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Resolving Taxonomic Uncertainties in Khaya Genus using Foliar Anatomic Traits

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Abstract
Identification of species in the genus khaya based on morphology has always been done with some level of uncertainties. Leaf is perhaps anatomically most varied organ of angiosperms and its anatomical variations often agree with generic and specific lines. Anatomic characters of vegetative parts of flowering plants have been successfully employed to solve taxonomic problems and for the elucidation of phylogenetic relationships. This research, therefore, was designed to reveal folia anatomic structure in the three species of *Khaya* in Ondo State, Nigeria and compare their folia anatomic characteristics towards resolving their taxonomic uncertainties. Leaflets of three khaya species (*K. grandifoliola,* K. *seneglensis* and *K. ivorensis*) were collected from different locations in Ondo State. Sizeable (7cm x 3cm) portions of the leaflets from each of the species were taken from the standard median portion and to evaluate their folia anatomic structure. Findings from the study revealed that the three species were dorsiventral and were similar for all the anatomical traits investigated except for upper cuticle thickness in *Khaya ivorensis* which was significantly lower (15.33µmm) than other two species, *Khaya grandifoliola* (18.00µmm) and *Khaya senegalensis* (17.33µmm). This is an indication that folia anatomic characteristics alone could not be sufficiently used to resolve these taxonomic uncertainties in Khaya genus in Ondo State. Therefore, combination of anatomic characteristics with morphological traits could resolve this uncertainty and as such, recommended.

Keywords: Foliar anatomy, Taxonomic uncertainty and Khaya genus

Introduction

There are about 900 tree species in Nigeria; some are easily recognized but most can only be identified with certainty when flower or fruits are available (Lawal *et al.*, 2016). Identification of most tree species particularly *Khaya* genus by taxonomists is a great task because their identification has long been based on morphological traits. Duminil and Dimichele (2009) pointed out that plant morphology is polymorphic and phenotypic characters may, in principle, allow plant species classification. However, different individual trees of the same species may present a variation in their morphology either naturally or in connection with local adaptations. According to Pratt and Clark (2004), this intra-species morphological variation could be the origin of inflated species delimitation. Alternatively, some species of the same genus can be very similar morphologically and may be grouped into the same species even though they represent separate taxonomic entities (Whittall *et. al.,* 2004). In the latter case, the taxonomic group may contain cryptic species. Duminil *et al.* (2006) pointed out that the major constraint of morphological characters for the differentiation of species is based on accessibility to the vegetative parts of the plants and absent of reproductive traits at certain time of the year. Indeed it is often difficult to have access to the vegetative part of adult woody individuals, especially in tropical forest ecosystems (Duminil *et al.,* 2006).

Khaya species (African mahogany) are valuable tropical hardwoods species with high economic value in the world due to its beauty, durability and colour. Ibrahim, *et al.,* (2006) reported the occurrence of five species of *Khaya* in Africa; *Khaya anthoteca* (Welw.) C. Dc., *Khaya senegalensis* (Desv.) A. Juss, *Khaya. ivoriensis* A. Chev. *Khaya grandifoliola* C. Dc. and *Khaya nyasica*. However, only three of these species, *K. ivorensis, K. grandifoliola and K. senegalensis* are most popular and have been found in South-West Nigeria. They all belong to the family Meliaceae, sub-family Swieteniodeae. *Khaya ivorensis* is among the most important economic hardwood

timber species in Africa (Ofori, *et. al.,* 2007). The species are distributed throughout the coast of West Africa, from Cote d'Ivoire through Ghana and Southern Nigeria to Cameroon, growing mostly in the rainforest but extending into dry forests (Irvine, 1961). *Khaya grandifoliola* occurs in more or less the transitional zone between savanna and closed forests, *K grandifoliola* (Kg) trees are predominant in the Ivory Coast, Ghana and Nigeria. *K senegalensis* has *a* very wide distribution and occurs in almost all areas of the savanna stretching from Senegal to Uganda. These areas cover Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Gabon, Gambia, Ghana, Guinea and Guinea Bissau. Others are Ivory Coast, Mali, Niger, Nigeria, Sierra Leone, Sudan, Togo and the Democratic Republic of Congo. In South West Nigeria, *K. senegalensis* is prevalent in the savanna zone.

The leaf is one of the most anatomically varied organs of angiosperms and its anatomical variations have been used to resolve taxonomic uncertainty in tree systematics (Lawal and Akinnusi, 2020). Although morphological traits were among the earliest markers used in germplasm management, they have several limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992). Fortunately, DNA markers do not have such limitations. They have been proven useful in detecting variation at the DNA level and provide effective tools for distinguishing between closely related genotypes (Beyena *et. al*., 2005) but very expensive. Thus, understanding folia anatomic features could be helpful to solve the problem with this identification. Hence, the need for this study

Methodology

Study Area

This study was carried out in Akure, Ondo State which lies within the latitude 5^{o} 45' and 7^{o} 52' North and Longitude 4^{o} 20' and 6^{o} 05' East. The state is located in the southwestern geopolitical zone of Nigeria and bounded in the North by Ekiti and Kogi state, in the East by Edo state, in the west by Osun and Ogun state and in the south by the Atlantic Ocean. The climate of the area is humid sub-tropical. The mean annual temperature is about 26 ^{0}C (minimum 19 ^{0}C and maximum 34 ^{0}C), 9 months annually for rainy season which is between March and November (2,500 mm bimodal rainfall pattern) while the dry seasons last for 3 months, between the month of December and February.

Method of Data Collection

This inventory was carried out using key informants, forest guards, farmers or tree finders who were very knowledgeable about the areas where different *Khaya* species could be found in the forest reserve. This method was adopted because it was found to be the most appropriate for a study that has to do with target trees. Three trees of each species were sampled in this study and their GPS locations were recorded and used to produce a map with the aid of QGIS software (Figure 1).

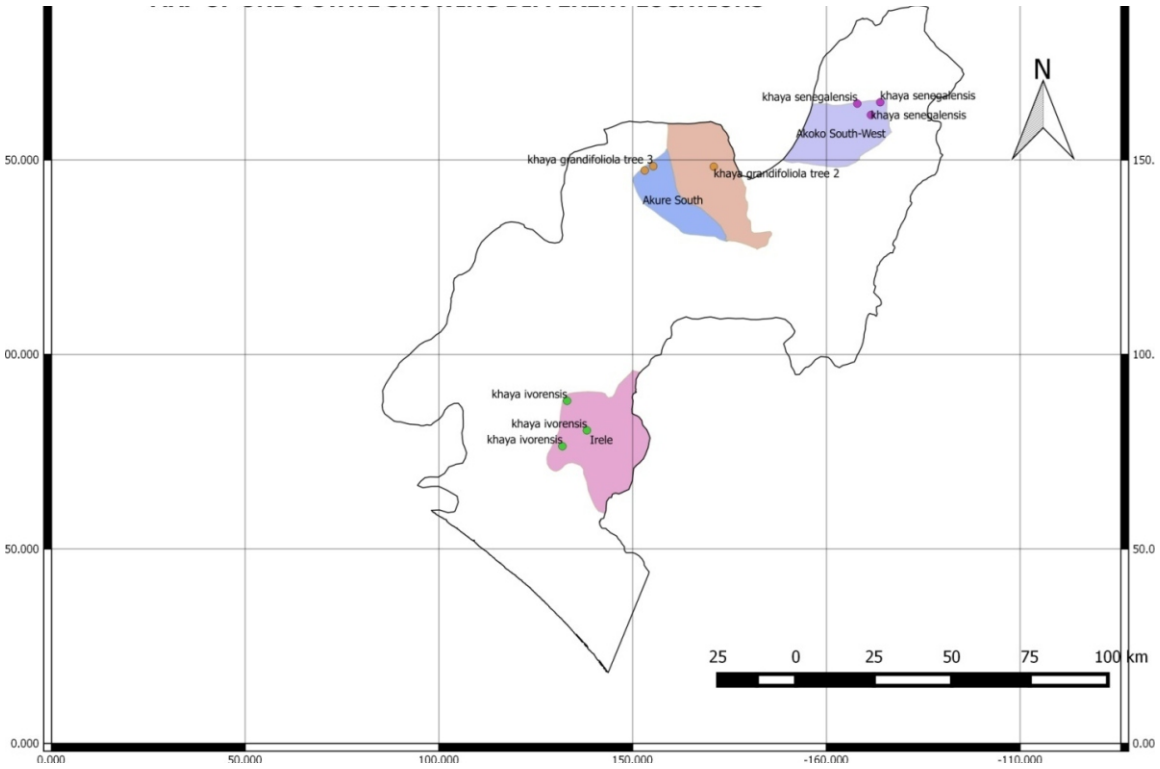


Figure 1: Map of Ondo state showing the study area.Leaflet Epidermal Study

Sizeable portions from the leaves of all the accessions of *Khaya* spp were taken from the standard median portion. The portions were put into Nitric Acid, in glass Petri-dishes, and kept in an oven at 60ºC for 20 minutes. Each sample was then washed thoroughly in 4-5 changes of water. The abaxial and the adaxial epidermis were separated using fine forceps and dissecting needle. The epidermis was then stained in Safranin O, and counterstained in Toluedene blue for five minutes, washed with 4-5 changes of water to remove excess stain and then mounted in 25% glycerol. The scraping method of Metcalfe (1968) was utilized in getting the epidermal peels from each sample. The entire samples were placed face down on a slide and flooded with a commercial bleaching agent (containing Sodium Hypochlorate). The samples were carefully scraped with a new razor blade until the epidermis was reached. The bleaching agent acted as a lubricant and at the same time helped to soften the cell layers as they were scraped off. After scraping, the scraped portion was carefully cut and the peels were stained in Safranin O and then mounted in 25% glycerol for examination under the light microscope. Photomicrographs of the epidermis were made for both the adaxial and abaxial surfaces. Trichome type(s), crystal type(s), shapes of the epidermal cells, numbers of epidermal cells, stomata types and stomata frequency were all recorded for both leaflet surfaces. Also, stomata frequency per square millimetre and stomata index (I) were estimated for the two leaflet surfaces using the formula proposed by Wilkinson (1979). The length and breadth of the stomata were measured using ocular micrometre and the measurements were converted to microns using the stage micrometre.

Stomata index (I) = S/(S+E)*100

Where: S = number of stomata per unit area,

E = number of ordinary epidermal cell in the same area.

Leaf Anatomy

The anatomy of each *Khaya* species was examined by cutting transverse sections of the

leaflets. All the sections were made with the aid of Reichert Sliding Microtome at a thickness of 8 - 10 microns. The sections were stained in Alcian blue for 3-5 minutes, rinsed thoroughly in water to remove excess stain and counterstained in Safranin O solution for 3-5 minutes. They were again washed with water and treated in a series of ethanol dilution: 50%, 70%, 80%, 90% and 100% to enhance dehydration process. The dehydrated sections were transferred into absolute xylene to remove any remaining trace of water and ethanol. These made sections clear and prevented cloudiness of the slide, as well as the drying of the slide. Sections were therefore mounted in 25% glycerol. Photomicrographs of all anatomical features were made with the aid of Accu-scope Trinocular Microscope (Accu-scope 33001 LED Trinocular Microscope with 3.2 MP CMOS digital camera) according to Lawal *et al.* (2018). All measurements including lower and upper cuticle thickness were made with the aid of ocular micrometre and final figures derived with ocular constant.

Results and Discussion

Results

The result of folia photomicrograph of *Khaya ivorensis* is presented in Plate 1. The leaflet of *Khaya ivorensis* was dorsiventral. On the adaxial, epidermal cells were polygonal with mostly straight anticlinal walls. Mean epidermal cells density was 56 per square millimeter. Stoma was absent on the adaxial surface. On the abaxial surface, the mean epidermal cell was 39.3 per square millimeter. The mean upper cuticle thickness was 15.4µmm. Palisade Mesophyll cell was a layer and composed of closely packed cylindrical cells, irregular in lengths and arrangement. Spongy mesophyll cells were largely irregular in shape with large intercellular air spaces with mean lower cuticle thickness of 15.7 µmm. The mean of stomata density was 31.0 per square millimeter while the stomata index was 15.76%.

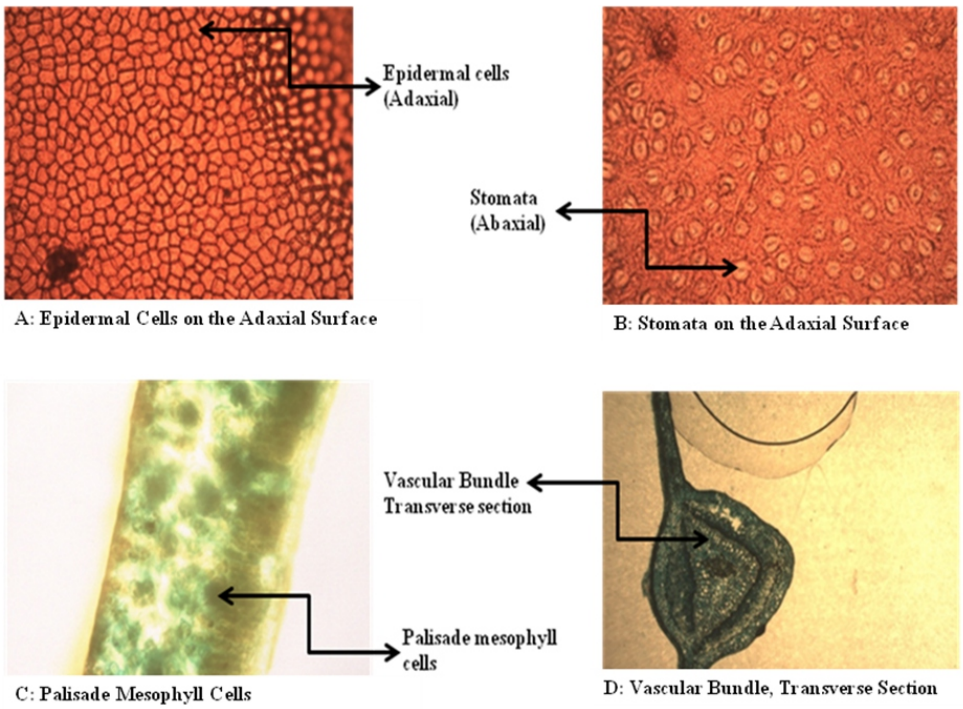
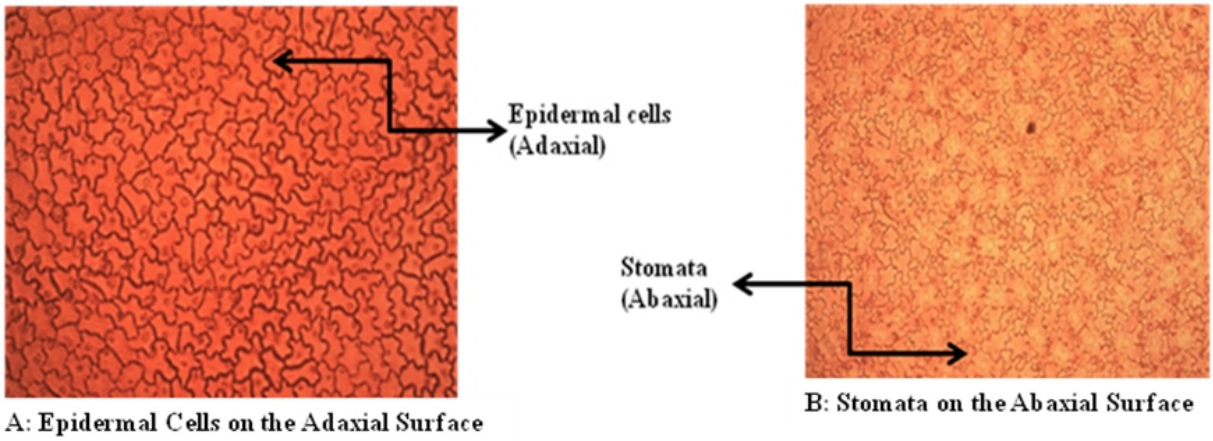


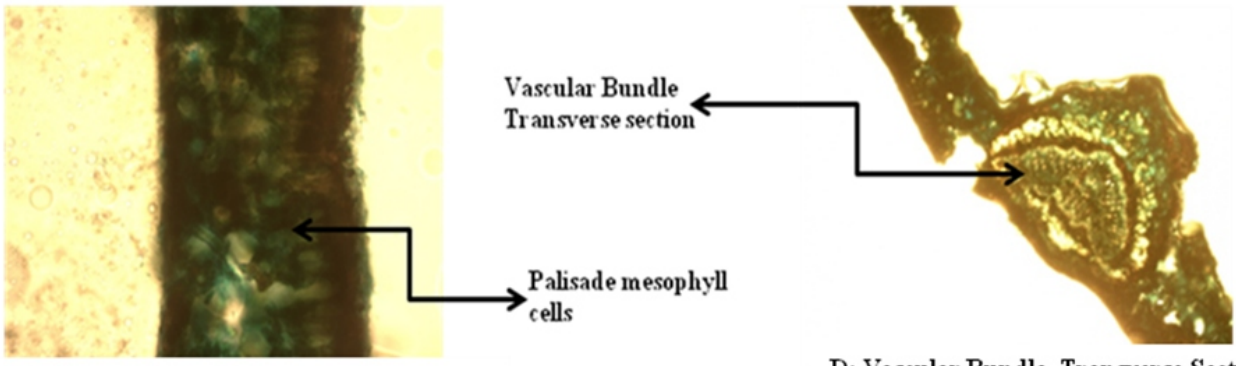
Plate 1: Folia Photomicrograph of *Khaya ivorensis*

The folia photomicrograph of *Khaya senegalensis* is presented in Plate 2. The leaflet of this species is bifacial. On the adaxial, epidermal cells were polygonal with mostly straight anticlinal walls. Mean epidermal cells density was 54.5 per square millimeter. Stoma was absent on the adaxial surface. On the abaxial surface, the mean epidermal cell was 37.0 per square millimeter. The mean upper cuticle thickness was 17.3µm. Palisade mesophyll cells was a layer, composed of closely packed cylindrical cells, irregular in lengths and arrangement. The spongy mesophyll cells were largely irregular in shape with large intercellular air spaces with mean lower cuticle thickness of 14.3 µm. The mean of stomata density was 27.7 per square millimeter, while the stomata index is 17.80%.



A: Epidermal Cells on the Adaxial Surface

B: Stomata on the Abaxial Surface



C: Palisade Mesophyll Cells

D: Vascular Bundle, Transverse Section

Plate 2: Folia photomicrographs of *Khaya senegalensis*

Leaflet of *Khaya grandifoliola* was bifacial on the adaxial surface. Also, epidermis was uniseriate and composed of rectangular or occasionally cylindrical as presented in Plate 3. Epidermal cells were polygonal with mostly straight anticlinal walls. Mean epidermal cells density was 43.3 per square millimeter. Stoma was absent. On the abaxial surface, mean epidermal cells were 35.8 per square millimeter. The mean upper cuticle thickness was 18.3µmm. Palisade mesophyll cells was one layered, composed of closely packed cylindrical cells, irregular in lengths and arrangement. The spongy mesophyll cells were largely irregular in shape with large intercellular air spaces. Mean lower cuticle thickness was 14.3 µmm and the mean of stomata density was 28.5 per square millimeter, while the stomata index was 15.22%.

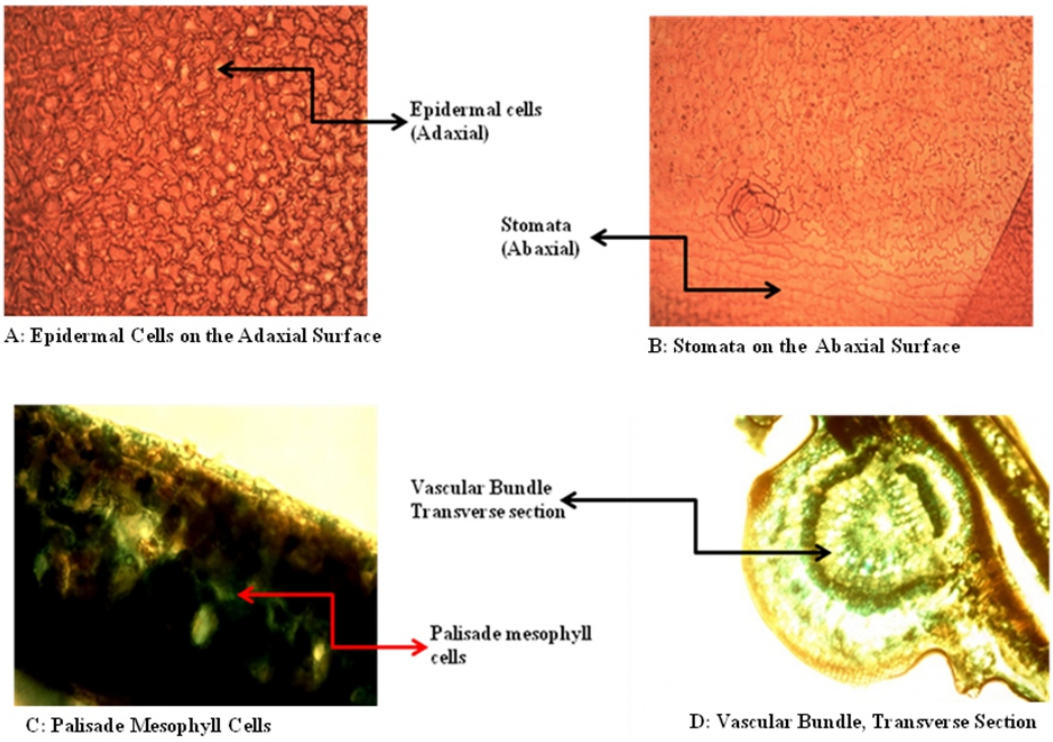


Plate 3: Folia photomicrographs of *Khaya grandifoliola*

The test for variation in the folia epidermal cell density among the genus is presented in Table 1. Though the means number of epidermal cells at both adaxial and abaxial surface were not the same for these species, they were not significantly different from each other. Lower cuticle thickness was not significantly different among the species. However, lower cuticle thickness was significantly higher in *Khaya grandifoliola and Khaya senegalensis* than in *Khaya iverensis* as presented in Table 2. Stomata index (SI) and stomata frequency on the abaxial surface was found to be the same for all the species (Table 3).

Table 1: Mean number of epidermal cells on adaxial and abaxial surfaces in *Khaya* species

Species	Adaxial surface	Abaxial surface
Khaya grandifoliola	43.33[a]	35.8333[a]
Khaya senegalensis	54.50[a]	37.000[a]
Khaya ivorensis	56.00[a]	39.3333[a]

Means with the same superscripts on the same column are not significantly different.

Table 2: Mean cuticle thickness in the studied *Khaya* species

	Upper Cuticle (µmm)	**Lower Cuticle** (µmm)
Species	Mean	Mean
Khaya grandifoliola	18.000[a]	12.966[a]
Khaya senegalensis	17.333[a]	14.300[a]
Khaya ivorensis	15.433[b]	15.663[a]

Means with the same superscripts on the same column are not significantly different.

Table 3: Mean stomata index and frequency in *Khay*a species

Species	Stomata Index (SI)	Stomata frequency on abaxial surface
Khaya grandifoliola	15.216[a]	27.6667[a]
Khaya senegalensis	17.796[a]	28.5000[a]
Khaya ivorensis	15.763[a]	31.0000[a]

Means with the same superscripts on the same column are not significantly different.

Discussion

Anatomic characters of vegetative parts of flowering plants could be employed to solve taxonomic problems and for the elucidation of phylogenetic relationships. According to Khan *et al.*, (2013), opined that stomata, Epidermal cell type, cuticular characteristics, trichome types and subsidiary cells are characteristics that have turned out to be efficient means of identification. Studies on leaf anatomic structures have been reported to be taxonomically useful at both the genus and species levels within Cyperaceae (Starr and Ford 2001). In another study, intra-specific variation was reported for anatomical traits (stomata density) of *Pinus ponderosa* (Cregg, 1993). Similarly, stomata density, epidermal cell density and the stomata index did not change in pecan trees of the same cultivar grown at different eco-geographical locations (Sagaram *et al*., 2007). This indicates the stability of certain leaf anatomical characteristics such as stomata density and epidermal cell density may be linked to the long-term climatic conditions of the location where the species (or population) developed rather than their current planting location (Sagaram *et al*., 2007).

The three tree species investigated in this study have some morphological similarities, particularly when found in the same environment making their identification using morphological traits very difficult (Lawal *et al.,* 2016). Similarly, findings from the research work revealed that the three species were dorsiventral (bifacial) and were similar for almost all the anatomic characteristics investigated. This is not expected as that different species should have different folia anatomic characteristics. Even research by Bruchi *et al.*, (2003) confirmed an inter-population variation about stomatal density, stomatal apparatus length and width, and the surface area of a stomatal aperture on a *Quercus petraea* leaf in natural populations, as well as intra-population variation for all traits, apart from the surface area of the stomatal aperture, for which a significant leaf position effect was confirmed. By comparison, the stomatal length of *Quercus coccinea* leaf was significantly smaller than the other two species (*Quercus rubra* and *Quercus velutina*) (Ashton and Berlyn, 1994).

Significant lower cuticle thickness was recorded for *Khaya ivorensis*. This may be due to the habitat where it usually occurs. While *Khaya ivorensis* is usually found in humid rainforest, *Khaya grandifoliola* occurs at the fringe of rainforest and savanna and *Khaya senegalensis* is a savanna species. According to Barbe *et al.* (2004), plant anatomical traits are good indicators of habitat quality, since they manifest variability about microclimatic conditions. Bala-sooriya *et al.* (2009) pointed out that the presents of heterogeneity of external factors (light, humidity, temperature and other) is a generator of intra-individual variation and directly influences leaf traits. Fahrny (1997) studied leaf anatomy of twenty (20) desert plants and its relation to eco-physiology and found leaf anatomic differences between plants in different habitats.

This study undoubtedly revealed that folia anatomic traits could not provide an efficient guide for appropriate identification of khaya species in Ondo State, Nigeria. Contrary to this observation, Lawal and Akinnusi (2020) provided an efficient guide for appropriate identification of *Entandrophragma* species where taxonomists are uncertain about the specific name of any tree in this genus using anatomical traits. In a study to compare their anatomical variations and identify the characteristic features which are potential markers for the identification of *Magnifera indica* cultivars, variations were noted in the thickness of

cuticle, length of epidermal cells in the abaxial and adaxial surfaces, length of palisade and spongy tissue (Sharma *et al.,* 2018).

Conclusion and Recommendation

This study revealed very similar anatomic characteristics among *Khaya ivorensis, Khaya grandifoliola* and *Khaya seneglensis* in Ondo State, Nigeria. The leaflet of all the species was dorsiventral. On the adaxial, epidermal cells were polygonal with mostly straight anticlinal walls in all the species. Their palisade mesophyll cell was single-layered and composed of closely packed cylindrical cells, irregular in lengths and arrangement. Of all the anatomical characteristics investigated, only upper cuticle thickness was discovered to be significantly lower in *Khaya ivorensis* but the same in *Khaya seneglensis* and *Khaya grandifolila*. This is an indication that folia anatomic characteristics could not be sufficiently used to resolve taxonomic uncertainties in Khaya genus in Ondo State. Therefore, combination of anatomic characteristics with morphological traits could resolve this uncertainty and as such, recommended. .

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