Micromorphology and Microchemical Evaluation of *Garcinia kola* and *Garcinia gerrardii*

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Abstract
The use of medicinal plants as alternative and complementary medicine is gaining more grounds on a daily basis globally.. Thus, this study is aimed at delimiting *Garcina kola* and *Garcinia gerrardii* leaves who are both members of the same genus and family by evaluating the fresh, powdered and anatomical sections of the leaves using standard procedures for the macromorphological, microscopy (quantity and quality), chemomicroscopic profiles. The result obtained for the unaided observation shows that both plant species look extremely similar with opposite leaf arrangement, ovate leaf shape, entire leaf margin and cuspidate apex but differ in the leaf size and stalk with *G. gerrardii* having bigger leaves and angular stalk while *G.kola* has smaller leaves and rounded leaf stalk. Qualitative microscopy revealed that *G.kola* cells are irregular shaped with wavy, undulating anticlinal walls while *G. gerrardii* has irregular shaped cells with straight anticlinal walls. Stomata were only found on the abaxial layers of both species with the same stomata type (paracytic). Also, trichomes were found absent in both layers of the two (2) plant species. Quantitative study shows Stomata index (13.35%, 6.54%) for the abaxial surfaces of *G. kola* and *G. grerrardii* respectively. *G. kola* gave a mean cell length of (61.60 µm, 58.72 µm) while *G. gerrardii* has a mean cell length of (30.29 µm, 31.40 µm) on both abaxial and adaxial surfaces. However, there is an issue with identification of some of these plants particularly for those ones that belong in the same genus and family. Therefore, there is a need to establish a standard profile for these plants in order to achieve proper identification Information obtained from this work can be used as good indicators and standards for the examined plant species which can serve as reference in herbal pharmacopoeias.
Key Word: Dioecious, Pharmacopoeias, ethnomedicine, folial epidermal.

Introduction
The genus *Garcinia* L. *Clusiaceae* comprises about 260 species of mostly dioecious small shrubs or trees up to 30 m tall which are predominantly found in lowland tropical forests worldwide (Stevens 2007). It is native to Asia, America, Australia, Tropical and Southern Africa (Asinelli *et al.*, 2001) and is often regarded as a genus with a difficult taxonomy (Sosef and Dauby, 2012). These groups of medicinal plants has been reported to have multiple applications in Culinary, pharmaceutical and Industrial fields (Hemshekhar *et al.*, 2011) Worldwide, the different plant parts have been used ethnomedicinally for the treatment of some health conditions such as oxidative stress, inflammation and diseases like obesity and cancer. (Padye *et al.*, 2009; Obolskey *et al.*, 2009; Acuna *et al.*, 2009). One of the renowned species of genus Garcinia is *Garcinia kola*. *G.kola* is a dicotyledonous plant occurring in rain forests and swamps and usually grows as a medium sized tree up to a height of about 12m and it produces reddish yellowish or orange coloured fruit (Okwu 2005; Adesanya *et al.*, 2007. The plant is found across Western and Central African countries such as Nigeria, Ghana, Cameroon, Sierra Leone, Togo, Congo Democratic Republic, Angola, Liberia, Gambia etc and it is humanly distributed around the towns and villages of these countries.

It is known by various names such as bitter kola, male kola (English name), orogbo (Yoruba), Aku ilu (Igbo) and Namijin goro (Hausa). It can also be called "wonder plant" because every part of the plant from the roots to the leaves have been found to have phyto-therapeutic purpose (Iwu *et al.*, 1990; Adesuyi *et al.*, 2012). *G. kola* has a bitter astringent taste similar to that of raw coffee, followed by a slight sweetness. It is highly valued in ethnomedicine of Africa because of its varied and numerous uses which are social and medicinal; thus, making the plant an essential ingredient in folk medicine. Some of these metabolites includes tannins, saponins, alkaloids, cardiac glycosides, protein, triterpenoids, sterols (Terashima *et al.*, 2002; Esimone *et al.*, 2007). Locally, the plant is being used in the treatment of skin infections in Liberia and Congo Democratic Republic while the bark is applied to malignant tumours, cancers among others. In Sierra Leone, the roots and bark are taken as a tonic for sexual dysfunction in men (Adesuyi *et al.*, 2012). Pharmacologically, it has been reported to have Antimalarial (Oluwatosin et al., 2014, Konziase, 2015), Antitrypanosomal (Ogbadoyi *et al.*,

2011; Ibikunle and Ogbadoyi, 2011), Anti-asthmatic (Ferguson, 2001; Hodek *et al.,* 2002), Antioxidant (Oloyede and Afolabi, 2012; Olatunde *et al.,* 2008), Antiinflammatory and Analgesic (Olaleye *et al.,* 2000) activities. Another important but not thoroughly explored species of the Clusiaceae family is *Garcinia gerrardii* Harv.ex.Sim. It is commonly called "Forest Mangosteen" and is a large shrub of about 4-5m tall or small tree of about 10-13m tall with dark green, shiny, leathery, simple leaves arranged in pairs or threes. It is indigenous to South Africa and found in forests, kloofs and woodlands in Eastern South Africa and Swaziland and mainly found in the Ngoye Forest in Zululand. Traditionally, the leaves are used to treat ear ache, the root sap is effective for killing snails and the bark is used in sprinkling charms to prevent lightning. It has been reported to have fungicidal activities against *Cladosporium cucumerinum* (Boon, 2010; Schmidt *et al.,* 2002; Palmer and Pitman., 1972; Hutchings, 1996)

Although *G. kola* and *G. gerrardii* are easily recognized by the naked eyes, they still have some striking and confusing similarities as a result of being members of the same genus. It is however important to provide alternative ways of delimiting/differentiating them. Therefore, this study is aimed at observing the similarities and differences through the assessment of their physical, folial epidermal characteristics and transverse section of both plants. The results obtained will help differentiate the two species as well as serve as reference standards for herbal pharmacopoeias.

Materials and Methods

Plant collection

Fresh leaves of *G. gerrardii* and *G. cola* were collected from the Herbal garden and Arboretum of the Forestry Research Institute of Nigeria, Ibadan respectively and carefully identified by a taxonomist resident at the Forest Herbarium Ibadan (FHI) of the same Institute.

Plant preparation

Fresh leaves were used for evaluation of the characters that are visibly seen with the naked eye (microscopic) as well as for the qualitative and quantitative micro examination done with the use of a microscope. Also, fresh leaves were collected and dried under standard conditions, after which it was blended and stored for later use. The powdered sample was then used for microchemical evaluation.

Macroscopic evaluation

The macroscopic characteristic of the leaf was described according to standard botanical method of Brain and Turner, (1975). Different macroscopic parameters of the leaves were noted. Leaf evaluation, namely leaf shape leaf arrangement, venations and apex types were observed.

Microscopic evaluation

This is used to identify the various microscopic characters of the plant such as stomata, cell length, cell width e.t.c (Radford *et al.,* 1974; Adedeji, 2004).

Epidermal section preparation

Pieces of about 1–5 cm^2 of the leaves of each specimen were cut and soaked in concentrated trioxonitrate (v) acid (HNO_3) in well covered Petri dishes for about two to four hours in order to macerate the mesophyll. Observation of bubbles is indicative of tissue disntegration and the epidermises were carefully peeled off using forceps. Tissue debris was carefully cleared off the epidermises with fine Carmel hair brush, and the isolated epidermal layers (adaxial and abaxial) were adequately rinsed in water. The epidermises were then transferred into another Petri dish containing 50 % ethanol for 1–2 minutes which is majorly for the hardening of cells. Afterwards, the tissues were transferred unto a clear-glass microscopic slide and stained with Safranine minutes and then rinsed again in distilled water to remove excess staining. They were mounted thereafter in 25 % glycerol on a microscopic slide, covered with coverslips and nail vanish was applied on the edges of the cover slip to help seal the slides by preventing dehydration and damage. Five slides were prepared for each epidermis of the two species, all of which were labelled appropriately and viewed under the microscope with X40 objective lens (Radford *et al.,* 1974); Brain and Turner, (1975); Khatijah & Zaharina (1998), Adedeji (2004), Metcalfe & Chalk (2004) ; Evans *et al.,* (2005). For each micro-morphological character, measurements were randomly taken from all slides prepared for each specimen. The mean value and standard deviation for all microscopic parameters were also calculated on the basis of occurrence of each examined character in a total of 10 fields of view.

The stomata index (SI) for the epidermises was calculated using the formula reported by Salisbury (1927):

Stomatal Index (SI) = S × 100 %, E + S

where S = number of stomata per unit area, and E = number of epidermal cells on the same area.

Transverse section preparation

The anatomical section of the leaves was prepared using standard procedures. The transverse section of the two species were cut using a sledge microtome, and preserved in 50% ethanol. They were then stained in aqueous solution of Safranin O for about 5 minutes and rinsed in distilled water to

remove excess stain. The sections were then put into a container containing 50:50 Alcohol/Xylene and rinsed, after which it is the cleared with chloralhydrate solution. They were thereafter mounted on a glass slide in 15% glycerol, covered with cover slips (Evans *et al.,* (2005); Brain and Turner, (1975).

All slides were labelled appropriately and examined under Olympus light microscope. Photomicrographic images of each specimen were taken with a 14 megapixels Amscopedigital camera mounted on Olympus photomicroscope at the Biomedicine Research Centre, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Observations and measurements were made with Amscope microscope software Version 3.7.7149 2016. For each micromorphological character, measurements were randomly taken from all slides prepared for each specimen. The mean value and standard deviation for all microscopic parameters were also calculated on the basis of occurrence of each examined character in a total of 10 fields of view as mentioned above.

Microchemical Tests

A Little amount of powdered leaf samples of the two (2) species was placed on the microscopic slide along with the drop of glycerin and water (1:1) and then it was covered with a clean cover slip. It was then observed under the microscope to reveal the presence or absence of chemical substances such as lignin, starch, calcium crystals etc using certain laboratory reagents (Trease and Evans, 1996; Taiwo and Omolola, 2015).

Lignin Test

The powdered plant was mounted in phloroglucinol followed by concentrated hydrochloric acid; a red coloration indicates lignifications.

Starch Test

The powdered plant was mounted in N/50 iodine. Bluish coloration indicates presence of starch.

Calcium Oxalate Crystals Test

The powdered plant was cleared in chloral hydrate solution; presence of calcium oxalate crystals is seen as bright structures of definite shapes and sizes. On addition of 80% hydrochloric acid and viewing under microscope, disappearance of calcium oxalate crystals confirms their presence.

Calcium Carbonate Test

The powdered plant was mounted in glycerol. The slide was irrigated with acetic acid solution. Evolution of gas indicates the presence of calcium carbonate.

Test for Oils (Fats)

The powdered plant was mounted in Sudan IV reagent. Pinkish coloration is an indication of the presence of oils.

Mucilage Test

The powdered leaf sample was mounted on the slide and 1 drop of Ruthenium red was added, a pink coloration shows the presence of mucilage.

Results and Discussion

In recent times, there has been an increasing interest in the use of herbal drugs because of the general assumption that they are safe and inexpensive (Prasad *et al.,* 2012). However, herbal treatment has not received overall acceptability because of the presumed deficient standardization. The subject of standardization is very important particularly in the area of identification because the misuse of medicine in general starts with wrong identification. Thus, it is essential to lay down standards starting with identification which in turn will give rise to an authentic product of good quality (Taiwo and Omolola, 2015).

The macroscopic features of the leaves of *G.kola* and *G.gerrardii* showed that the both leaves are ovate shaped with opposite leaf arrangement with simple and entire margin and pinnate venation as well as cuspidate apex (Plate 1a and Plate 1b). The two species only differ in size, thickness and stalk type, *G.gerrardii* is bigger in size, is thicker and an angular leaf stalk while *G.kola* has a rounded stalk as clearly differerntiated in Table 2. The simplest, correct, reliable and cheapest means of establishing identification which is proper and accurate is through macroscopic means (Patel and Zaveri, 2011). The qualitative microscopic characters examined using a light microscope revealed the presence of cell descriptions like shape, stomata, trichomes, microcrystals and anticlinal walls. The cell shape of both species are irregularly shaped on both the adaxial and abaxial layers but to distinguish them, *G.kola* has wavy, undulating anticlinal walls while *G. gerrardii* has straight anticlinal walls(Fig 2a, 2b, 3a, 3b). Also, it was observed that both species only had stomata present on the abaxial layers, and it was observed that they both have the same stomata type (paracytic) (Fig 2a, 3a). Trichomes were evidently absent in both species while microcrystals were only discovered on the adaxial layer of *G.gerrardii*. Quantitatively, as shown in Table 3, overall, there is statistical difference (using standard deviation at $p<0.05$) in the values obtained for both species understudied. Values depicts that there is a wide variation in the cell length of both species with *G.kola* having

a mean cell length of (61.60 µm, 58.73 µm) while *G.gerrardii* has a mean cell length of (30.20 µm, 31.40 µm) on the lower and upper epidermis, we can therefore infer that the cell length of *G. kola* is double that of *G. gerrardii*. However, with respect to the Cell density, the average number of cells for *G. gerrardii* ranged from 163 on the adaxial surface to 208 on the abaxial surface while for *G. kola*, the average number of cells ranged from 66 on the abaxial surface to 67 on the adaxial surface thus, we can say that the smaller the cell length/width, the higher the number of epidermal cells (i.e cell density). Also, as shown on the table, *G.kola* has a higher mean stomata length and width (31.54 µm, 24.13 µm) than that of *G. gerrardii* (22.71 µm , 16.11 µm).

This result is similar to that of *Centrosema pubescence* and *Clitoria ternatea* (Chukwuma *et al.,* 2014). We have therefore established from the result that the cell length/width is inversely proportional to the frequency of cells, stomata length and width which agrees with the findings of Chukwuma *et al.*, (2014). *G. gerradii* recorded a mean stomata number of 14.50 and 10.10 for *G.kola* while the cell density is 208.20 and 66.40 for *G. gerrardii* and *G. kola* respectively. This finding does not agree with the fact that the number of stomata is higher when the number of epidermal cells is low and vice versa (Salisbury 1927, Chukwuma *et al.,* 2014) as the reverse is the case in this study. The transverse section of the leaves of both plants studied shows the presence of collenchyma, parenchyma, sclerenchyma, xylem, phloem and cambium.

The microchemical examination result as presented in Table 4 revealed the presence of Fats, Starch and Calcium carbonate. This result is in contrast with the result obtained for *Garcinia latissima* (a member of the same Genus) indicating the presence of Calcium oxalate as reported by Ambarwati *et al.,* 2017).

1A

Plate 1A: Picture of *Garcinia kola* leaves

Plate 1B: Picture of *Garcinia gerrardii*

Table 1: Macroscopic features of *G. kola* and *G. gerrardii* leaves

S/N	Features	*G. kola*	*G. gerrardii*
1.	Leaf shape	Ovate	Ovate
2.	Leaf arrangement	Opposite	Opposite
3.	Leaf venation	Pinnate	Pinnate
4.	Leaf margin	Entire	Entire
5.	Leaf apex	Cuspidate	Cuspidate
6.	Leaf thickness	Thick	Thicker
7.	Leaf Stalk	Rounded	Angular
8.	Leaf size	Big	Bigger

Table 2: Qualitative Microscopic Leaf Characteristics of *G. kola* and *G. gerrardii*

Characters	*G. kola*		*G. gerrardii*	
	Abaxial	**Adaxial**	**Abaxial**	**Adaxial**
Cell shape	Irregular	Irregular	Irregular	Irregular
Anticlinal walls	Wavy	Wavy	Straight	Straight
Stomata Type	Paracytic	Absent	Paracytic	Absent
Microcrystal	Absent	Present	Absent	Present
Trichomes	Absent	Absent	Absent	Absent

Table 2 shows the results for the qualitative microscopy of the leaves epidermal section of *G.kola* and *G.gerrardii*. The result reveals the presence of some characteristic features on both the adaxial and abaxial layers of both species understudied such as irregular and straight cell shape, wavy to straight anticlinal walls and the presence of stomata on only the abaxial layers of the two species. Trichomes are absent on both layers of the two species. Crystals are present only in *G.gerrardii* leaves.

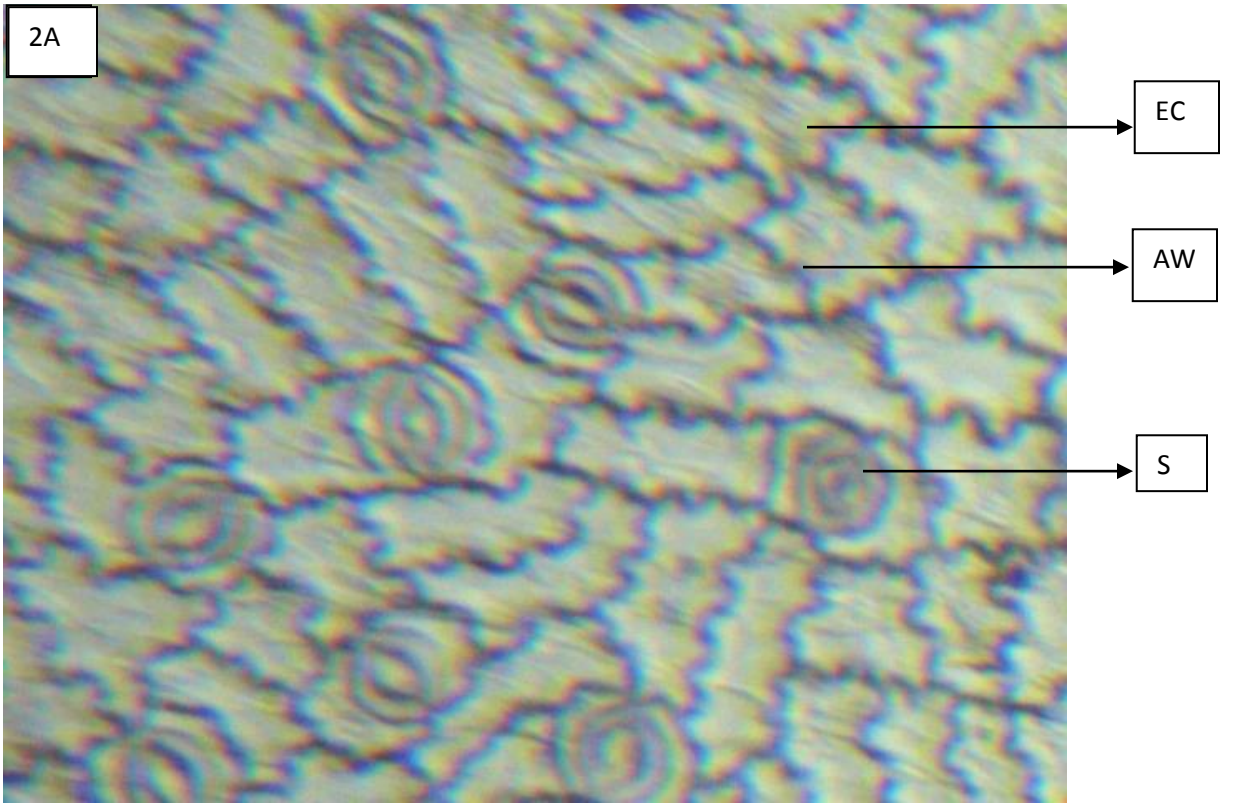
Table 3 shows the comparative result of the quantitative leaf micromorphology of *G.kola* and *G.gerrardii*. The result shows that cell length and width on both the abaxial and adaxial epidermis for both species ranges from (30.29-61.60) µm. The cell density values shows that *G.gerrardii* has more epidermal cells than *G.kola*. The mean number of stomata for *G.gerrardii* (14.50) is also more than that of *G.kola* (10.10)

Table 3: **Quantitative Microscopic Leaf Characteristics of *G. kola* and *G. gerrardii***

Characters	*G. kola*		*G. gerrardii*	
	Abaxial	**Adaxial**	**Abaxial**	**Adaxial**
Cell length (µm)	61.60±7.53	58.72±7.04	30.29±4.86	31.40±2.23
Cell width (µm)	29.80±6.04	27.63±5.64	15.99±8.86	19.38±2.03
Cell density (µm)	66.50±7.23	67.70±4.67	208.00±21.22	163.00±10.66
Stomata length (µm)	31.50±3.69	-	22.71±2.47	-
Stomata width (µm)	24.13±4.47	-	16.11±4.68	-
Stomatal no(µm)	10.10±1.85	-	14.50±3.13	-
Stomatal index (%)	13.35±2.94	-	6.54±1.12	-

The pictorial view of the Qualitative micromorphological examination of the two Garcinia species is as presented in Fig 2 (*G. kola*) and Fig 3 (*G. gerrardii*) below where the epidermal cells, anticlinal walls, stomata types have been clearly distinguished between the two species. *G. kola* has irregular cell shapes, wavy anticlinal walls on both leaf surfaces while *G. gerrardii* has straight cell shapes and straight anticlinal walls. Also, it was found that the same stomata type (Paracytic) exists on the abaxial layers of the two species. However, trichomes were found absent in both understudied species.

The micro chemical screening of the powdered leaf samples of *G. kola* and *G. gerrardii* as shown in Table 4 reveals the presence of fats, starch and calcium carbonate crystals while lignified cells, mucilage and calcium oxalates are absent in the two Garcinia species


2A
EC
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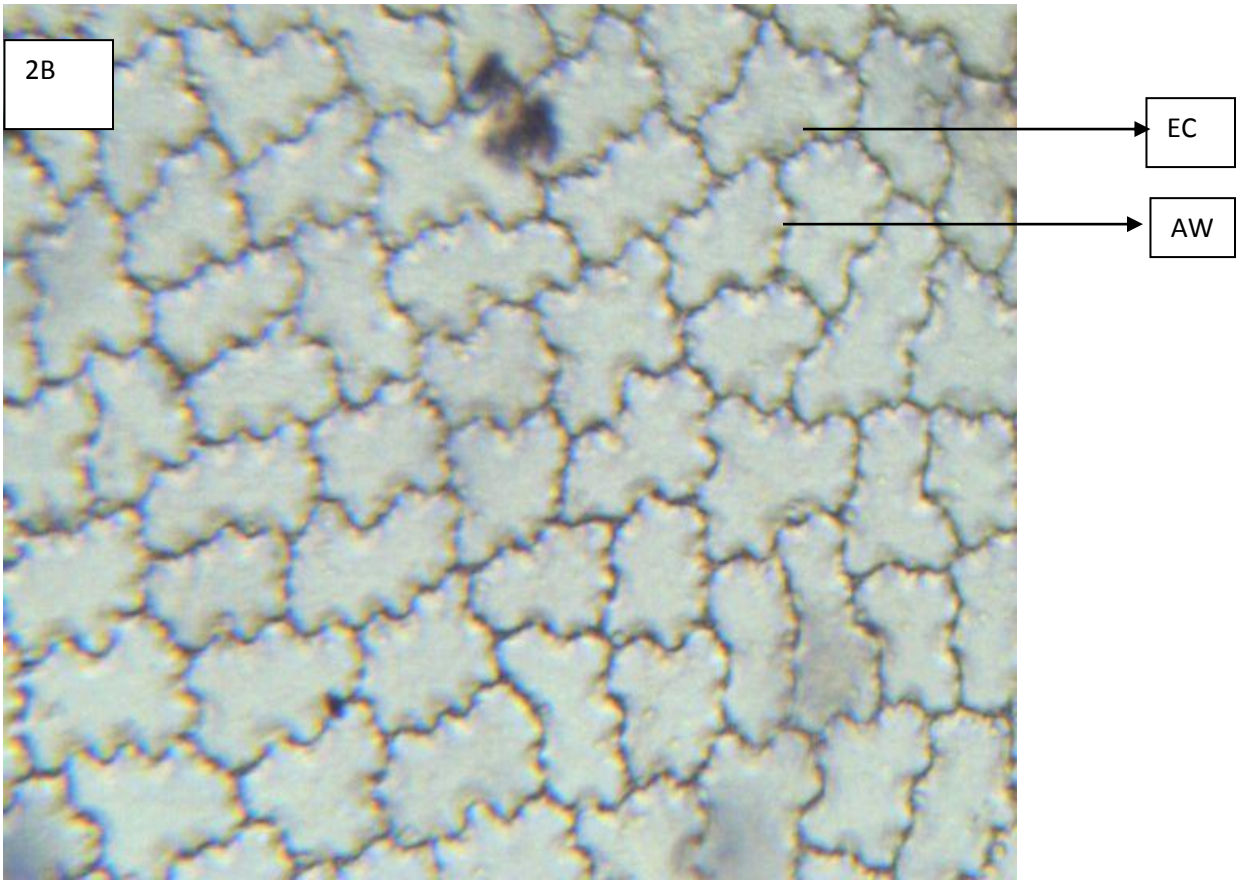
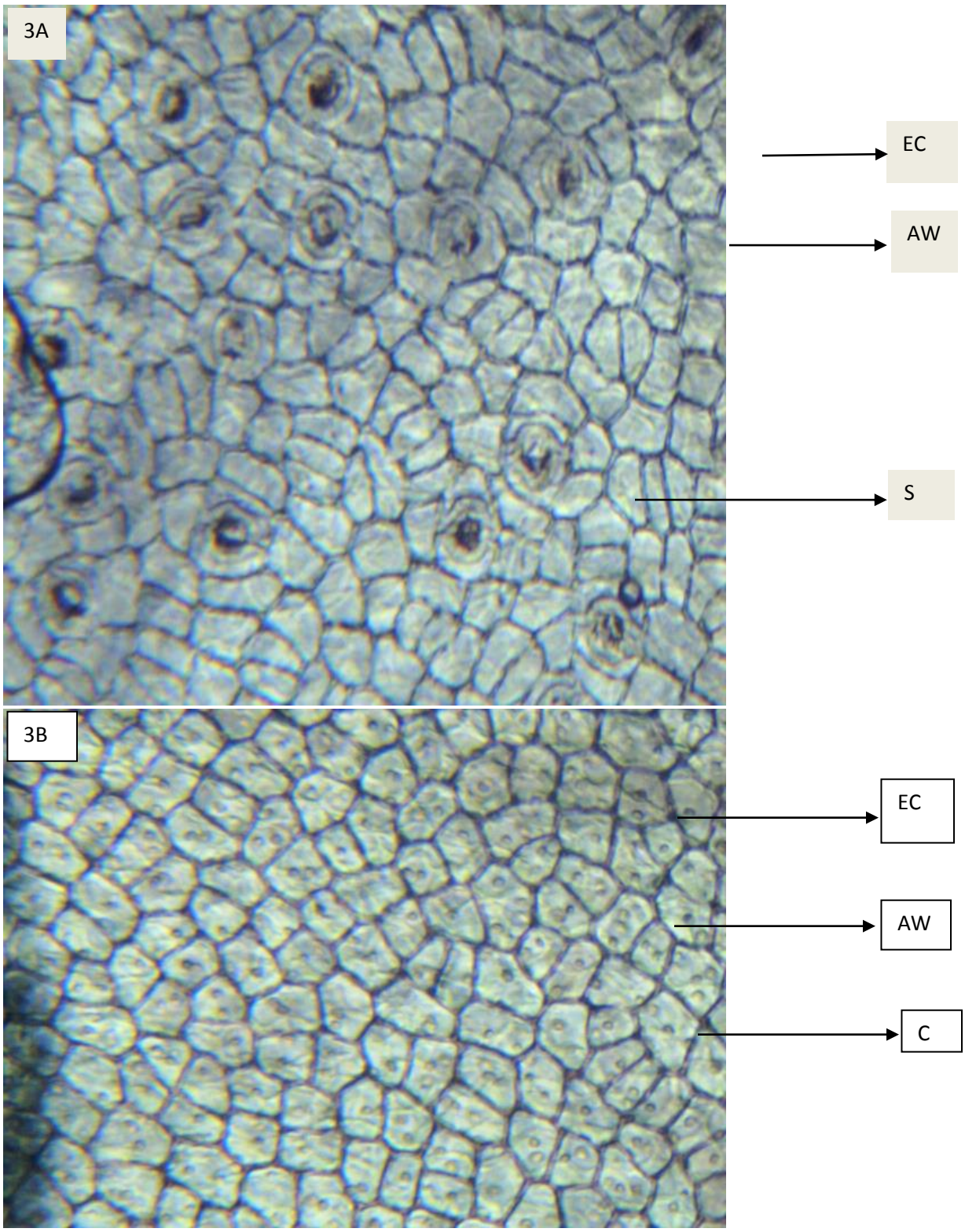

2B
EC
AW

Figure 2: Photomicrographs of the epidermal layers of *Garcinia kola*. (A-Abaxial; B-Adaxial; C- Transverse section). **A** – Irregular cell shape, wavy undulating anticlinal walls, paracytic stomata type. **B** – Irregular cell shape, wavy undulating anticlinal; **ec** = epidermal cell; aw=anticlinal walls; **s** = stomata; **c** = crystals; px- protoxylem; mx- metaxylem; pc- parenchyma; co- collenchyma; ph- phloem; ic- inner cambium; oc- outer cambium; oe – outer epidermis; ipc- internal parenchyma; op- outer phloem; oc- outer cambium; ip- inner phloem).


3A
EC
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3B
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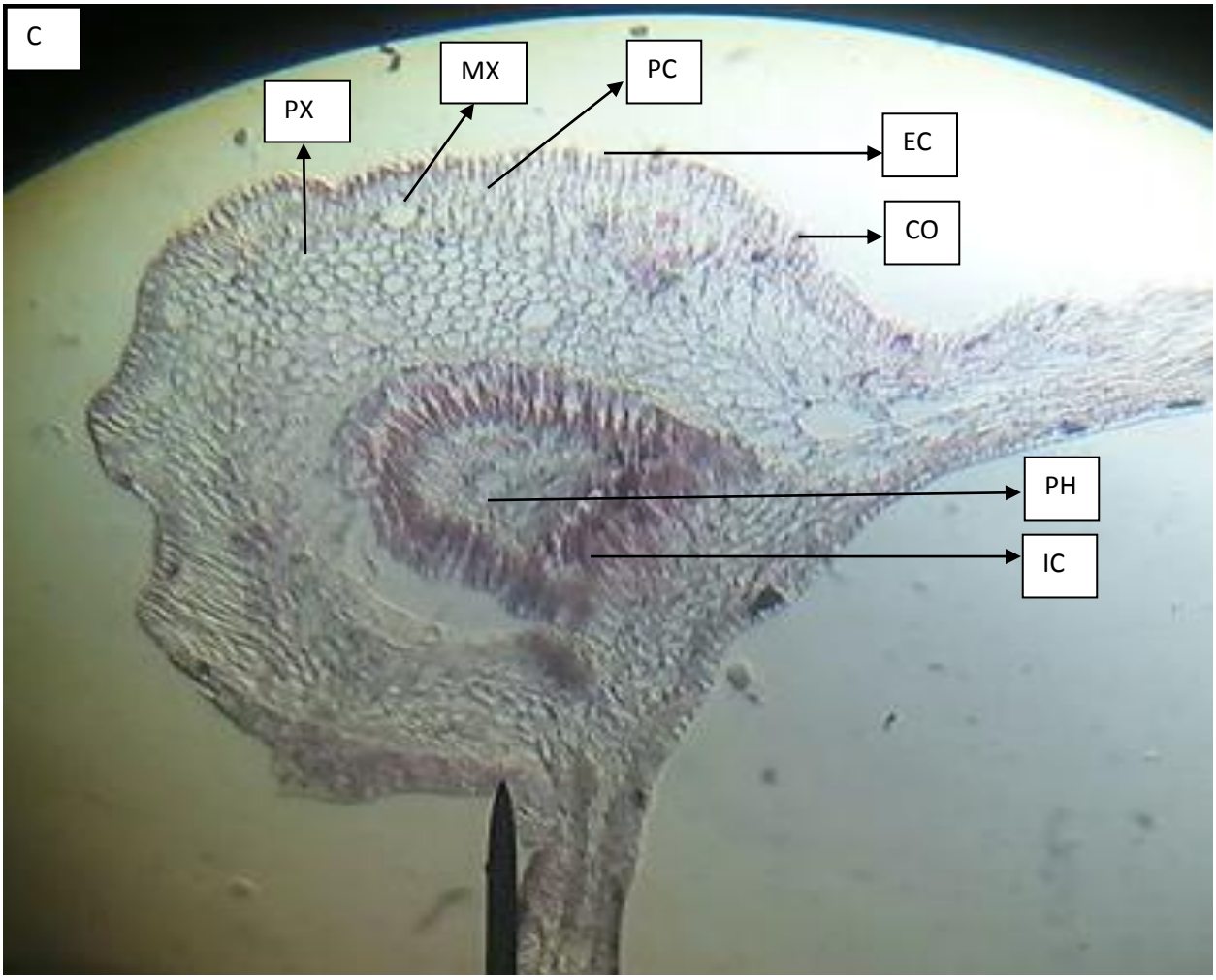

Figure 3: Photomicrographs of the epidermal layers of *Garcinia gerrardii*. (**A** – abaxial; **B**- adaxial; **C**- Transverse section). **A** – Irregular cell shape, straight anticlinal walls, paracytic stomata type. **B** – Irregular cell shape, straight anticlinal; **ec** = epidermal cell; aw=anticlinal walls; **c** = crystals **s** = stomata; px- protoxylem; mx- metaxylem; pc- parenchyma; co- collenchyma; ph- phloem; ic- inner cambium; oc- outer cambium; oe – outer epidermis).

Table 4. Microchemical evaluation of the powdered Leaves of *G.kola* and *G.gerrardii*

Parameter	Observation	Result *G. kola*	*G. gerrardii*
Lignin	No red coloration seen	-	-
Starch grains	Dark blue coloration observed	+	+
Fats	Pink coloration seen	+	+
Calcium Oxalate Crystals	No effervescence	-	-
Calcium Carbonate	Effervescence	+	+
Mucilage	No pink coloration seen	-	-
Crystals	Blue coloration observed	+	+

Conclusion

The findings of this study revealed similarity, difference and peculiarity in the leaf macromorphology and micromorphology of *G. kola* in comparism with that of *G. gerrardii*. The details as presented in this study can be used to provide distinguishing taxonomic information for the two species since there are dearth reports in that area, the presence of irregularly shaped cells with wavy, undulating anticlinal walls in *G. kola* can serve as a diagnostic character in distinguishing this species from *G. gerrardi* that also has irregularly shaped cells but straight anticlinal walls. This report has also contributed to the existing taxonomic information about *G. kola* and *G. gerrardi* which may be used in distinguishing the species in the absence of their inflorescences as well as serve as a reference standard for future use. Further ethnopharmacological studies is encouraged on *G. gerrardii* because it is an underutilized specie in Nigeria.

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