

Pollen Morphology of Some Members of Nigerian Clusiaceae and Its Taxonomic Significance

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Abstract: The palynological features of three members of Nigerian Clusiaceae were assessed by light microscopy (LM) after acetolysis in order to determine the observed external and internal peculiarities of these species, with respect to their taxonomic implications. These species were *Harungana madagascariensis* (Lam.) ex Poir., *Garcinia kola* Heckel. and *Pentadesma butyracea* Sabine. Results revealed many interesting palynological features with significant taxonomic values. Pollen grains are radially symmetrical, shed in monads and isopolar in all the taxa. Apertural types are tetracolporate and zonocolporate for *G. kola* and *P. butyracea* but tricolporate in *H. madagascariensis*. Pollen form indices vary significantly from 0.55 ± 0.02 , 1.59 ± 0.12 and 1.18 ± 0.01 to give pollen shapes of oblate spheroidal, spheroidal and subspheroidal for *H. madagascariensis*, *G. kola* and *P. butyracea*, respectively. Pore orientation is angulaperturate in all while the exine ornamentations were coarsely psilate in *G. kola* and *P. butyracea* but reticulate in *H. madagascariensis*. The above features are of high taxonomic value in the classification of these species. Their taxonomic implications were discussed.

Key Words: Taxonomic Values, Pollen Features, Nigerian, Clusiaceae,

I. Introduction

The Clusiaceae include herbs, shrubs and trees with sap resinous and abundant oil glands. Leaves are opposite or whorled, rarely alternate and exstipulate while flowers are usually unisexual, sometimes bisexual on the same plant (Keay 1954, Lawrence, 1968) and functionally polygamodioecious, actinomorphic with sepals 2-10 or more. Petals range from 4-12, usually imbricate, subvalvate or contorted. Stamens are few or numerous, hypogynous, distinct and variously united. Anther is 2-celled and dehiscing longitudinally. Pistil is 1, ovary superior, 1 to many loculed; carpels are usually 3 to 5 or as many as the locule (Robson, 1961, Matig *et al.*, 2007).

This family is very important economically, with some members having very high socio-economic, industrial, ethno medicinal and pharmacological values.

The Igbo and Efik cultures in Southeastern Nigeria use *H. madagascariensis* medicinally for the treatment of ulcer and asthma (Inyang, 2003). Leaves are mixed with water, made into a paste and applied topically to treat ringworm, conjunctivitis, rashes and fevers (Madubunyi, 1995). Fresh leaf juice is applied topically to treat abscesses, while young leaves are boiled in water and the resulting "tea" is administered orally three times a day as an analgesic and for whooping cough. In addition, dried leaf infusion is administered orally in Nigeria to treat stomach problems, leprosy and it equally serves as an oxytocic and abortifacient (Bisby *et al.*, 2007).

Economically, a mature tree of *G. kola* can yield about 500 fruits, providing 1700 nuts per year, with an average of N50- 60,000.00 proceed annually. It serves as a very good source of income for women and men in Igbo land (Okafor, 2001, Adebisi, 2002, Okeke, personal opinion, 2010,). The seeds are widely eaten in Igbo land as masticant.

G. kola is used in natural dental care to prevent dental caries (Eyogi *et al.*, 2007). The bark is used for tanning while in the last 3- 4 years, the seeds have also been used as an industrial bittering agent in some Nigerian breweries (Aniche and Uwakwe, 2010). the fruit pulp is used for the treatment of jaundice or high fever (Esimone *et al.*, 2002).The nuts are dried, ground and mixed with honey to make a traditional cough mixture (Adebisi, 2002).

In Southern Nigeria a cold water extract of root-bark with salt is administered to ease off bronchial asthma or cough and vomiting (Farombi *et al.*, 2005), while the fruits are eaten in Nigeria as a cure for general aches in the head, back and as well as a vermifuge. Igbo medicine-men prescribe the fruit for arthritic conditions while the latex from the stem is applied externally to treat parasitic skin diseases and for tropical sores (Liu *et al.*, 2005).

In line with the above remedies ASICUMPON (2005) opined that the seed is an aphrodisiac and that palm wine tapers use it when tapping to scar away snakes. They concluded that, it also serves as antidote to poison while the leaves that have bitter taste are used as purgative.

Aduradola (1999) opined that the biflavonoid compound found in *G. kola* has anti-inflammatory, antimicrobial, anti-diabetic and antiviral properties. He further stated that the extract from seeds have been formulated into various dosage forms including tablets, lozenges, creams, vials and toothpaste.

A recent study reported a reduction in subchondral pressure and pain in knee osteoarthritis due to *G. kola* intake (Adegbehingbe et al., 2008) it has also proven to be safe in human eyes and its systemic blood pressure lowering effects has been published in previous studies (Adefule et al., 2004).

The fat acid composition from *P. butyracea* seed has been analyzed for its chemical and physical constancy and when compared with those of the known cocoa butter and shea butter; it was discovered to contain 50.0, 52.1, and 53.4% of fat, respectively. Their fats are similar in many of their characteristics, particularly in their slip point, saponification number and solidification point. Butter tree fat has a much lower unsaponification matter content of 1.5 - 1.8% than Shea butter that has 7.3- 9.0%. They further stated that the profile aesthetically gives the butter a distinct texture and their replacement fats in respect to their melting points are quite unique (Tchobo et al., 2007).

However, the inclusion of *H. madagascariensis* in the Clusiaceae has been a source of confusion to both botanist and other researchers, this because it shears many morphological features with members of this family. It therefore becomes imperative to carry out palynological assessment of some members of these taxa to aid their proper classification. The main objectives of this work were to 1) assess the palynological feature of these species, 2) determine external and internal peculiarities of these features and 3) evaluate their taxonomic values.

II. Materials and Methods

Sample collection

Specimens were collected from their natural habitats in the various study sites in Southern and Southeastern parts of Nigeria, Enugu State Forest Reserve, Enugu, Odenigbo in Nsukka Local Government Area of Enugu State, Adada Forest along Adada River at Nkpologu, Uzo Uwani Local Government Area of Enugu State. Others were from Staff farm in the Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Forestry Research Institute of Nigeria (FRIN) at Benin in Edo State and Forestry Research Institute of Nigeria (FRIN) at Ikom in Cross River State.

Specimens were identified with the aid of Keay, 1954; Benson, 1957; Keay, 1989). Voucher specimens of these plants are deposited in the Herbaria of the Department of Botany, University of Nigeria, Nsukka (UNH) and Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

Preparation of sample

Fresh polleniferous buds from *H. madagascariensis*, *G. kola* and *P. butyracea* were obtained from the field. Mature buds which had not undergone anthesis were removed from these shoots. Anthers were teased off from the flower buds into five ml of 70 % alcohol in 25 ml beakers. They were squashed with a glass rod. This process breaks the anthers to release the pollen grains. The solutions were sieved with fine network mesh of wire gauze (sterilized under the Bunsen burner to avoid contaminations). This process removes all the plant tissues.

The solutions were then centrifuged at 2000 rev/min for a minute (R P M) for 5 minutes. They were washed twice with distilled water, centrifuged and then decanted again. **Acetolysis**

The precipitates were acetolyzed according to a modified version of Erdtman's (1971) acetolysis method of 9:1 conc. sulphuric acid and acetic anhydride for ten minutes. They were centrifuged, decanted and washed twice with distilled water. The recovered precipitates were stored in vials with ten drops of glycerin, for subsequent light microscopy.

Temporary slides were prepared, pollen grains were mounted in glycerin jelly and examined using light Olympus CH Trinocular microscope (LM) fitted 650 IS Cannon Digital Camera at x 400 and x 100 magnifications.

The following parameters were assessed qualitatively and quantitatively: polar diameter, equatorial diameter, pollen form index, exine thickness, shape in polar view, shape in equatorial view, sculpturing type, aperture type, dimension of pore at polar and equatorial views. Terminology for pollen descriptions were adopted from Erdtman (1971), and Punt et al., (1994). All measurements and photomicrographs were taken under x 1000 magnification.

Analyses for palynological data were based on 20 measurements for a feature per slide for the four slides per a sample. These values were tabulated with the species description citing Mean Standard Errors only for some of the important pollen features.

III. Results

Polarity and Symmetry of pollen grains

Pollen grains are radially symmetrical, isopolar, generally shed in monads and rarely in tetrads.

Shapes

Pollen shapes are oblate- spheroidal; amb in polar view is triangular, concave with an oblate outline in equatorial view for *H. madagascariensis* while it was spheroidal equatorially, amb in polar view is circular in outline for *G. kola* and subspheroidal with circular outline in polar view for *P. butyracea*.

The pollen form indices were 0.55 ± 0.02 , 1.59 ± 0.12 and 1.18 ± 0.01 for *H. madagascariensis*, *G. kola* and *P. butyracea*, respectively, while polar and equatorial axis 116.65 ± 0.10 , 859.04 ± 0.65 , 868.8 ± 1.30 , 211.19 ± 0.25 , 539.94 ± 0.75 and 737.14 ± 0.70 , respectively (Table 1).

Aperture

Pollen grains are tricolporate, zonocolporate and non lophate while pore orientation was angulaperturate (Table 2) with pore length and breadth of 121.09 ± 0.10 and 12.22 ± 0.01 , respectively for *H. madagascariensis*; and apoporium was 34.44 ± 0.1 μm while mesocolpium is 23.31 ± 0.01 μm . In *G. kola* pollen grains are tetracolporate, zonocolporate, and non lophate (Table 2). Pore orientation was angulaperturate with apertural length and breadth of 424.4 ± 0.30 and 14.44 ± 0.25 (Table 2). Apocolpium is 292.19 ± 0.50 μm while mesocolpium is 199.98 ± 0.1 μm , respectively (Fig. 2). *P. butyracea* grains are tetracolporate, zonocolporate and non lophate (Table 2). Apertural length and breadth are 836.58 ± 0.5 and 83.33 ± 0.35 , respectively, while pore orientation is angulaperturate. Pore diameter at equatorial view is 24.18 ± 0.45 while apocolpium is 264.42 ± 0.83 μm and mesocolpium of 384.41 ± 0.13 μm , respectively (Fig. 2).

The Exine

Pollen ornamentation is reticulate, tectate, without carvae and a thickness of 22.22 ± 0.05 μm (Table 2 and Fig. 3) while pollen ornamentation is psilate *G. kola*, tectate with an exine thickness of 43.33 ± 0.10 μm , a thick margo and a carvae of about 0.42 ± 0.10 . Apoporium and mesocolpium are about 292.19 ± 0.50 and 199.98 ± 0.1 , respectively (Figure 2).

In *P. butyracea* pollen ornamentation is psilate, tectate with an exine thickness of 83.32 ± 0.46 μm . (Figures 2, 3, and Table 1). There is the presence of a thick carvae of about 0.22 ± 0.05 μm with apocolpium and mesocolpium of 264.42 ± 0.83 and 384.41 ± 0.13 , respectively (Table 1 and Figure 2).

Table 1: Quantitative Data on Pollen Features of the Three Clusiaceae Species (mean standard error in μm , x 1000).

Pollen Character	<i>H. madagascariensis</i>	<i>G. kola</i>	<i>P. butyracea</i>
Polar diameter	116.65 ± 0.10	859.04 ± 0.65	868.8 ± 1.30
Equatorial diameter	211.19 ± 0.25	539.94 ± 0.75	737.14 ± 0.70
Exine Thickness	18.22 ± 0.05	43.33 ± 0.10	83.32 ± 0.50
P D E V	0.22 ± 0.05	14.44 ± 0.05	24.18 ± 0.45
Pollen Form Index	0.55 ± 0.02	1.59 ± 0.12	1.18 ± 0.01
Carvae	-	0.42 ± 0.10	0.22 ± 0.05

P D E V- pore diameter at equatorial view

Table 2: Apertural and Sculptural Features of the Three Species.

Species	APT	NAP	SCPT	SPPV	SPEV
<i>H. madagascariensis</i>	Tricolporate	3	Reticulate	Angular	Oblate Spheroidal
<i>G. kola</i>	Tetracolporate	4	Psilate	Circular	Spheroidal
<i>P. butyracea</i>	Tetracolporate	4	Psilate	Circular	Subspheroidal

Legend :A PT- Apertural type, NAP- No of apertures, SCPT- Sculpturing type, SPPV-Shape of pollen at polar view, SPEV- Shape of pollen at equatorial view

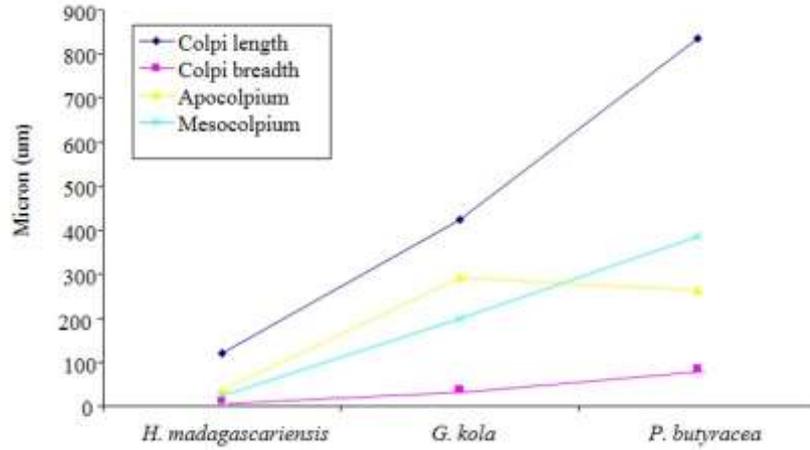


Figure 2: Pollen and Apertural Features for the Four Species

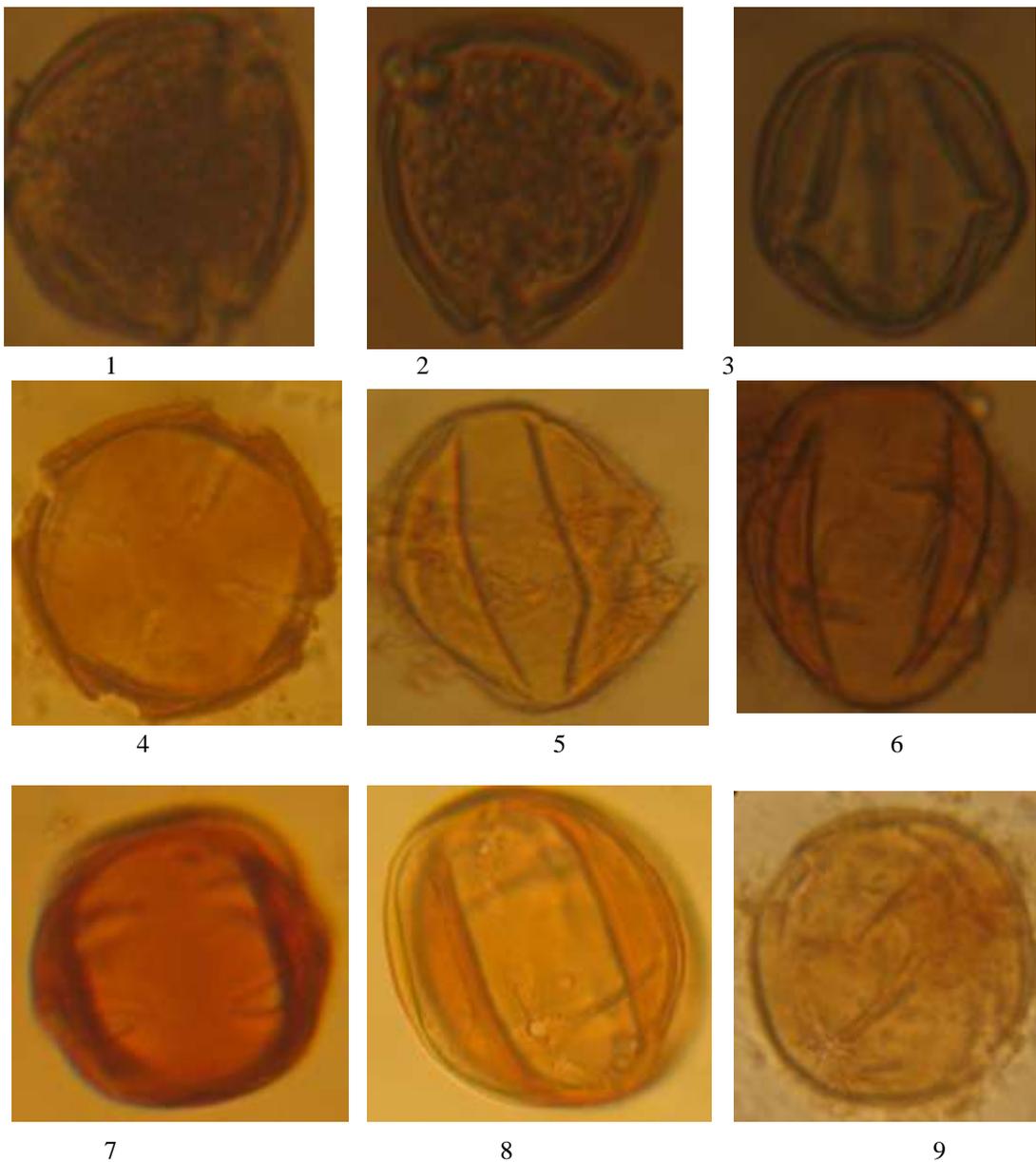


Figure 3: Pollen Features of the Nigerian Clusiaceae- 1-3 *H. madagascariensis*. 4-6. *G. kola* and 7-9. *P. butyracea*

IV. Discussion

This palynological study sought to provide more useful information on the taxonomy of these taxa. The three species assessed show interesting features with significant taxonomic values. These variations actually reinforce the morphological and anatomical data obtained from earlier work.

Apertural and Sculpturing type

This study recorded two apertural types, the tetracolporate type with psilate sculpturing ornamentation in both *G. kola* and *P. butyracea* with tricolporate and reticulate type found in *H. madagascariensis* (Figures 3 and Table 2). The above results were in line with the findings of Passarelli *et al.*, (2010) who in their work reported of psilate ornamentation in *Podostemum* spp in the family Podostemumaceae, a basal sister group of the Clusiaceae. This is equally in conformity with the report of Furness *et al.*, (2004) ; Perveen and Kaiser, (2007) who reported that pollen grains do not differ within most families and as such can be of great value in establishing affinity or otherwise.

These distinctive pollen apertural and sculpturing types could be used in the taxonomic delimitation of *H. madagascariensis* from the rest of the genera. It equally affirms the excluded taxonomic status of *H. madagascariensis*, as well as the close affinity of *G. kola* and *P. butyracea*.

The absence of reticulate ornamentation pattern in *G. kola* and *P. butyracea* (except in the *H. madagascariensis*) is in accordance with the observations of Punt *et al.*, (1994), who found coarsely psilate sculpturing pattern with distinctively large pollen in the exotic species of this Clusiaceae. This result is in agreement with the reports of Bashir and Khan (2003), Mbagwu and Edeoga (2006) and Mbagwu *et al.*, (2009) who in their various works utilized apertural attributes of pollen grains to establish probable evidence of relationships among some species in many genera of flowering plants. They suggested that pollen morphology can be useful in supporting taxonomic evidence and could be used in solving taxonomic controversies in the classification of problematic taxa.

Dessein *et al.*, (2005) however, noted that the number of apertures are positively correlated with pollen size, since the larger the pollen grain, the larger the surface area where the colpi may be initiated. They stressed that more colpi may facilitate higher germination. They further, reported that a possible explanation may be found in the harmomegathy function of the colpi, a process by which the pollen and spores change in shape, to accommodate variations in the volume of the cytoplasm caused by the changes in dehydration.

However, the present observations conform totally with this report by Dessein *et al.*, (2005), because all the examined taxa except *H. madagascariensis* are large with larger number of colpi but on the other hand, they do not germinate easily from all the available literature.

Shape of pollen

Pollen shapes in polar view are angular in *H. madagascariensis*, circular in *G. kola* and *P. butyracea* while the equatorial shapes were oblate spheroidal, spheroidal and subspheroidal in *H. madagascariensis*, *G. kola* and *P. butyracea*, respectively. The above variations could be attributed to their intergeneric differences.

However, Lowe and Soladoye (1990) and Ogwal (1990) suggested that, it is likely that the nature or shape of pollen grains could be an evolutionary modification often inherited to determine the mode of pollination and thereby perpetuate a particular group of plant in a given environment.

In the same line, Mbagwu and Edeoga (2006), were of the opinion that, where the pollen grain is longer than wide, it may be explained as a structural adaptation for effective dispersal by wind while the circular nature of some of the pollen grains were related to structural adaptation for effective pollination by insects. The above opinions if right may not apply to the above shapes found in all these species. This inference is deduced because based on the floral morphology of these species, they are all entomophilous plants.

Size, Symmetry and Dispersal Unit

Pollen size of these species varied considerably within the taxa. These variations between polar and equatorial diameter gave an explanation to the oblate spheroidal, spheroidal and subspheroidal shapes found in *H. madagascariensis*, *G. kola* and *P. butyracea*, respectively. The pollen grains are shed in monads in all the species; they are radially symmetrical and isopolar. The above features are not taxonomically informative, but rather show evidence of their levels of advancement.

Exine Thickness

The exine of these species varies significantly in *H. madagascariensis*, *G. kola* and *P. butyracea*. *H. madagascariensis* had the smallest exine thickness while *G. kola* had the thickest exine walls. *H. madagascariensis* with its thin exine wall is considered more primitive than *G. kola* and *P. butyracea* which are more advanced by possessing pollen grains with thicker exine walls. This is in line with the report of Tomsovie (1997) and Sahreen *et al.*, (2008) who observed that the presence of thin exine with reduced columellae is a characteristic feature of primitiveness in Angiosperms.

References

- [1] Adebisi, A. A. (2002). A Case Study of *Garcinia kola* Nut Production to Consumption System in J4. Area of Omo Forest Reserve. In: Sunderland, T. and Ndoye, O. (eds). *Forest Products, Livelihood and Conservation*. Africa Publishers. 139 p p.
- [2] Adebukunola, O., Adefule-Ositelu, B. O., Adegbehingbe, A. K. Adefule, O. O., Adegbehingbe, E. S. and Kehinde O. (2010). Efficacy of *Garcinia kola* 0.5% aqueous eye drops in patients with primary open-angle glaucoma or ocular hypertension. *East African Journal of Ophthalmology*. 17:1,88-93.
- [3] Adefule, A. K., Oosa, B. O. and Onyenafa, P. C. (2004). Antifungal Activities of *Garcinia kola* Extracts on Purulent Discharges in Lagos University Teaching Hospital, Lagos, Nigeria. *Quarterly Journal Hospital Medicine*. 14: 112-114.
- [4] Adegbehingbe, O. O., Adesanya, S. A., Idowu, T. O., Okimi, O. C., Oyelami, O. A., Iwalewa, E. O. (2008). Clinical Effects of *Garcinia kola* in Knee Osteoarthritis. *Journal of Orthop Surgone*; 3:34-38.
- [5] Aduradola, A. M. (1999). Preliminary Investigation of some Factors Affecting Germination in Seeds of *Garcinia kola*. *Journal of Tropical Ethnobotany* 2(1): 47-46.
- [6] Aniche, G. N. and Uwakwe, G. U. (2010). Potential Use of *G. kola* as Hop Substitute in Large Beer Brewing. *World Journal of Microbiology and Biotechnology*: 6: 3, 87-82.
- [7] ASICUMPON, (2005). *Check List of Medicinal Plants of Nigeria and their Uses*. Trinity Biz Publishers, Nigeria, 135, pp.
- [8] Bashir, S. and Khan, M. A. (2003). Pollen Morphology as an Aid to the Identification of Medicinal Plants: *Trianthema portulacastrum* L., *Boerhaavia procumbens* Banks ex Roxb. and *Alternanthera pungens* Kunth. *J. Hamdard Medicus*, XLVI: 7-10.
- [9] Bisby, F. A., Roskov, Y. R., Ruggiero, M. A., Orrell, T. M., Paglinawan, L. E., Brewer, P. W. and Bailly, N. (2007). *Species 2000 and ITIS Catalogue of Life: In: Hertum, J. V. (ed) 2007 Annual Checklist*. Reading, U.K.
- [10] Bonsen, K. J. M. and Kucera, L. J. (1990). Vessel Occlusion in Plants: Morphological and evolutionary Aspects. *International Association of Wood Anatomists Bulletin II* (4): 393-399.
- [11] Burkill, H. M. (1994). *The Useful Plants of West Tropical Africa*. Vol. 4. Royal Botanic Gardens Kew. 969, pp.
- [12] Dessein, S., Harwood, R., Smmets, E. and Robbrecht, E. (2005). Pollen of the Spermaceoe (Rubiaceae) Species from the Northern Territory of Australian: Morphology and Taxonomic Significance. *Australia Systematic Botany*, 18: 367-382.
- [13] Erdtman, G. (1971). *Pollen Morphology and Plant Taxonomy (Angiosperm)*. Hafner Publishing Company, New York.
- [14] Esimone, C. O., Nwafor, S. O. V., Okoli, C. O., Chah, K. F., Uzuegbu, D. B., Chibundu, C. and Adikwu, M. U. (2002). In Vivo Evaluation of Interaction between Aqueous Seed Extract of *Garcinia kola* and Ciprofloxacin Hydrochloride. *American Journal of Therapeutics*. 9 (4): 275-280.
- [15] Eyogi, O. M., Aoudji, A. K. and Linsoussi, C. (2007). *Garcinia kola* Seed Dormancy Breaking. *Applied Ecology and Environmental Research*. 5 (1): 63-71.
- [16] Farombi, E. O., Akanni, O. O. and Emerole, G. O. (2005). Antioxidant and Scavenging Activities of Flavonoid Extract (Kolaviron) of *Garcinia kola* Seeds in Vitro. *Pharmacological Biology*. 40 (2) I: 107-116.
- [17] Furness, C. A. and Rudall, P. J. (2004). Pollen Apertural Evolution: A Crucial Factor for Eudicot Success? *Trends in Plant Science* 9:1360-1385.
- [18] Inyang, E. (2003). *Ethnobotany, Conventional and Traditional Uses of Plants*. Verdict Press, Nigeria.,191, pp.
- [19] Liu, Y., Qiu, Y. P., Hang, L. Z. and Chen, I. (2005). Dormancy Breaking and Storage Behaviour of *Garcinia kola* Seeds: Implications for Ecological Functions and Germplasm Conservation. *Journal of Integrated Biology*. 471(1): 38-49.
- [20] Lowe, J. and Soladoye, M. O. (1990). Some Changes and Corrections to Names of Nigerian Plants and Nigerian Trees since the Publication of *Flora of West Tropical Africa*. 2nd Ed. *Nigerian Journal of Botany*, 3:1-24.
- [21] Madubuuyi, I. I. (1995). Antimicrobial Activities of the Constituent of *G. kola* Seeds *Int Journal of Pharmacognocny* 33: 232-237.
- [22] Matig, A. L., Aoudji, A. K. N. and Linsoussi, A. (2007). *G. kola* Seed Dormancy- Breaking Applied Ecology and Environmental Research 5(1): 63-71.
- [23] Mbagwu, F. N. and Edeoga, H. O. (2006). Palynological Studies on Some Nigerian Species of *Vigna* spp. *Journal Biological Science* 6 (6): 1122-1125.
- [24] Mbagwu, F. N., Chime, E. G. and Unamba, C. I. N. (2009). Palynological Studies on Five Species of Asteraceae. *African Journal of Biotechnology* Vol. 8 (7), 1222-1225.
- [25] Metcalfe, C. R. and Chalk, L. (1957). *Anatomy of the Dicotyledons, Vol 1*, Oxford University Clarendon Press, London. 726 pp.
- [26] (1965). *Anatomy of the Dicotyledons. Vol. 2*. Clarendon Press, Oxford, London. pp. 726-1460.
- [27] Nasreen, S., Yousa, F. M., Mohmand, A. S. and Ashraf, M. (2002). Study of Seed Dormancy
- [28] Nnamani, C. V. and Agwu, C. O. C. (2007). Pollen Analysis of Honey Samples from Ebonyi State Nigeria. *Bio-Research*: 5 (1) 184-188.
- [29] (2008). Index of Phytoecological Indicator Species in Five Honey Samples from Ebonyi State Nigeria. *Nigerian Journal of Botany* 21 (1) : 129-135.
- [30] Nzegebule, E. and Mbakwe, R. (2001). Effect of Pre-Sowing and Incubation Treatment on Germination of *Garcinia kola* Seed. *Fruits*, 56: 437-442.
- [31] Okafor, J. C. (2001). Case Study: Role of Conservation and Domestication of Minor Woody Forest Plants for Local Communities in Nigeria. In: F. T Last (ed) *Ecosystems of the World* (19), *Tree Crop Ecosystem*, Elevier, Amsterdam-London, pp.7-17.
- [32] Ogwal, E. N. K. (1990). A Taxonomic Investigation of the Genus *Commelina* in Uganda. *Proceedings AETFAT*. Hamburg, 236: 573 -592.
- [33] Perveen, A. and M. Qaiser, (2007). Pollen Floral of Pakistan - Malvaceae - Grewioideae - LII. *Pakistan Journal of Botany*. 39 (1): 1-7
- [34] Passarelli, L. M., Tur, N. M. and Girarde, S. B. (2010). Morphology of Pollen of the Species of Neo-Tropical of Podostemum (Podostemaceae). *International Journal of Tropical Biology and Conservation*:58 (1), 82-88.
- [35] Punt. W. W.W., Nilsson, S., Blackmore, S. and Thomas, L. A. (1994). *Glossary of Pollen and Spore Terminology*, -L P P Foundation, Utrecht, L P P Contribution Series No. 1.
- [36] Sahreen, S., Khan, M. A., Meo, A. A. and Jabeen, A. (2008). Pollen Morphology of the Genus *Silene* (Sileneae-Caryophyllaceae) from Pakistan. *Biological Diversity and Conservation* 1 (2): 74-85.
- [37] Tchobo, F. P., Natta, A. K, Barea, B., Barouh, N. , Piombo, G. P. M, Villeneuve, P., Soumanou, M. M. and Sohounhloe, D. C. K. (2007). Characterization of *Pentadesma butyracea* Sabine Butters of Different Production Regions in Benin. [Journal of the American Oil Chemists' Society](#) 84 (8) 755-780.
- [38] Tomsovic, P. (1997). Some Palynological Observations on the Genus *Echinops* (Asteraceae) and their Taxonomic Implications. *Preslia, Praha*, 69: 31-33.
- [39] Zafar, M., Khan, M. A., Ahmad, M. and Sultana, S. (2006). Palynological and Taxonomic Studies of Some Weeds from Flora of Rawalpindi, *Pakistan Journal of Weed Science Research*. 12 (1-2): 99-109.