be the evidence of the closeness in the relationship of both species (Tables 2 and 3). Mbagwu *et al*., (2008) proposed that the preponderance of stomata on the adaxial surface is probably an adaptation to water loss. The variation noted in the stomata index among species, which is often a reflection of physiological responses, together with the combination of environmental factors can be of help in delimitation at the species level (Adegbite, 2008).

The presence of undulating anticlinal wall on the abaxial surface of the three species and perhaps not arranged in specific pattern was another important and unique epidermal marker. These patterns of anticlinal walls are the characteristics of the plants growing in humid condition (Ayodele and Olowokudejo, 2006).

Specific type of polygonal epidermal cells were maximum, followed by irregular epidermal cell, dominant on both the surfaces. Therefore, the epidermal and stomata markers which are similar among the species could be translated as the affinity that exists within the family, while those that were variable could be termed as diagnostic features among the species. This corresponds to the reports of Olowokudejo (1990), Ayodele and Olowokudejo, (2006), Ibrahim *et al*., (2006) and, Ibrahim *et al*., (2009) in which stomata and epidermal cells were used to solve taxonomic problems among medicinal plants. According to a report by (Olowokudejo and Nyananyo, (1990), the stomatal index is very reliable and useful in delimiting some medicinal tree species.

CONCLUSION

The research reveals that studying leaf epidermal features is crucial for accurate species identification. In this study, various epidermal markers were identified to be useful for the discrimination of the medicinal tree species in the taxa. This, therefore, calls for further study at the molecular level to complement the existing findings generated from the epidermal markers and consideration for separating the species from the current family.

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