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Research

Foliar epidermal and Pollen characters in the genus *Cola* Schott. & Endl. in Nigeria

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Abstract

The leaf epidermal and pollen characters of eight Cola species in Nigeria were studied by light microscopy for the purpose of identification of the species even in fragmentary and sterile state. The epidermal cells were polygonal in shape except in C. glabra where they were irregular. The anticlinal walls were straight (C. gigantea), undulate (C. glabra) and straight to slightly curved in the others. C. acuminata, C. laurifolia and C. nitida were amphistomatic while the others were hypostomatic. Stomatal types were anisocytic (C. flaviflora), anomocytic (C. glabra), laterocytic (C. hispida) but staurocytic in the others. Crystal sands were present in C. hispida. C. flaviflora (24-99) and C. hispida (42-110) had the lowest number of epidermal cells/mm^2 while the highest occurred in C. millenii (720-930). Conversely, C. flaviflora (24-50μm) and C. hispida (29-60μm) had the widest cells. The highest stomatal densities occurred likewise in C. hispida and C. gigantea (72-121) but the lowest were found in C. flaviflora (24-70). Whereas, C. acuminata recorded the largest stomata (24-26 x 15-17μm), it had the lowest stomatal index (15.7%). The highest index (60.6%) was found in C. hispida while the smallest stomata belonged to C. laurifolia (9-12 x 9-11μm). Trichomes, though absent in all the taxa, the bases were present in them and very prominent in C. gigantea. The pollen grains were either subprolate or prolate spheroidal. The presence and number of pores and furrows varied in the species, tricolporate (C. acuminata, C. gigantea and C. hispida), dicolporate (C. flaviflora, C. millenii and C. nitida) and inaperturate (C. glabra and C. laurifolia). The combination of these various micro-morphological characters is useful for the delimitation and identification of the species even in their fragmentary state.

Keywords: Leaf epidermis, Stomata, Pollen, *Cola*, Nigeria

Introduction

Cola Schott. & Endl. is a large genus of about 125 species restricted to the forest regions of Africa (Burkill, 2000). About 42 species of the genus are recognized in West Tropical Africa (Hutchinson and Dalziel, 1954), 31 of which species occur in Nigeria. All the Nigerian species are found in lowland rainforest and forest outliers and are predominantly small to large trees with a few shrubs (Keay *et al.*, 1960).

The economic importance of *Cola* stems from its social and cultural uses. *C. acuminata* and *C. nitida* are valued for their nuts which contain caffeine (Kochlar, 1986; Langenhein & Thiman, 1982).

However, *C. acuminata* is more prominently used in cultural activities while *C. nitida* is preferred for chewing because of its higher caffeine content. *Cola* seeds have been used in tropical Africa for centuries as a masticatory in much the same way as coca leaves in South America. Kolanut aids the digestive system (Akinbode, 1982) and has nutritive value which sustains life for a while even without food.

It may be boiled or pulverized into a beverage that inhibits fatigue and forestall hunger (Langenhein & Thimann, 1982). The chewing of *Cola* is known to cause mild stimulation of the central nervous system and produces a temporary feeling of increased physical strength, often associated with a reduction of hunger and fatigue (Kochler, 1986; Langenhein & Thiman, 1982). Small doses of kolanut

increase mental activities and thus, reduce the need for sleep (Akinbode, 1982). Medicinally, the powdered bark of *Cola gigantea* is applied to sores and ulcers and decoction taken internally as a remedy for pile (Irvine, 1961). The juice from leaves of *Cola gigantea* macerated in water is applied to the eye for ophthalmia while the powdered leaves with tobacco are used as snuff (Irvine, 1961). A decoction of *Cola acuminata* has healing properties in cleansing sores (Burkill, 2000). The root of *Cola digitata* is pulped and given as a sedative in cases of trembling and convulsions while the oily seed is used as a vermifuge (Burkill, 2000). A decoction from *Cola gigantea* with wood-ash is a linimeny put on oedema and the bark is used for headache, intestinal and lumbar pain (Burkill, 2000). A leaf decoction of *Cola hispida* is used to ease cough and stomach trouble and the sap from fresh leaves is dripped into the ear for inflammation of the outer ear tract. The powdered root mixed with palm oil is applied to the skin for skin infection and to kill body lice (Burkill, 2000).

Takhtajan (1973) noted that the micro molecular approach to plant classification, though useful at generic and lower levels, is of very limited value at the higher taxonomic levels. Chemical data are being used either by recording the presence or absence of various compounds in different taxa or by comparing structural features and biosynthetic pathways of common or related compounds. According to Cronquist (1973), the taxonomic system is supposed to reflect the totality of similarities and differences among organisms. Therefore, all kinds of data including chemical data, insofar as it is reasonably possible are used in classification. According to Metcalfe and Chalk (1950), the systematic anatomists rely on those characters which are less plastic. The classification of plants based on stomata type, number and position of subsidiaries as well as ontogeny had been proposed (Van Cotthen, 1970).

The present study is a contribution to the taxonomy of the genus *Cola* in Nigeria. The aim is to provide tools for quick identification of specimens of the genus even if in fragmentary state. Eight species of the genus were selected based on their availability and economic importance.

Material and methods

Eight species of *Cola*, *C. acuminata*, *C. flaviflora*, *C. gigantea*, *C. glabra*, *C. hispida*, *C. laurifolia*, *C. millenii*, and *C. nitida*, were selected for the study. Fresh samples and dried herbarium specimens were employed for this study. Specimens were studied at the Herbaria of the Forestry Research Institute of Nigeria (FHI) Ibadan and the University of Ibadan (UIH), Ibadan, Nigeria. Fresh specimens were collected from the forest south of the country. *C. acuminata* and *C. glabra* were collected from Owena, Ondo State, *C. acuminata* and *C. millenii* from the Botanical garden, University of Ibadan, *C. hispida* from Ibusogboro, Oyo State, *C. nitida* from Gambari Forest Reserve, Ibadan, Oyo State and *C. flaviflora* from Olokemeji Forest reserve, Ogun State.

Epidermal Preparation

Fresh leaves of each species were preserved in 50% ethanol while the dried leaves were irrigated by boiling in water. The preserved leaves were rinsed in ordinary water. A $1cm^2$ area was cut from the standard median portion of the leaf of each species, soaked in concentrated trioxonitrate V acid (HNO_3) and left in the sun to hasten the action of the acid. The formation of air bubbles in the leaves indicated the separation of the upper and the lower epidermides from the mesophyll layer. The specimens were transferred into new Petri dishes and rinsed in water three times. The epidermides were separated carefully with a pair of forceps. The layers were brushed with camel hairbrush to remove residual mesophyll layer. The peels were stained in Toluidine blue for about 3 min, rinsed in water and mounted in glycerol on clean glass slides. The edges of the cover slips were sealed with nail varnish to prevent dehydration. A NIKON AFX-DX photomicroscope was used to take photomicrographs of the specimens. 25 measurements of each character were taken at random using a micrometer eyepiece graticule and, the mean and standard error calculated. The Stomatal Index (SI) was calculated using the formula of Salisbury (1927).

$$SI = \frac{S}{S+E} x100\%$$

where

S = number of stomata per unit area

E = number of epidermal cells of the same unit area.

Pollen Preparation

The method of acetolysis by Erdtman (1952) was used for pollen analysis. Dried flower buds were crushed with a glass rod in a centrifuge tube. $3cm^3$ of freshly prepared acetolysis mixture (9:1 acetic anhydride and concentrated tetraoxosulphate VI [H_2SO_4]) was added to each pollen sample and heated in water bath from 70°C to boiling point. The content was stirred intermitently and left in boiling water for 3min and centrifuged while hot at 4000 rpm for 5min. The supernatant was decanted. Water was added to the precipitate and shaken vigorously using whirl mixer, centrifuged and decanted to wash off the acetolysis mixture. The foam formed was dispersed using few

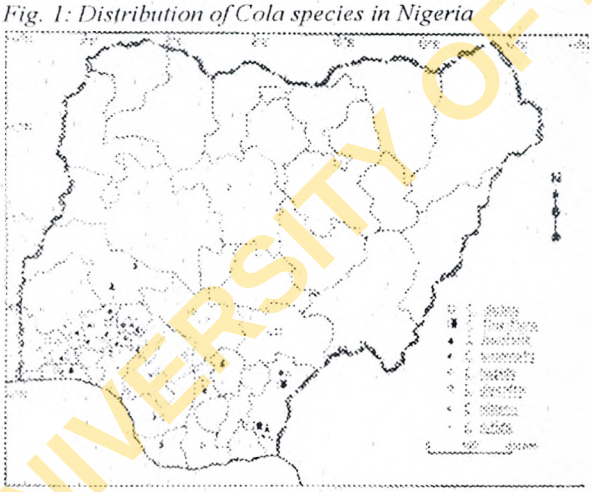
Table 1: Qualitative micro-morphological characters of leaves of Cola species in Nigeria

	Cell shape		Anticlinal wall pattern		Stomatal type	
Species	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Cola acuminata	Polygonal	Polygonal	Straight/slightly curved	Straight/slightly curved	Staurocytic	Staurocytic
C. flaviflora	Polygonal	Polygonal	Straight/slightly curved	Straight/slightly curved	Absent	Anisocytic
C. gigantea	Polygonal	Polygonal	Straight	Straight	Absent	Staurocytic
C. glabra	Irregular	Irregular	Undulating	Undulating	Absent	Anomocytic
C. hispida	Polygonal	Polygonal	Straight/slightly curved	Straight/slightly curved	Absent	Laterocytic
C. laurifolia	Polygonal	Polygonal	Straight/slightly curved	Straight/slightly curved	Staurocytic	Staurocytic
C. millenii	Polygonal	Polygonal	Straight	Straight	Absent	Staurocytic
C. nitida	Polygonal	Polygonal	Straight/slightly curved	Straight/slightly curved	Staurocytic	Staurocytic

Table 2: Quantitative micro-morphological characters of leaves of Cola species in Nigeria

	No of cells/mm^2		Epidermal cell width (µm)		Cell wall thickness (µm)	
Species	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
C. acuminata	676-840	156-418	6-17	8-39	2-3	2-3
	762 ± 42	291 ± 63	13.1± 3.5	22.1± 5.8	2.4 ± 0.4	2.4 ± 0.4
C. flaviflora	121-169	24-99	24-50	19-45	1.4-2	1-1.1
	146 ± 15	58 ± 18	36.2 ± 6.8	32.3 ± 7.2	1.7 ± 0.4	1.0 ± 0.1
C. gigantea	224-342	58-144	10-40	12-50	2-3	1.1-2.0
	279 ± 31	99 ± 23	23.4 ± 8.7	27 ± 8.2	2.5 ± 0.3	1.8 ± 0.4
C. glabra	272-440	72-168	13-31	19-48	1.5-2.2	1-1.4
	349 ± 41	111± 26	20.9 ± 4.5	30.4 ± 6.6	1.8 ± 0.2	1.2 ± 0.1
C. hispida	90-156	42-110	29-60	19-40	1.0 -1.2	1.0-1.2
	133 ± 17	65 ± 15	38.2 ± 7.5	29.1 ± 5.1	1.1 ± 0.1	1.1 ± 0.2
C. laurifolia	552-729	306-504	7-17	10-21	1.8-2.5	2-2.5
	622 ± 44	401 ± 61	12.6 ± 2.9	14.5 ± 2.6	2.2 ± 0.2	2.3 ± 0.2
C. millenii	720-930	56-208	17-40	12-46	1.1-26	1.0-13
	784 ± 57	110 ± 33	31.8 ± 6.3	31.0 ± 8.9	1.7 ± 0.3	1.1 ± 0.1
C. nitida	169-306	132-256	9-21	7-32	1.8-2.6	1.8-2.3
	216 ± 40	204 ± 37	14.8 ± 4.1	23.4 ± 6.5	2.3 ± 0.3	2.1 ± 0.2

Fig. 1: Distribution of Cola species in Nigeria



drops of methylated spirit. The washing of the aceto-lysis mixture and dispersing of the foam was repeated six times. 50% glycerol was added and left overnight. The content was shaken vigorously, centrifuged for 10 minutes at 4000 rpm, decanted and inverted over a piece of filter paper and left overnight. 100% glycerol was added to the content and stored in glass vials. Few drops of the pollen were mounted on clean glass slides. The terminologies used in the description are according to Moore *et al.* (1991) and Erdtman (1952).

Results

The results are presented in Tables 1-4. Figure 1 shows the geographical distribution of *Cola* species in Nigeria while Figs. 2-7 show the micro-morphological characters observed.

Micro-morphological Characters

The epidermal cells were polygonal on both the adaxial and abaxial surfaces in all the species (Figs. 2-5) except *C. glabra* which had irregular cells (Fig. 3). Anticlinal wall pattern may be straight (*C. gigantea*), undulate (*C. glabra*) or straight to slightly curve walls in the other taxa (Figs. 2-5, Table 1). *C. acuminata, C. laurifolia* and *C. nitida* were amphistomatic while other taxa were hypostomatic. In *C. acuminata, C. gigantea, C. laurifolia, C. millenii,* and *C. nitida,* stomata were staurocytic and anisocytic in *C. flaviflora* but anomocytic and laterocytic *in C. glabra* and *C. hispida* respectively

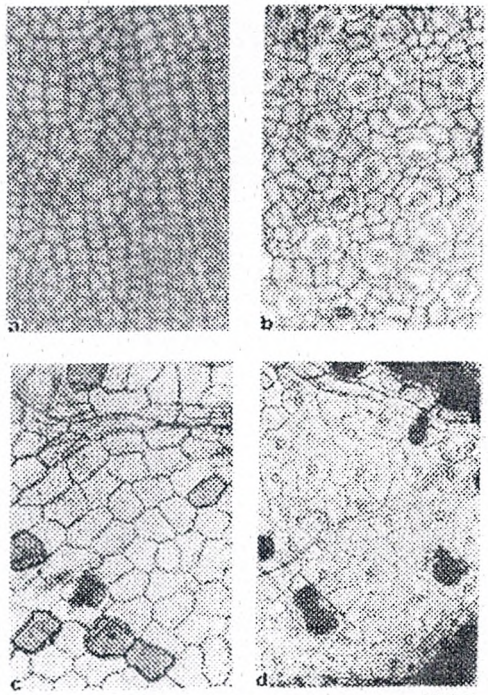


Fig. 2: Leaf epidermis of C. acuminata (a. adaxial, b. abaxial) and C. flaviflora (c. adaxial, d. abaxial)

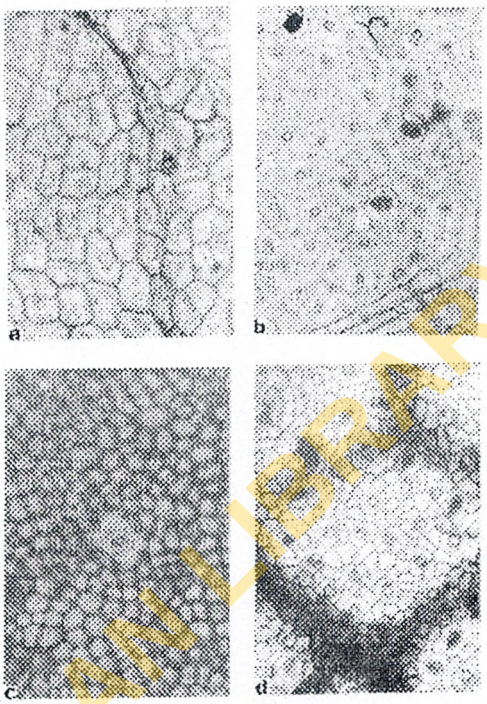


Fig. 4: Leaf epidermis of C. hispida (a. adaxial, b. abaxial) and C. laurifolia (c. adaxial, d. abaxial)

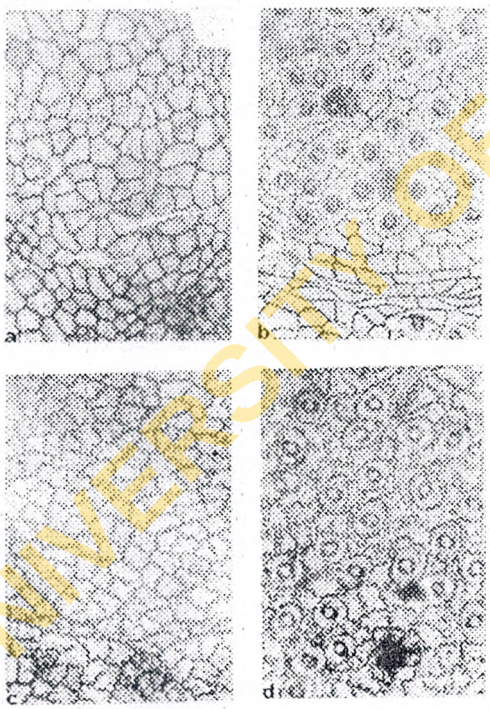


Fig. 3: Leaf epidermis of C. gigantea (a. adaxial, b. abaxial) and C. glabra (c. adaxial, d. abaxial)

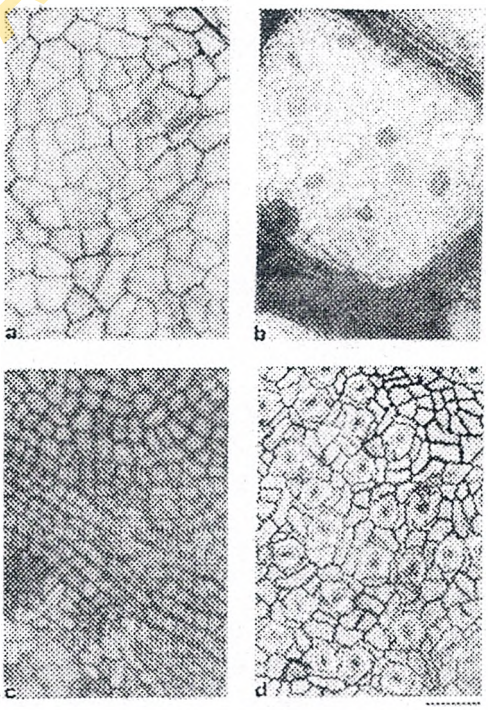


Fig. 5: Leaf epidermis of C. millenni (a. adaxial, b. abaxial) and C. nitida (c. adaxial, d. abaxial). Scale bar = 25μm (Figs. 1-4 same scale)

Table 3: Stomatal characters of leaves of Cola species in Nigeria

	Stomatal density		Stomatal length (µm)		Stomatal width (µm)		Stomatal index (%)	
Species	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
C. acuminata	0-1.0	44-61	24-26	17-21	15-17	14-17	0.2	15.7
	1.0±0.3	54± 4.0	24.7±1.2	19.5±1.2	16 ±1	15.1± 0.8		
C. flaviflora	-	24-70	-	9-15	-	12-16	-	44.1
		45±12,7		13.1±1.8		14.6±1.1		
C. gigantea	-	72-121	-	14-17	-	16-18	-	49.1
		96±13.7		15.0± 0.8		17.4±0.7		
C. glabra	-	36-58	-	15-19	-	14-18	-	30.4
		49±6		17.1±1.1		16.2±1.4		
C. hispida	-	72-121	-	15-18	-	14-16	-	60.6
		100±15.2		15.9±0.8		14.9±0.6		
C. laurifolia	0-1.0	64-121	22-25	9-12	14-15	9-11	0.01	18.4
	1.0±0.3	91±16.6	23.5±2.1	10.0±0.8	14.5±0.7	9.7±0.6		
C. millenii	-	54-61	-	15-18	-	11-18	-	34.5
		58±2		16.4±0.4		15.7±1.7		
C. nitida	0-1.0	36-72	25-25	16-20	15-15	11-15	0.02	21.8
	1.0±0.2	57±9.4	25.1±2.0	18.4±1.3	15.0±0.2	12.9±1.2		

Table 4: Pollen morphological features of Cola species in Nigeria

Species	Class	Shape	Exine thick-ness (µm)	Polar axis (P) (µm)	Equitorial (E) diam. (µm)	P/E
C. acuminata	Subprolate	Circular/elliptic	1.1	32.7	26.8	1.22
C. flaviflora	Prolate spheroidal	Ovate/circular	1.1	35.1	31.6	1.11
C. gigantea	Prolate spheroidal	Circular	1.1	19.7	19.6	1.01
C. glabra	Prolate spheroidal	Ovate/circular	1.0	38.9	37.4	1.04
C. hispida	Subprolate	Elliptic	1.1	39.6	32.6	1.21
C. laurifolia	Prolate/ spheroidal	Circular	1.6	20.1	18.7	1.07
C. millenii	Prolate/ spheroidal	Circular	1.2	35.2	34.4	1.02
C. nitida	Subprolate	Oblong/circular	1.5	30.0	25.9	1.16

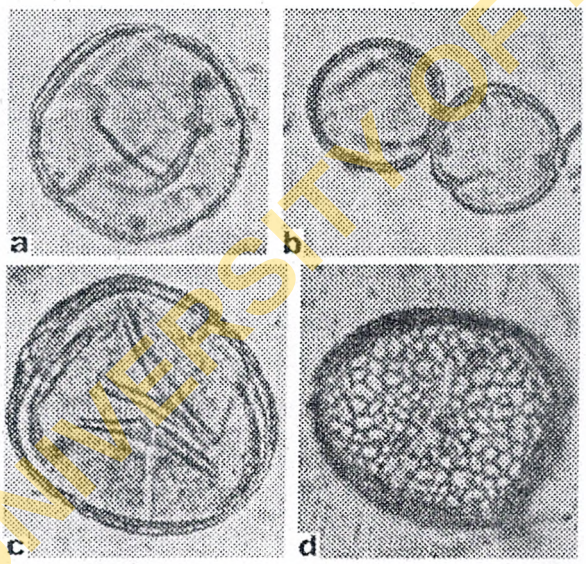


Fig. 6: Pollen grains of a. Cola acuminata, b. C. flaviflora, c. C. gigantea and d. C. glabra

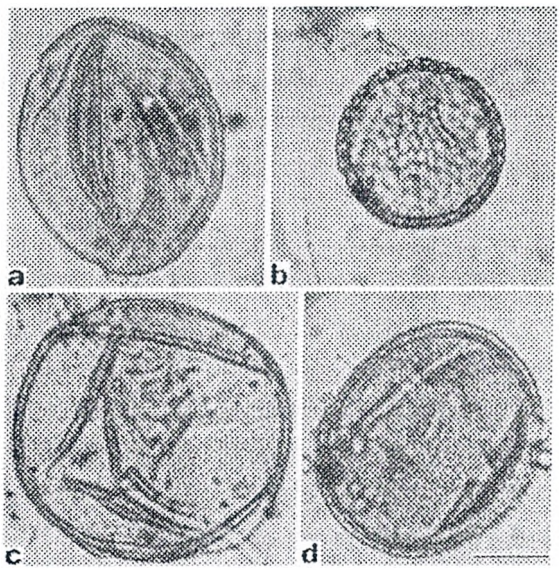


Fig. 7: Pollen grains of a. Cola hispida, b. C. laurifolia, c. C. millennii and C. nitida. Scale bar = 25µm (Figs. 5 & 6 same scale)

(Table 1). Crystal sands were observed in *C. hispida* (Fig. 4a) and predominantly on the adaxial surface. The number of epidermal cells/mm^2 on abaxial leaf was lowest in *C. flaviflora* (24-99) and *C. hispida* (42-110) but highest in *C. millenii* (720-930) on the adaxial surface. Conversely, the cells were widest on adaxial surface in *C. flaviflora* (24-50μm) and *C. hispida* (29-60μm), Table 2. The highest stomatal densities on abaxial surface were found in *C. gigantea* and *C. hispida* (72-121) while the lowest occurred in *C. flaviflora* (24-70), Table 3. The largest stomata occurred in *C. acuminata* (24-26 x 15-17μm) and the smallest in *C. laurifolia* (9-12 x 9-11μm). Stomatal indices on the abaxial surface were highest in *C. hispida* (60.6%) and lowest in *C. acuminata* (15.7%), Table 3. The thickness of the cell walls ranged from 1-3μm on both surfaces in all the taxa (Table 2). Trichomes were absent but trichome bases were observed in all the taxa. The bases were prominent in *C. gigantea* but fewer in *C. nitida* (Figs. 3 & 5).

Pollen morphology

Pollen grains were subprolate, prolate or spheroidal in shape. Exine was 1-1.6μm thick. The pollen sizes ranged from 19.7 x 19.6μm in *C. gigantea* to 39.6 x 32.6μm in *C. hispida*. They could be circular (*C. acuminata, C. gigantea, C. millenii* and *C. laurifolia*), oblong (*C. nitida*), elliptic (*C. hispida)* or ovate (*C. flaviflora* and *C. glabra*), Table 4. *C. acuminata* and *C. hispida* were tricolporate, *C. flaviflora, C. millenii* and *C. nitida* were dicolpate, *C. gigantea* triporate while *C. glabra* and *C. laurifolia* were inaperturate (Figs. 6 & 7).

Discussion

The micro-morphological features of the *Cola* species studied were of taxonomic significance. *C. glabra* could be separated from the others on the basis of its irregularly shaped epidermal cells and undulating anticlinal walls. Plants inhabiting high humidity dense area often possess undulating cell walls while those in drier areas tend to have straight to curve walls (Stace, 1965). Thus, the stress caused by high humidity hardened the cuticular membrane making the plastic cell wall to undulate and at maturity walls become too rigid to permit further undulation (Watson, 1942).

The cell wall thickness values overlap considerably and hence not reliable taxonomically. The number of cells/mm^2 was inversely proportional to the cell width in some species and hence useful for systematic application. *C. flaviflora, C. hispida, C. acuminata*, and *C. laurifolia* were good examples. All the taxa were hypostomatic except *C. acuminata, C. laurifolia* and *C. nitida* which were amphistomatic. The stomatal sizes exhibited complex relationships and overlap hence could only be used carefully. However, *C. acuminata* had one of the largest stomata (24.7 x 16μm, adaxial and 19.5 x 15.1μm, abaxial) but the lowest stomatal indicies (15.7). *C. flaviflora* was distinct for its lowest stomatal density occurred only along the veins on the adaxial surface unlike the other two amphistomatic taxa. *C. acuminata, C. gigantea, C. laurifolia, C. millenii* and *C. nitida* had staurocytic stomata on their abaxial surface while *C. flaviflora, C. glabra* and *C. hispida* had anisocytic, anomocytic and laterocytic stomata respectively.

The pollen were either subprolate or prolate spheroidal. The pollen of related genera usually show more or less the same type with few exceptions (Erdtman (1952). *C. gigantea* had the smallest pollen (19.7 x 17.6μm) while *C. glabra* possessed the largest (38.9 x 37.4μm). The importance of pollen in plant taxonomy had been amply demonstrated by Nair, 1970. In *Cola*, the presence and number of pores and furrows were diagnostic. *C. acuminata, C. gigantea* and *C. hispida* were tricolporate while *C. flaviflora, C. millenii* and *C. nitida* were dicolporate. *C. glabra and C. laurifolia* were distinctly inaperturate. The combination of various micro-morphological characters of *Cola* species in Nigeria is useful for the delimitation and identification of the species even in their fragmentary or sterile state.

References

Akinbode. A. 1982. *Kolanut Production and Trade in Nigeria*. Nigeria Institute of Social and Economic Research (NISER) Ibadan, Nigeria.

Burkill H. M. 2000. *The Useful plants of West Tropical Africa.* Vol. 5. 2nd ed. Royal Botanic Gardens, Kew. 686pp

Cronquist, A. 1973. Chemical plant taxonomy. In: G. Bendz, & J. Santesson (eds.), Chemistry in Botanical Classification. Proceedings of the 25th Nobel Symposium, August 20-25. Academic Press, New York & London.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy. Almqvist & Wiksell, Stockholm, Sweden. 487pp.

Hutchinson, J. & Dalziel, J. M. 1954. *Floral of West Tropical Africa Vol. 1, part 3.* The Crown Agents for the Colonies, London. 828pp

Irvine, F. R. 1961. *Woody plants of Ghana.* Oxford University Press, London. 868pp.

Keay, R. W. J, Onochie C. F. A & Stanfield D. P. 1960. *Nigerian Trees.* Federal Department of Forest Research, Ibadan. 349pp.

Kochler S. L. 1986. *Tropical Crops. A Textbook of Economic Botany.* Macmillan Publishers. Pp. 301-302.

Langenheim, J. H. & Thiman, K. V. 1982. *Plant Biology*

and its Relation to Human Affairs. John Willy & Sons International.

Metcalfe, C. R. & Chalk, L. 1950. *Anatomy of the Dicotyledons*. Vol. I. Oxford University Press, Oxford.724pp.

Metcalfe, C. R. & Chalk, L. 1979. *Anatomy of the Dicotyledons*. Vol. I. Clarendon Press, Oxford. 279pp.

Moore, P. D., Webb, J. A. & Collinson, M. E. 1991. Pollen Analysis. 2nd ed. Blackwell Scientific Publication. Oxford, 216pp.

Nair, P. K. 1970. *Pollen morphology of Angiosperms: A Historical and Phylogenetic study*. Barness & Noble Inc. New York pp. 22-26.

Salisbury, E. J. 1927. On the cause and ecological significance of stomatal frequency with special reference to the woodland flora. *Phil. Trans. Roy. Soc. Lond. Ser. B.* **216**: 1-65.

Stace, C. A. 1965. Cuticular studies as an aid to plant taxonomy. *Bulletin of* the museum (Natural History). *Botany* **4**: 1-78.

Takhtajan, A. 1973. *The chemical approach to plant classification with special references to higher taxa of Magnoliophyta*. In: G. Bendz & J. Santesson (eds.). Proceedings of the 25th Nobel Symposium held August 20-25, Academic Press, New York & London.

Van Cotthem W. R. J. 1970. A classification of stomatal types. *Bot. J. Linn. Soc.* **63**: 235-246.

Watson, R. W. 1942. The effect of cuticular hardening on the form of epidermal cells. *New phytol.* **41**: 223–229.