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Taxonomic value of leaf epidermal markers in discriminating some medicinal tree species of *Apocynaceae* Juss. †

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Abstract: Apocynaceae is a useful family comprising of trees notable for different medicinal remedies. Consequent to their importance vis-à-vis scarcity in the forest, they are being sold in various Nigerian markets by herb sellers mostly in sterile and fragmentary forms. Hence, the medicinal plants are subjected to adulteration and substitution. Frequently, identification of the plants by users is basically with the aid of floristic markers, which are not readily available for such purpose. It, therefore, becomes pertinent to carry out the taxonomic revision of these trees to provide additional markers that will contribute to their effective identification for correct use. Various documentations have been made on members of apocynaceae and are properly placed on their respective taxa using epidermal traits. However, such information is scarce for *Alstonia boonei*, *Holarrhena floribunda, Rauvolfia vomitoria*, *Thevetia nerifolia* and *Vocanga africana.* This study therefore aimed at providing epidermal taxonomic markers that could be employed in delimiting the species as an alternative when the fruit or floral parts are wanting. Leaf epidermises of five (5) species of apocynaceae representing 5 genera were studied under a Biological microscope with a camera attachment. Data obtained were statistically analyzed. The epidermal cell was penta or hexagonal in *A. boonei* and *V. africana*. The stomatal length varied from 20.88 µm (*R. vomitoria*) to 25.92 µm (*T. nerifolia*) and 18.96 µm (*R. vomitoria*) to 29.28 µm (*V. africana*) on the abaxial and adaxial layers respectively. All the epidermal characters on the adaxial layer were significantly different ($p<0.05$) among the species. Anticlinal walls were sinuated in *H. floribunda* and *T. nerifolia* while in *R. vomitoria*, it was straight to wavy. *V. africana* and *A. boonei* anticlinal walls were straight. This study represents the first account of epidermal characterization of the members of apocynaceae in Nigeria and is of taxonomic importance in setting boundaries among the species.

Keywords: Medicinal trees; taxonomy; leaf epidermis; apocynaceae; stomatal crypt

1. Introduction

Apocynaceae Juss. is one of the important families in angiosperm founded in 1789. About 1900 species representing 215 genera have been identified worldwide [1]. According to [2], the family was delimited into five subfamilies, comprising of Secamonoideae, Apocynoideae, Rauvolfioideae, Periplocoideae and Asclepiadoideae. Plants of this family are greatly diversified in lifeform to trees, shrubs, climbers and rarely herbs [3–5]. Notable diagnostic morphological feature of the family is the production of pod-like fruits. The leaves are usually sessile or petiolate having variable shapes of lanceolate, ovate, linear, obovate, elliptic or oblong [1, 6–7].

Various works have been published on the epidermal characterization of many taxa of the family apocynaceae. *Wriohtia tinctoria, Ervatamia divaricata* and *Catharanthus* spp. were delimited based on

their amphistomatic leaves from six other apocynaceous species after studying the foliar epidermis of ten Indian species comprising of nine genera by [8]. Seven species representing seven genera were investigated by [1] and recommended the merging of Apocynaceae and Asclepiadaceae to one big family. *Nerium indicum* was distinguished from *Thevetia peruviana, Catharanthus roseus*, and *Tabernaemontana divaricata* by having stomatal crypt [9]. Despite these wonderful documentations, the taxonomic relationship among the members of *apocynaceae* remains unsettled and incomplete. This can be deduced from the fact that some of the African species in the family such as *Astonia boonei*, *Holarrhena floribunda, Rauvolfia vomitoria*, *Thevetia neriifolia* and *Vocanga africana* are still being left out. These species are among the trees commonly used for traditional medicine in Nigeria. The bark of *Alstonia boonei* in a tincture with the bark of *Enantia chlorantha* for the treatment of malaria and yellow fever [10]. According to [11], the decoction of root and bark of *Rauvolfia vomitoria* in tandem with some species of *meliaceae* is efficacious in the treatment of coated tongue disease.

Often time, identification of these medicinal trees is achieved using the floral and fruiting components. However, the existence of flowers is seasonal and is therefore useless for taxonomic discrimination purpose during off-flowering and fruiting seasons. Due to their wide application in ailment treatment, they are usually sold in the market either fragmentary or sterile conditions [12]. Hence, they are highly prone to substitution and adulteration, which is very inimical to effective application. Given the various medicinal uses of these species, there is a need for thorough taxonomic revision to provide additional markers for proper discrimination of the taxa. This study therefore aimed at providing important epidermal taxonomic markers that could be employed in delimiting the species as an alternative when the fruit or floral parts are wanting.

2. Materials and Methods

2.1. Sources of plant samples and Epidermal Peels preparation

Fresh leaf samples of the species were collected from Onigambari Forest reserve and University of Ibadan, Nigeria. Identification and authentication of the samples were carried out the Forest Herbarium, Ibadan. According to [13], leaf samples were first preserved in 50% ethanol before subjection to epidermal characterization. Mature leaves were randomly selected, cut into sizeable sections and soaked in concentrated nitic acid ranging from 8 to 24 hours depending on the leaf texture [14].

Swollen of the 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 [13–14].

2.2. Preparation of Slides, Assessment of epidermal characters and Data Analysis

Epidermal peels were first washed in water before staining them with appreciable drops of safranine [13]. For clear visibility, peels were counterstained using toluidine blue and the excess stains were removed by washing with water twice. The samples were subjected to a series of ethanol concentrations of 50%, 70%, 80%, 90% and 100% for approximately 3 minutes to dehydrate To completely remove all traces of stains, water and ethanol, the peels were treated using absolute xylene. Each epidermal peel was then mounted on a slide using 25% glycerol for the feasibility of the internal structure. The slides were studied under a CIWA XSP-35TV biological microscope. Photomicrographs were taken using with 200W Electronic Eyepiece. Quantitative variables such aslength and breadth of the epidermal cell, stomata were measured using an ocular micrometre.

Guard cell area (GCA) and Stomal index (SI) were estimated according to [15]. Data were subjected to analysis of variance (ANOVA).

3. Results

Qualitative epidermal markers of the Nigerian species of apocynaceae are shown in Table 1. Stomata were present on both the abaxial and adaxial surfaces of all the species except for *Alstonia boonei*, which lacked stomata on the adaxial layer (hypostomatic). Paracytic stomata were identified in all the species except in *A. boonei,* where stomatal crypts were found (Figure 1). Generally, stomata were more distributed in the abaxial surface compared to the adaxial layer of all the species. Stomata were abundant in an abaxial layer of *R. vomitoria, H. floribunda* and *T. nerifolia* but were scanty in *V. africana* and *A. boonei.*

Epidermal cells were generally polygonal (Table 1). The epidermal cell was penta or hexagonal in *A. boonei* and *V. africana* (Figure 1). Anticlinal walls were sinuated in *H. floribunda* and *T. nerifolia* while in *R. vomitoria*; it was straight to wavy (Table 1). *V. africana* and *A. boonei* anticlinal walls were straight. Crystal was present in the epidermal cell of all the species on both leaf surfaces. However, crystal druses were discovered in *R. vomitoria, H. floribunda* and *T. nerifolia,* whereas, crystal raphides were discovered *V. africana* and *A. boonei.*

Table 1. Qualitative epidermal markers of the Nigerian species of apocynaceae.

Epidermal Markers	**Leaf surfaces**	***Rauvofia vomitoria***	***Holarhena floribunda***	***Vocanga africana***	***Thevetia nerifolia***	***Alstonia boonei***
Stomata (P/A)	Abaxial	Present	Present	Present	Present	Present
Stomatal type	Abaxial	Paracytic	Paracytic	Paracytic	Paracytic	Stomatal crypts
Stomata abundance	Abaxial	Many	Many	Few	Many	Few
Cell shape	Abaxial	Polygonal	Polygonal	Polygonal	Polygonal	Polygonal
Anticlinal wall	Abaxial	Straight-wavy	Sinuated	Straight	Sinuated	Straight
Crystal type	Abaxial	Druses	Druses	Raphides	Druses	Raphides
Stomata (P/A)	Adaxial	Present	Present	Present	Present	Absent
Stomatal type	Adaxial	Paracytic	Paracytic	Paracytic	Paracytic	Nil
Stomata abundance	Adaxial	Few	Few	Few	Few	None
Cell shape	Adaxial	Polygonal	Polygonal	Polygonal	Polygonal	Polygonal
Anticlinal wall	Adaxial	Straight-wavy	Sinuated	Straight	Sinuated	Straight
Crystal type	Adaxial	Druses	Druses	Raphides	Druses	Raphides

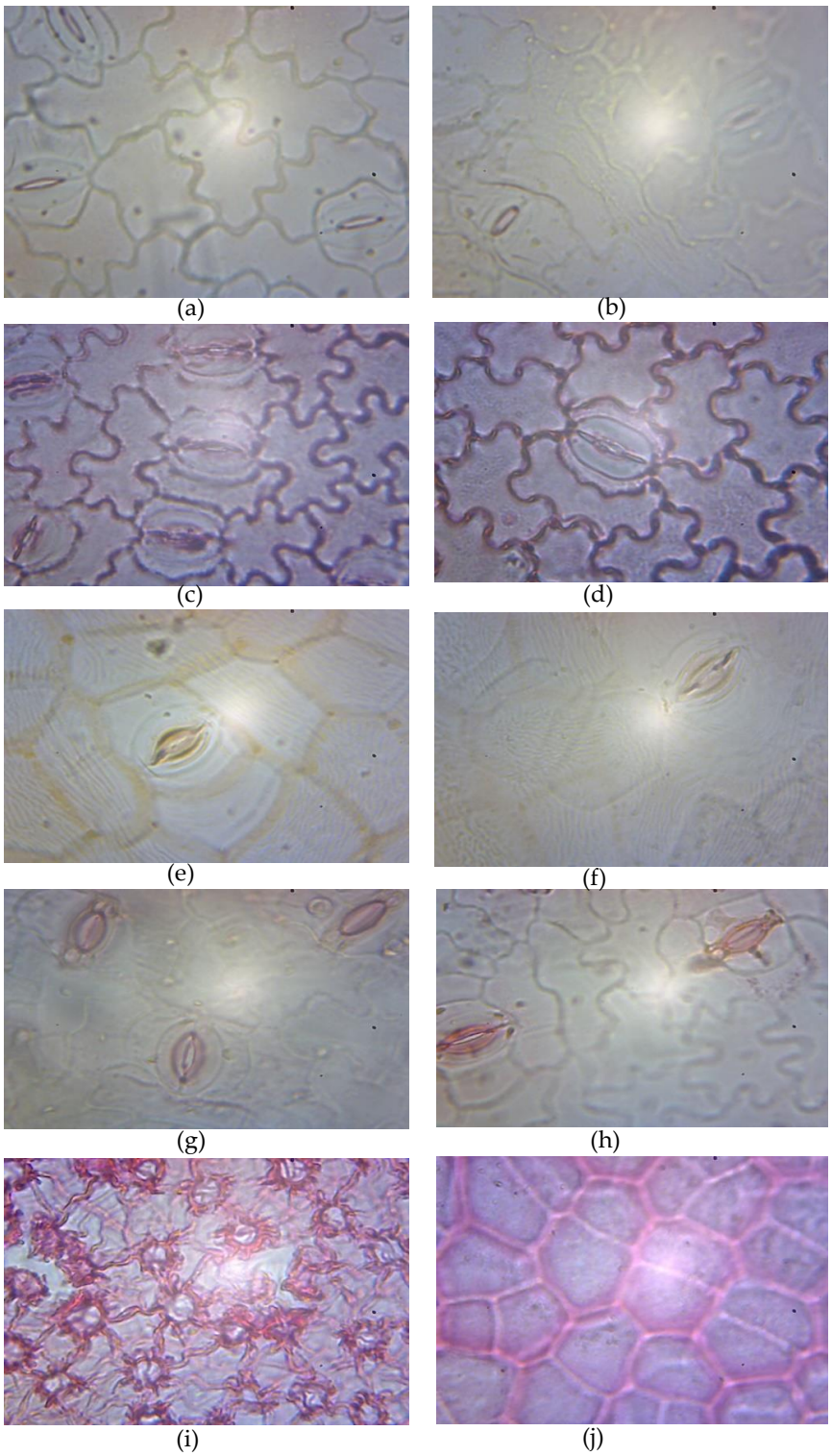

Figure 1. Photomicrograph of the species: (a) *Rauvolfia vomitoria* (abaxial); (b) *Rauvolfia vomitoria* (adaxial); (c) *Holarhena floribunda* (abaxial); (d) *Holarhena floribunda* (adaxial); (e) *Vocanga africana* (abaxial); (f) *Vocanga africana* (adaxial); (g) *Thevetia nerifolia* (abaxial); (h) *Thevetia nerifolia* (adaxial); (i) *Alstonia boonei* (abaxial); (j) *Alstonia boonei* (adaxial)

Quantitative epidermal markers of the species on the epidermal are presented in Table 2. Length of stomata varied from 20.88 μm (*R. vomitoria*) to 25.92 μm (*T. nerifolia*). Highest length of the epidermal cell (34.32 μm), breadth of the epidermal cell (20.64 μm), breadth of stomata (21.6 μm), the width of guard cell (9.12 μm) and guard cell area (144.66 μm^2) were found in *Holarhena floribunda* while the least was (19.44 μm), (10.08 μm), (9.60 μm), (4.08 μm), (65.73 μm^2) respectively discovered

in *Alstonia boonei*. Stomatal density ranged from 85.95 mm² (*A. boonei*) to 137.32 mm² (*R. vomitoria*) while stomatal index varied from 28.57 % (*Vocanga* africana) to 46.34 % (*T. nerifolia*). *A. boonei* (19.87 μm) and *R. vomitoria* (20.88 μm) had the least length of guard cell and length of the epidermal cell respectively while *T. nerifolia* had the highest values.

All the epidermal markers were significantly different ($p<0.05$) among the species except for the length of the guard cell and length of stomata on the abaxial leaf surface (Table 2).

Table 2. Quantitative epidermal markers (abaxial layer) of the Nigerian species of apocynaceae

Tree species	LE (μm)	BE (μm)	LS (μm)	BS (μm)	LGC (μm)	WGC (μm)	GCA (μm²)	SD (mm²)	SI (%)
Rauvolfia vomitoria	24.00	11.04	20.88	11.76	20.4	4.32	68.98	137.32	36.84
Hollarhena floribunda	34.32	20.64	22.08	21.6	20.35	9.12	146.66	88.41	30.00
Vocanga africana	31.44	20.63	21.93	12.72	21.84	4.44	80.75	122.79	28.57
Thevetia neriifolia	29.52	18.00	25.92	12.00	24.48	4.80	93.19	110.51	46.34
Alstonia boonei	19.44	10.08	22.80	9.60	19.87	4.08	65.73	85.95	35.35
p-value	0.000*	0.000*	0.182^{ns}	0.000*	0.217^{ns}	0.000*	0.008*	0.000*	0.041*

LE = length of epidermal cell, BE = breadth of epidermal cell, LS = Length of stomata, BS = breadth of stomata, LGC = length of guard cell, WGC = width of guard cell, GCA = guard cell area, SD = Stomatal density, SI = Stomatal index; * = significant at 5% probability level; ns = not significant at 5% probability level.

On the adaxial leaf surface, highest length of the epidermal cell (30.48 μm), breadth of stomata (19.68 μm) and guard cell area (249.04 μm²) were discovered in *Holarhena floribunda* while the least values; 24.00 μm, 10.46 μm and 95.77 μm² were respectively obtained in *Rauvolfia vomitoria* (Table 3). Length of stomata and guard cell ranged from 18.97 μm and 18.96 μm in *Rauvolfia vomitoria* to 29.28 μm and 28.32 μm respectively in *Vocanga africana* (Table 3). The least stomatal density (29.47 mm²) and stomatal index (23.07 %) were recorded in *H. floribunda* while the highest; 186.64 mm²and 44.44 % were found in *T. nerifolia respectively*. The breadth of the epidermal cell increased from *V. africana* (12.48 μm) to *H. floribunda* (21.12 μm), whereas, the width of guard cell varied from *T. nerifolia* (5.66 μm) to *H. floribunda* (14.64 μm). Quantitative epidermal markers of the adaxial layer are significantly different ($p<0.05$) among the species.

Table 3. Quantitative epidermal markers (adaxial layer) of the Nigerian species of apocynaceae

Tree species	LE (μm)	BE (μm)	LS (μm)	BS (μm)	LGC (μm)	WGC (μm)	GCA (μm²)	SD (mm²)	SI (%)
Rauvolfia vomitoria	24.00	13.68	18.97	10.46	18.96	6.38	95.77	98.23	38.83
Hollarhena floribunda	30.48	21.12	24.00	19.68	21.36	14.64	249.04	29.47	23.07
Vocanga africana	26.16	12.48	29.28	12.96	28.32	6.48	142.95	39.29	29.00
Thevetia neriifolia	30.00	18.24	26.64	16.56	26.16	5.66	116.26	186.64	44.44
Alstonia boonei	-	-	-	-	-	-	-	-	-
p-value	0.030*	0.001*	0.001*	0.000*	0.002*	0.012*	0.049*	0.000*	0.000*

LE= length of epidermal cell, BE=breadth of epidermal cell, LS= Length of stomata, BS=breadth of stomata, LGC=length of guard cell, WGC=width of guard cell, GCA=guard cell area, SD= Stomatal density, SI= Stomatal index; *=significant at 5% probability level.

4. Discussion

There has been some documentation in which epidermal markers were used to discriminate plant taxa [14, 16–19]. Results obtained from this study clearly demonstrated that epidermal markers could provide a reasonable value for discriminating the selected medicinal tree species of

apocynaceae. For instance, only *Alstonia boonei* of all the medicinal species considered was hypostomatic. This marker could be used as a veritable means of discriminating it from other taxa. The result agrees with [20], in which hypostomatic distribution of stomata was used as a marker to set the boundary between the species of East African apocynaceae.

Another important and unique epidermal marker peculiar to *A. boonei* is the stomatal crypt. Stomatal scripts are also known as sunken stomata that are located in depressed portions of the epidermis, which forms a narrow-mouthed or deep longitudinal groove [21]. The stomatal crypt can contain one or more stomata and at times with trichomes or wax accumulations. The presence of sunken stomata in *A. boonei* can be linked to its wide range of ecological distribution, which occurs both in the savannah and wet regions [22]. The effectiveness of stomatal crypt for apocynaceae taxa delimitation has been reported. *Nerium indicum* was said to be taxonomically distinct from *Catharanthus roseus* and *Tabernaemontana divaricata* by having stomatal crypts [23]. *N. indicum* was the first species of apocynaceae to be reported with stomatal crypts characteristics while *A. boonei* observed in the present study happened to be the second species with such important taxonomic trait.

Other useful qualitative markers with taxonomic value based on the result include stomatal abundance, anticlinal wall and crystal types, which varied among the species. Such markers could be employed at both specific and generic level of the medicinal species as previously reported in some plant's families [13, 17]. The overlap in the epidermal cell shape of these species makes it unsuitable as a marker for taxonomic purpose in the taxa.

Holarhena floribunda was separated from other species by having the largest value for some of the stomata and epidermal quantitative markers, especially on the abaxial leaf surface. Also, *Thevetia nerifolia* was singled out with the highest value of the stomata index on both the abaxial and adaxial layers. Therefore, the epidermal and stomatal 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 literature [12, 24–26] in which stomatal and epidermal markers were used to solve taxonomic problems among the medicinal plants. According to a report by [12], the stomatal index is very reliable and useful in delimiting some medicinal tree species.

5. Conclusion

Aside from the fact that this study is the first account of epidermal characterization of the members of apocynaceae in Nigeria, various epidermal markers have been identified to be useful for the discrimination of the medicinal tree species in the taxa. Of worthy of note is the stomatal crypt, which is being reported for the first time in Nigerian *Alstonia boonei*. This, therefore, calls for further study at the molecular level to complement the existing finding generated from the epidermal markers and consideration for separating the species from the current family.

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