

Comparative epidermal morphology of Nigerian species of *Alchornea* (Euphorbiaceae)

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Abstract

The leaf epidermal morphology of the three Nigerian species of *Alchornea* (Euphorbiaceae) has been studied using light microscopy. Epidermal cells are irregular with undulate to sinuate anticlinal walls. The abaxial and adaxial walls of *A. cordifolia* and *A. floribunda* respectively are characterized by the presence of knobs on them. All taxa have paracytic stomata. However, while *A. cordifolia* and *A. laxiflora* are amphistomatic, *A. floribunda* is hypostomatic. Trichomes are simple, unicellular and uniseriate in *A. floribunda* and *A. laxiflora* while stellate trichomes with two to eight radiating arms are present in *A. cordifolia*. All taxa except *A. laxiflora* are entirely smooth on the adaxial surface. An indented dichotomous key based on the features of light microscopy is presented for the identification of the species.

Keywords: *Alchornea*, morphology, Nigeria.

Introduction

Alchornea Sw. is represented by three species in Nigeria. *A. cordifolia* (Schum. and Thonn) Mull Arg., *A. floribunda* Mull Arg., and *A. laxiflora* (Beith.) Pax and K. Hoffm. [2, 3]. It is a genus of high medicinal value in Nigeria. The extract of the leaf of *A. cordifolia* has a high level of anti-bacterial and anti-fungal activities [5]. It is applied as a mouthwash for toothache and dental caries [1]. The twigs and stems are chewed often with salt as a remedy for diarrhoea and cough and to keep the mouth free of diseases [1]. The powdered leaves are used to treat skin infections such as ringworm while the pulped root is taken as a remedy for gonorrhoea in Nigeria [1]. A cold infusion of the dry and crushed leaves is commonly used in the treatment of venereal diseases.

The roots of *A. floribunda* are used as toothpicks and aphrodisiac and in the treatment of ophthalmia [2]. The leaf sap of the same plant is used in the treatment of skin infections and circumcision wounds while the leaves are commonly eaten as a vegetable with meat or fish as an antidote to poison [1]. *A. floribunda* has been reported to have hallucinogenic potentials [7]. The stem and branchlets of *A. laxiflora* are used in Nigeria as chew sticks. The plant is also used in a Yoruba incantation to achieve bad medicine rebound onto the sender.

This work is a contribution to the programme of research into the taxonomy of medicinal plants which aims at providing useful taxonomic characters that will facilitate an accurate and rapid identification of specimens through a detailed systematic examination of the leaf epidermal surfaces. This paper reports the epidermal characters of the three taxa of *Alchornea* represented in Nigeria as observed by light microscopy. It highlights the significance of these characters and discusses the extent to which they may be used in the identification of the *Alchornea* species even under such conditions in which they are purchased from the local herbal markets.

Materials and methods

The plant materials used for this investigation were herbarium specimens collected from wide geographical ecological zones in Nigeria. All specimens of the genus *Alchornea* represented in the Forestry Research Herbarium (FHI) Ibadan and the Herbarium of the Department of Botany and Microbiology, University of Ibadan, Ibadan (UIH) were studied.

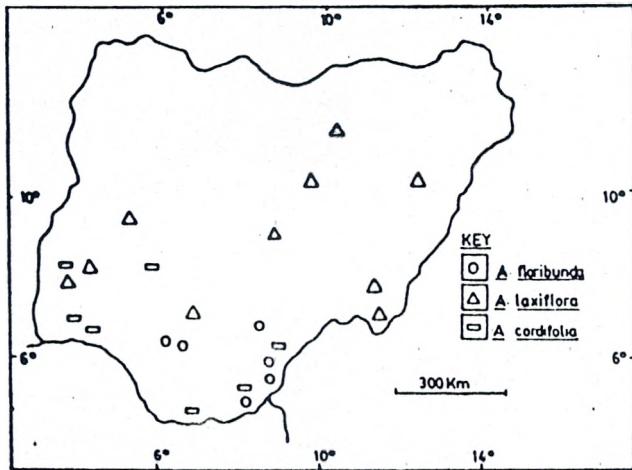


Fig. 1 : Map showing the distribution of *Alchornea* species in Nigeria.

Epidermal preparation

An area of about 2cm² was cut from the standard median position on each leaf specimen of the three species. Two of three specimens were used for each species depending on geographical spread. Each portion was then soaked in boiling water for 10-20 minutes. Each of the specimens was transferred to a Petri-dish containing 15% Sodium hypochlorite (NaOCl) over night after which the specimen

was completely bleached and the epidermal layers separated from the mesophyll. This was indicated by bubbles appearing on the leaf fragments.

Each sample was transferred to a Petri-dish containing distilled water to which two drops of Acetic acid has been added to neutralise the effect of the Sodium hypochlorite. The adaxial and abaxial membranes of each specimen were subsequently teased from the mesophyll using a pair of fine forceps and dissecting needles. The membranes were cleaned with a camel hair brush, rinsed in water and transferred to 50% ethanol for 5 minutes to harden them. They were stained in aqueous safranin for 5 minutes before being dehydrated by passing through 50%, 70%, 90%, absolute ethanol series and a mixture of equal parts of absolute ethanol and xylene (about 5 minutes in each). The membranes were cleared in xylene for 2 minutes and mounted in Canada balsam and the slides were dried on a hot plate.

For statistical analysis, 50 epidermal cells and stomata were chosen randomly from each taxon and measured using a micrometer eye-piece. All drawings were made using a wild M12 microscope with camera lucida attachment.

Results

A. laxiflora is a dryland and savanna species. *A. floribunda* is abundant in very wet areas but usually in unsalty vegetation while *A. cordifolia* is intermediate between the two species which may inhabit both wet and dry lands.

Table 1: Epidermal characters of the genus *Alchornea* in Nigeria

Taxa	Leaf surface	No. of cells/mm ²	Cell wall thickness	Cell width	Anticlinal wall pattern	Cell shape	Trochome type	Trichome Frequency /mm ²
<i>A. cordifolia</i>	Adaxial	624 - 870 731 ± 14.8	1.5 - 2.5 1.8 ± 0.6	20.0 - 25.0 21.4 ± 0.3	Undulate	Irregular	Absent	Absent
	Abaxial	572 - 864 719 ± 14.4	1.0 - 2.5 1.6 ± 0.1	12.5 - 32.5 23.5 ± 1.0	Undulate with Knobs	Irregular	Stellate	2 - 5 3 ± 0.1
<i>A. floribunda</i>	Adaxial	504 - 950 811 ± 19.7	2.0 - 2.5 2.3 ± 0.1	16.3 - 27.5 18.8 ± 0.9	Undulate with Knobs	Irregular	Absent	Absent
	Abaxial	440 - 621 555 ± 8.0	1.0 - 2.0 1.4 ± 0.1	27.5 - 42.5 32.5 ± 0.7	Undulate sinuate	Irregular	Simple, Unicellular, Uniseriate,	4 - 6 5 ± 0.1
<i>A. laxiflora</i>	Adaxial	270 - 374 315 ± 5.8	1.5 - 2.5 2.1 ± 0.1	27.5 - 45.0 31.6 ± 1.0	Sinuate	Irregular	Simple, Unicellular, Uniseriate,	0 - 2 1 ± 0.1
	Abaxial	462 - 690 587 ± 9.6	1.0 - 2.0 1.3 ± 0.1	20.0 - 35.0 28.5 ± 0.9	Sinuate	Irregular	Simple, Unicellular, Uniseriate,	1 - 4 3 ± 0.3

All measurements in microns = $\frac{\text{Range}}{\text{Mean} \pm \text{Standard Error}}$

Table 2: Stomata characters of the genus *Alchornea* in Nigeria

Taxa	Leaf Surface	Stomatal Frequency mm ²	Stomatal Length	Stomatal Width	Stomatal type	Standard Index %
<i>A. cordifolia</i>	Adaxial	9 - 15 11 ± 0.4	20.0 - 32.5 25.0 ± 0.7	17.5 - 37.5 26.7 ± 1.0	Paracytic	1.5
	Abaxial	40 - 69 57 ± 1.6	12.5 - 25.0 18.9 ± 0.8	12.5 - 20.0 15.8 ± 0.6	Paracytic	7.4
<i>A. floribunda</i>	Adaxial	Absent	Absent	Absent	Absent	Absent
	Abaxial	25 - 43 34 ± 1.2	17.5 - 25.0 20.6 ± 0.4	8.8 - 21.3 11.8 ± 0.6	Paracytic	5.8
<i>A. laxiflora</i>	Adaxial	0 - 2 1 ± 0.2	17.5 - 30.0 9.09 ± 0.6	7.5 - 12.5 9.1 ± 0.3	Paracytic	0.3
	Abaxial	14 - 20 17 ± 0.3	17.5 - 27.5 20.5 ± 0.5	10.0 - 12.5 10.6 ± 0.3	Paracytic	2.8

All measurements in microns = $\frac{\text{Range}}{\text{Mean} \pm \text{Standard Error}}$

Epidermal morphology

Epidermal cells are irregular with undulate to sinuate anticlinal walls on both surfaces of the leaves in all taxa. The epidermal walls of *A. cordifolia* and *A. floribunda* have knobs on their abaxial and adaxial surface respectively

Fig. 2: Drawings of adaxial and abaxial epidermal surfaces of *Alchornea* species.

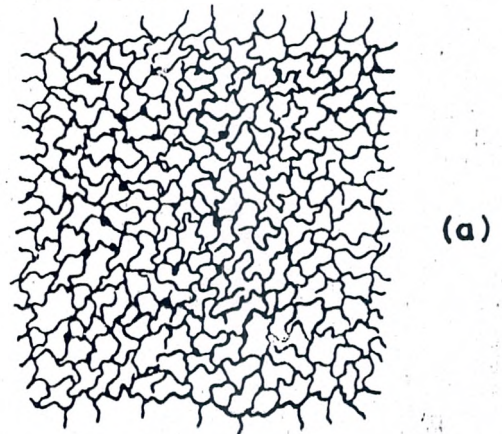


(a) *A. cordifolia* adaxial surface showing irregular cells with undulate anticlinal walls and paracytic stomata.



(b) *A. cordifolia* abaxial surface showing irregular cells with undulate anticlinal walls, paracytic stomata, stellate hairs and knobs on walls.

Fig. 3 Drawings of adaxial and abaxial epidermal surfaces of *Alchornea* species.



(a) *A. floribunda* adaxial surface showing irregular cells with undulate anticlinal walls and knobs on walls.

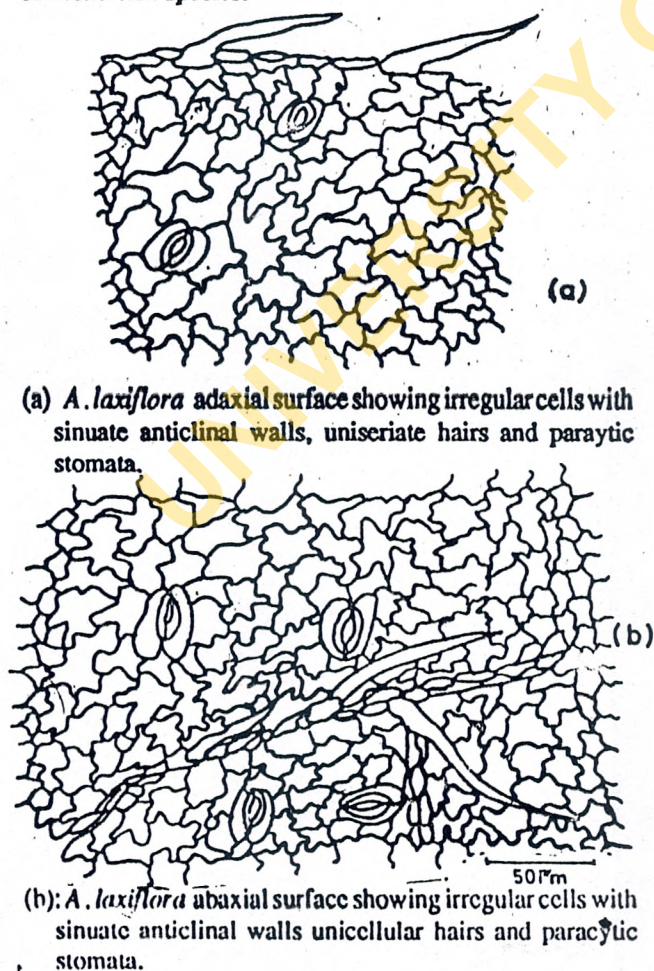


(b) *A. floribunda*, adaxial surface showing irregular cells with undulate to sinuate anticlinal walls, uniseriate hairs and paracytic stomata.

(Figs 2b & 3a). The number of epidermal cells vary within the genus. On the adaxial surface, the highest mean number of 811 was recorded for *A. floribunda* while the lowest mean value of 315 was recorded for *A. laxiflora* (Table 1). However, on the abaxial surface, the highest mean value of 719 was recorded in *A. cordifolia* while *A. floribunda* with a mean value of 555 had the lowest value. Except for *A. laxiflora*, epidermal cells are usually more per unit area on the adaxial surfaces (Table 1). The largest epidermal cells on the adaxial surface are found in *A. laxiflora* with a mean value of 31.6 μ m while the smallest cells occur in *A. floribunda* with a mean value of 18.8 μ m. On the abaxial surface *A. floribunda* with a mean value of 32.5 μ m has the largest cells while *A. cordifolia* with a mean value of 23.5 μ m has the smallest cells. The cell walls are much thicker in *A. floribunda* than in any other species especially on the adaxial surface (Table 1). The upper epidermal walls are generally thicker than those of the lower surface (Table 1).

All taxa except *A. laxiflora* are smooth on the adaxial surfaces (Fig 2-4). In *A. laxiflora*, the trichomes are mainly confined to the veins (Fig. 4a). On the abaxial surfaces

Fig. 4: Drawings of adaxial and abaxial epidermal surfaces of *Alchornea* species.



however simple unicellular trichomes occur in *A. floribunda* (Fig. 3b) and *A. laxiflora* (Fig. 4b) while stellate trichomes with 2-8 radiating arms are present in *A. cordifolia*. (Fig. 2b). It is noteworthy to state that more trichomes were observed per unit area in *A. floribunda* than in *A. laxiflora* with which it possesses the same type on the abaxial surface. The presence of trichomes on the epidermal cells on the adaxial surfaces of *A. laxiflora* is noteworthy.

Stomata type is paracytic in all taxa with the stomatal pore surrounded by two subsidiary cells parallel to the guard cells. While *A. floribunda* is hypostomatic with stomata occurring on the abaxial surface only, *A. cordifolia* and *A. laxiflora* are amphistomatic with stomata on both surfaces of the leaves. (Table 2, Figs 2-4). The stomata frequency per unit area varies within the genus with the highest and lowest values of 11 and 1 recorded for *A. cordifolia* and *A. laxiflora* respectively on the adaxial surface. On the abaxial surface, the highest frequency was found in *A. cordifolia* while the lowest frequency was found in *A. laxiflora*. The stomatal complex varies considerably among the taxa on the abaxial surface. The stomatal size of 20.6 x 11.8 μ m found in *A. floribunda* is the largest while the smallest stomata of 18.9 x 15.8 μ m is recorded in *A. cordifolia*. On the adaxial surface, *A. cordifolia* with a stomata size of 25 x 26.7 μ m is the largest. The stomatal index also varies from 0.3 to 1.5 on the adaxial surface in *A. laxiflora* and *A. cordifolia* respectively while on the abaxial surface *A. cordifolia* with a value of 7.4 and *A. laxiflora* with a value of 2.8 are the highest and lowest indices respectively (Table 2).

Discussion

The preceding observations and the summaries of character variation indicate the taxonomic significance of the micromorphological characters employed in this study. The occurrence of irregular cells with usually undulate to sinuate anticlinal walls on both surfaces of all species and the paracytic stomata type show the affinity of these taxa in the genus. Nonetheless, the presence of knobs on the walls of *A. cordifolia* and *A. floribunda* is noteworthy and can be used to separate both species from *A. laxiflora*. The variation in the number of epidermal cells and cell size particularly on the adaxial surface is a useful taxonomic character. *A. floribunda* with the smallest but highest number of cells on the adaxial surface can be separated from *A. cordifolia*. The presence of stellate hairs on the abaxial surface of *A. cordifolia* distinguishes it from other species with simple unicellular hairs. This observation agrees with Nyawuame *et al.* [6] who reported stellate hairs in *A. cordifolia*. Stace [8] has already noted that hairs are constant in a species when present and showed a constant range of form and distribution useful in diagnosis. Nevertheless, Metcalfe and Chalk [4] hold that trichome frequency and size are environmentally controlled, hence extreme caution is required if they are to be used taxonomically. This may probably provide explanation for

the presence of trichomes on the adaxial surface of *A. laxiflora* which tends to regulate water loss in the dry environment.

Stomata type is of no diagnostic importance in the taxa because of the paracytic type present in all the Nigerian species. This is however in contrast to the claim by Nyawuame *et al* [6] that the stomata in that *A. laxiflora* are anomocytic. While *A. cordifolia* and *A. laxiflora* are amphistomatic, *A. floribunda* is hypostomatic. This further serves as a useful character separating *A. floribunda* from the two species. This type of stomatal distribution has been found to be a diagnostic character because of its restricted occurrence [9]. The latter [9] has also observed that high altitude appears to induce an increase in the proportion of species with amphistomatic leaves. In *A. cordifolia*, the stomata are comparatively larger than in other species. This according to Wilkinson [9] is often correlated with density. Shade, humid atmosphere and moist soil conditions are also known to be coincidental with smaller stomata while full light and drier conditions seem to produce larger stomata. In all the species *A. laxiflora* has the least number of stomata on both the adaxial and abaxial surfaces. It should be noted that this species has a preference for high altitudes among the Nigerian species of the genus. Stomatal index is of taxonomic importance in the genus. The abaxial values clearly distinguish *A. cordifolia* with a comparatively high index of 7.4% and *A. laxiflora* with a very low value of 2.8%.

The indented dichotomous key presented below allows easy separation of the species based on features of light microscopy.

Key to Species of *Alchornea* in Nigeria.

1. Stomata few, less than 20 on abaxial surface, abaxial stomatal index less than 3%, anticlinal walls usually sinuate, knobs absent on walls, hairs present on both surfaces ... *A. laxiflora*.
1. Stomata many, more than 30 on abaxial surface,

abaxial stomatal index more than 5%, anticlinal walls undulate to sinuate, knobs present on walls, hairs absent on adaxial surface, ... 2.

2. Leaf hypostomatic, hairs simple, unicellular, stomata larger 20.6 x 11.8 μm on abaxial surface... *A. floribunda*
2. Leaf amphistomatic, hairs stellate, stomata smaller, 18.9 x 15.8 μm on abaxial surface... *A. cordifolia*.

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