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Epidermal Studies of the Leaves and Phytochemical Analysis of the Seeds of *Irvingia gabonensis* and *Irvingia wombolu* in Anyigba Nigeria

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ABSTRACT

African bush mangoes (Irvingiaceae) are important food trees local to the west and central Africa. There are seven species in this family and two have been distinguished as the bitter and sweet fruited trees (*Irvingia gabonensis* and *Irvingia wombolu*). These trees are difficult to distinguish based on morphological characters alone, hence, in this study, the leaf epidermal attributes as well as the quantitative composition of selected phytochemicals of the seeds were studied and compared with the aim of providing extra taxonomic characters for the classification of the species. Results obtained revealed significant differences in the epidermal layer of the studied species. Differences were observed in the number, length, and width of the epidermal and stomata cells, the shapes and wall of the epidermal cells varied significantly on the adaxial surface, while that of *Irvingia gabonensis* was polygonal and straight, that of *Irvingia wombolu* was irregular and curved. Both species possessed the following similarities on the epidermal layer: irregular and curved epidermal cells on the abaxial, hypostomatic (possession of stomata only on the abaxial) and paracytic stomata types. The phytochemical composition varied as well, while there was no significant difference in the quantity of alkaloid, flavonoid, tannins and phenols, the quantity of saponin and sterol varied significantly with Irvingia gabonensis having 0.80±0.01 and 0.21±0.02 and *Irvingia wombolu* having 0.73±0.00 and 0.15±0.01 respectively. The differences observed were significant therefore supporting the idea that the two plants are of different species and should be accorded different taxonomic classifications.

Keywords: Epidermal, Phytochemicals, Stomata, Seeds, Taxonomic and Abaxial

INTRODUCTION

The vast majorities of useful plant Species from a variety of ecosystems are exploited only locally and are known to the scientific community under various names among which “Non Timber Forest Products (NTFPs)” remains the most commonly used one [1]. The evaluation of the diversity of tropical plant Species exploited as NTFPs already started during colonial times and this shows the very old symbiotic relationship between local communities and the environment [1,2]. Lowe *et al*. [3] reported that many Species may become widely used across their natural distribution area or even transported, marketed and eventually cultivated beyond their original habitat, one of such is the African bush mango trees (ABMTs)) they are widely used and economically important among the multipurpose trees Species in Central and West Africa. African bush mango trees (ABMTs) belong to the family of Irvingiaceae with two economically important Speciesnamely:*Irvingia gabonensis* and *Irvingia wombolu* [3,4]. Before now *Irvingia gabonensis* and *Irvingia wombolu* were grouped together as one Species, *Irvingia gabonensis*, (Aubry-Lecomteex O’Rorke) this was because of the similarities in the morphology of the trees, leaves, fruits and seeds of the Species. However, a distinction was made between the two forms of *Irvingia gabonensis* with *I. gabonensis*var. *Gabonensis*as having a sweet edible pulp, and *I. gabonensis*var. *wombolu*, as having a bitter inedible pulp [1]. *Irvingia gabonensis* and *Irvingia wombolu* are commonly called bush mango or African mango because the trees bear mango-like fruits. The fruits are

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broadly ellipsoid, green when unripe and yellow to orange when ripe, with a fleshy meso carp [5]. Several research works have been carried out to assess the presence of antioxidants and phytochemicals in *Irvingia* seeds with their corresponding nutritional, economic and health benefits [6, 7]. The domestication of the plants as an indigenous fruit trees and their integration into diverse agro forests have been identified as important strategy for the improvement of land use in Africa [8]. Previous studies on the fruit and seed characteristics of the plant which would stimulate domestication of the trees did not take into account the potential differences in the phytochemical composition of the fruit of the two Species, *I. gabonensis* and *I. wombolu* [3]. Since the two Species of African Bush Mango Trees (ABMT) have made their way into the list of high valued economic plants in Africa and beyond. In the light of their domestication and cultivation potentials, the specific morphological characteristics of both taxa as well as the phytochemical variations present within each taxon are important research questions to be addressed [3]. The epidermal study of their leaves is valuable in order to assess the key differences between them which will assist in the determination of the appropriate level of their taxonomic distinction. In addition, the kernels of both Species are mixed for marketing, while it seems likely that there are differences in their organic properties as well as phytochemical composition, indicating different economic potential for sweet and bitter trees [1]. This study reveals the differences between *Irvingia gabonensis* and *Irvingia wombolu* through epidermal study of the leaves and phytochemical analysis of the seeds.

MATERIALS AND METHODS

Study Area

This study was carried out in Anyigba, a town in Dekina Local Government of Kogi State. Kogi state is located on latitude 7.4^0 N and longitudes 6.45^0E. The state occupies an area of 28,312.6 square kilometers and is found in the derived savanna vegetation of Nigeria. Hence, this area is co-habited by savanna and forest Species. Agriculture especially fishing and farming is the major occupation of this area.

Leaf Epidermal Study

Materials and Reagents: Knife, razor blade, petri dishes, Scalpel, Leica binocular Microscope, Slides and cover slips, Safranin stain, Glycerine jelly.

Preparation of Samples: The trees of both species were identified based on the morphological attributes described by Vihotogbe R. [1]. Fresh leaves of both Species were collected from the field and were taken to the Biological Sciences Laboratory, Kogi State University, Anyigba, Kogi State. They were preserved in a polythene bag to prevent drying out.

Procedure: The fresh leaves were soaked in water for few minutes to soften them. Mechanical peeling was done by holding the leaves between fingers and carefully scraping with a razor blade to get the lower cuticle of the side which was peeled. The abaxial and adaxial surfaces of the leaves were obtained by scraping the upper and lower surfaces of the leaves respectively. The cuticles were stained in Safranin for 10 minutes, rinsed in water to remove excess stain. Temporary mounts were made with a drop of glycerol and covered with clean cover slips. Each prepared specimen was viewed with Leica binocular microscope at x40 objective. The number, size and type of stomata and epidermal cells were observed and recorded. Epidermal strips from both the adaxial and abaxial surfaces were prepared and mounted separately. Photographs of good preparations were taken at x40 objective for photomicrograph. The length and width of epidermal cells and stomata apparatus were measured with micrometer eye piece graticule. The number, shape and size of stomata and epidermal cells were observed and recorded. Slides mounted for each leaf surface, observations and measurements were made from several microscope fields of focus at a magnification of ×400.

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Phytochemical Analysis

Dried fruits of both Species were bought from the domestic farms where the trees have already been correctly identified based on the morphological attributes described by [1]. Twenty five (25) fruits were collected from both trees, making a total of fifty samples. The seeds were extracted from the dried seed coat and were then dried under the sun for two days. The dried seeds were ground to fine powder. The phytochemical analysis of the order Alkaloids, Flavonoids, Tannins, Saponins, Phenols, and Sterols was carried out according to the standard method of Association of Analytical Chemistry [9].

Statistical Data Analysis

The data obtained were analyzed on Statistical Package for Social Sciences (SPSS, Version 20.0) using Descriptive statistics to test for significance difference (P>0.05) in the phytochemical compositions (Alkaloids, Flavonoids, Tannins, Saponins, Phenol, Sterol) of the studied plant species (*Irvingia gabonensis* and *Irvingia wombolu*). The result was summarized on a table as mean ± standard error. The results on the leaf epidermal study were analyzed for similarities and differences on a simple bar chart.

RESULTS

Leaf Epidermal Study: The figures below show the various differences that occur on the abaxial and adaxial epidermal surfaces of the leaves of the studied plants for all parameters considered (number, length and width of epidermal cells, number, length and width of stomata cells) represented on simple bar chart. It is seen in Figure 1.1 that *Irvingia gabonensis* has higher number of epidermal cells **(143)** than *Irvingia wombolu* (**115**) on the abaxial.

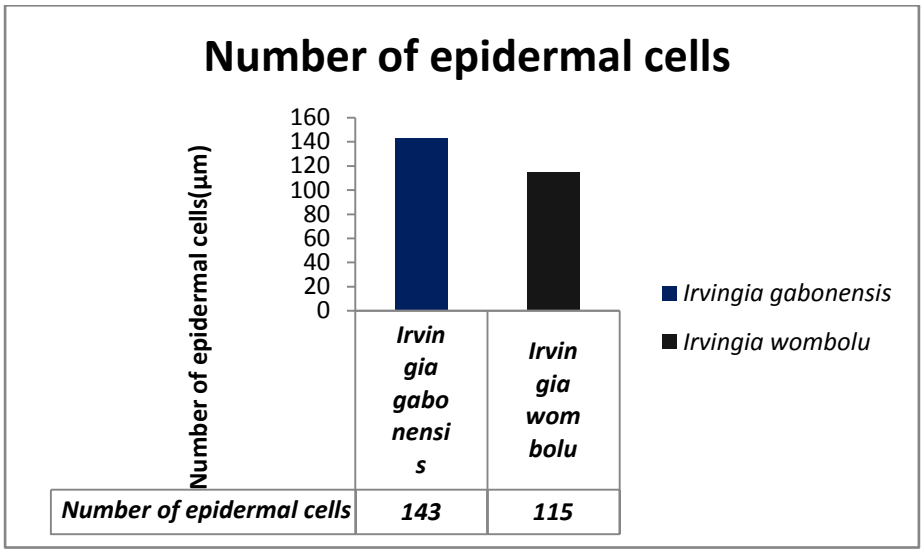

Figure 1.1: bar chart showing the relationship between the number of epidermal cells of the abaxial of *Irvingia gabonensis* and *Irvingia wombolu*

Figure 1.2 compares the length of epidermal cells on the two species studied showing *Irvingia wombolu* (**1.50µm**) to be longer than *Irvingia gabonensis* (**1.10µm**).

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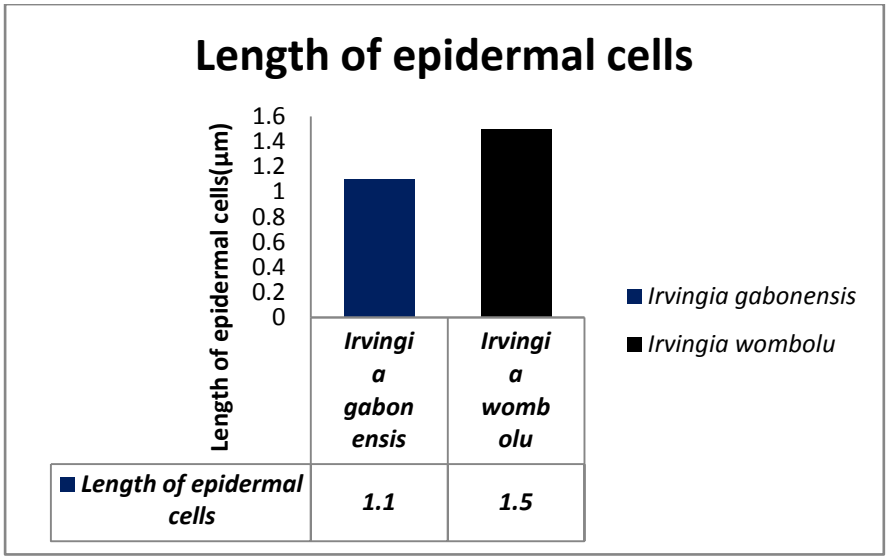

Figure 1.2: Bar chart showing the relationship between the length of epidermal cells of the abaxial of *Irvingia gabonensis* and *Irvingia wombolu*

In Figure 1.3, the bar chart shows that *Irvingia wombolu* (**1.49µM**) is wider than *Irvingia gabonensis*(**1.49µM**)on the abaxial.

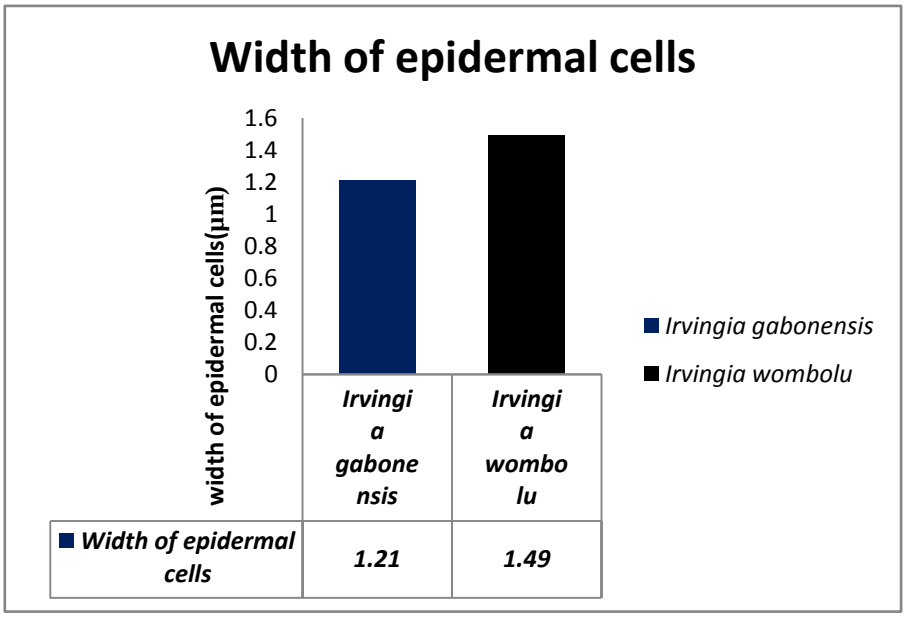

Figure 1.3: bar chart showing the relationship between the width of epidermal cells of the abaxial of *Irvingia gabonensis* and *Irvingia wombolu*

The bar chart below shows the differences in the number of epidermal cells on the adaxial surfaces. The cells of*Irvingia wombolu* (**132**) are observed to be longer than *Irvingia gabonensis* (**137**).

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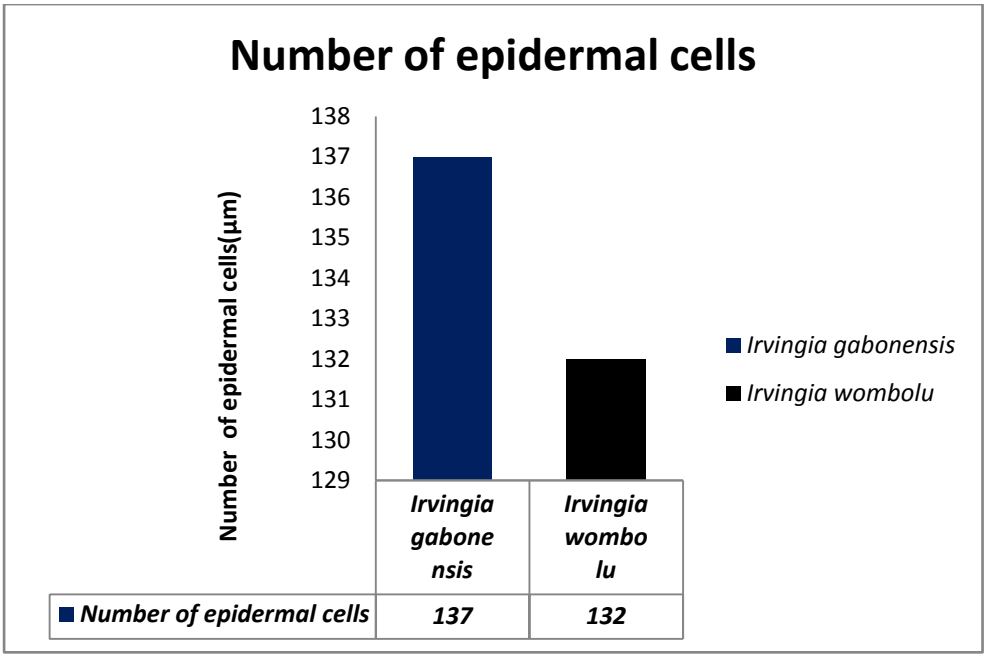

Figure 1.4: bar chart showing the relationship between the number of epidermal cells of the adaxial of *Irvingia gabonensis* and *Irvingia wombolu*

Figure 1.5 shows the length of the epidermal cells on the adaxial; *Irvingia wombolu* epidermal cells appear to be longer than that of *Irvingia gabonensis* (*Irvingia wombolu*=**1.87µm** while *Irvingia gabonensis*=**1.83µm**)

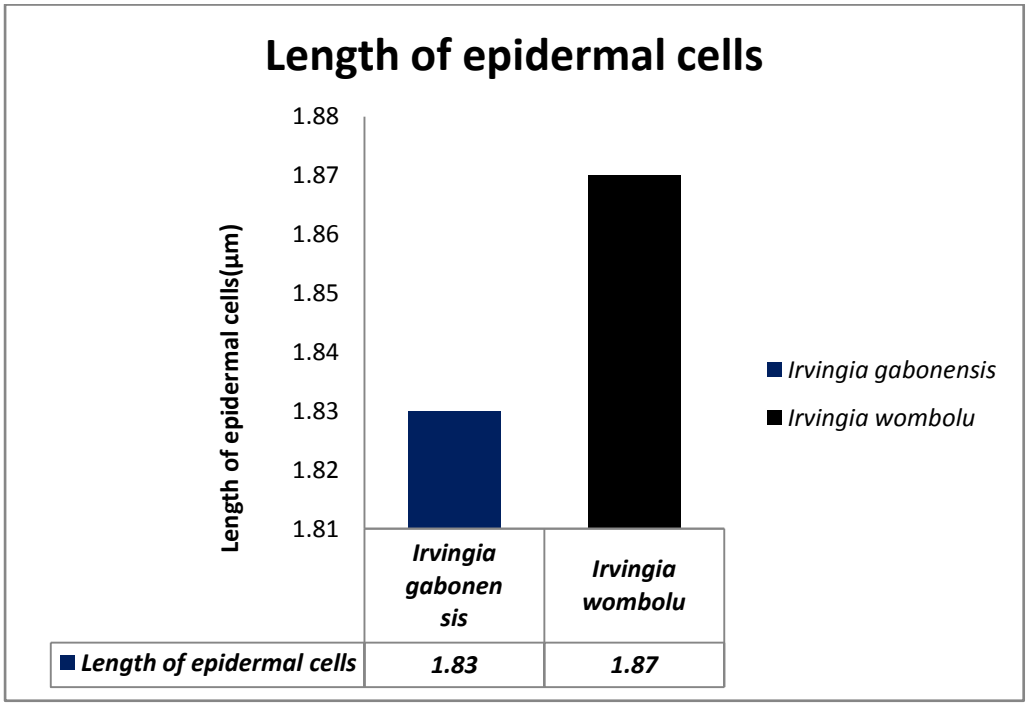

Figure 1.5: bar chart showing the relationship between the length of epidermal cells of the adaxial of *Irvingia gabonensis* and *Irvingia wombolu*

On Figure 1.6, the bar chart shows that there is no difference in the width of the epidermal cells. Both species have a mean length of **2.03µm**.

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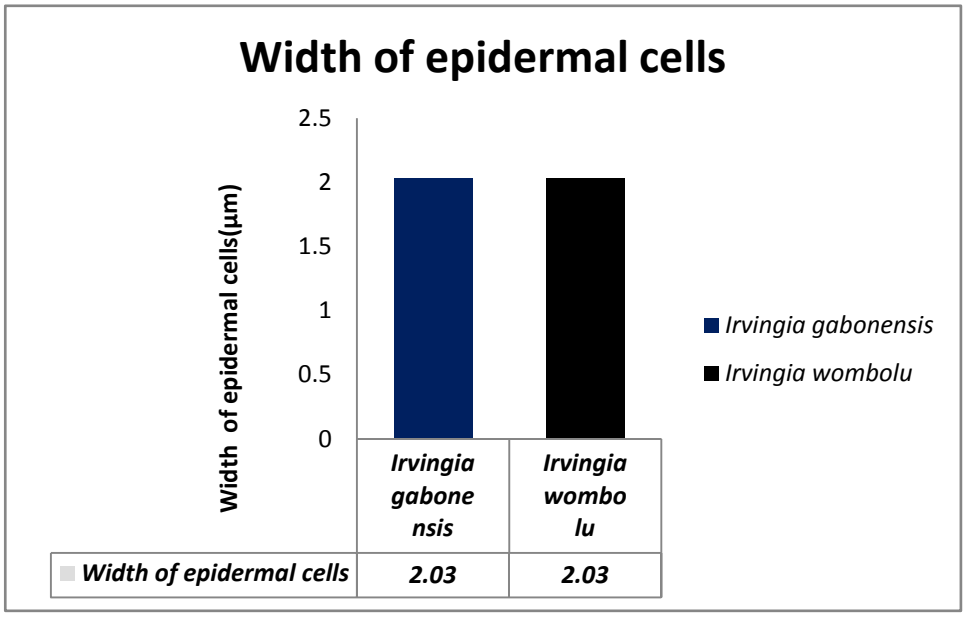

Figure 1.6: Bar chart showing the relationship between the width of epidermal cells of the adaxial of *Irvingia gabonensis* and *Irvingia wombolu*

The chart (Figure 1.7) below shows the distribution of stomata cells on the studied species. *Irvingia gabonensis* (**32**) has more stomata on the abaxial than *Irvingia* wombolu (**25**).

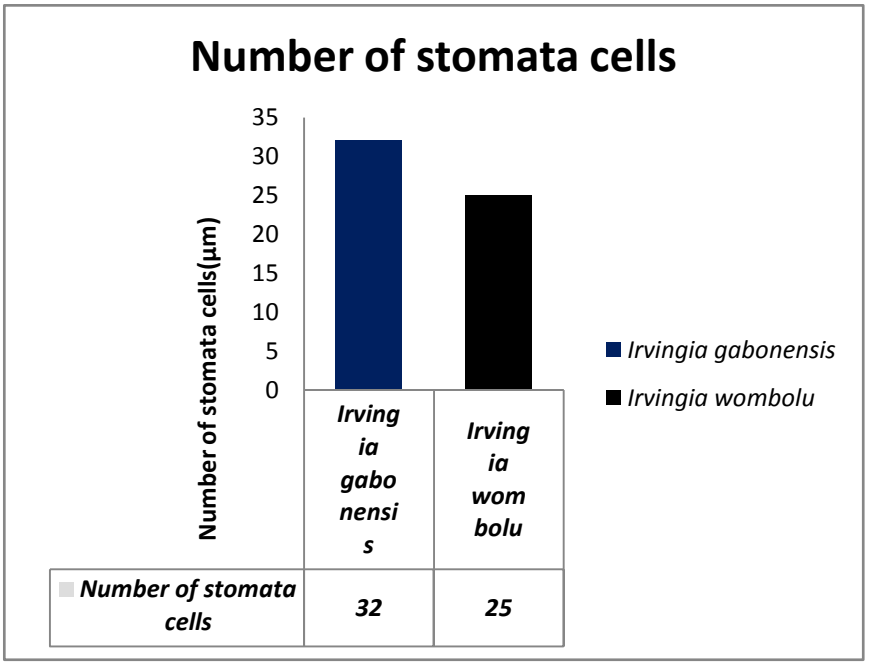

Figure 1.7: bar chart showing the relationship between the number of stomata cells of the abaxial of *Irvingia Gabonensis* and *Irvingia Wombolu*

In Figure 1.8, it is seen that the stomata cells are longer in *Irvingia wombolu* (**1.34µm**) than in *Irvingia gabonensis* (**1.16µm**).

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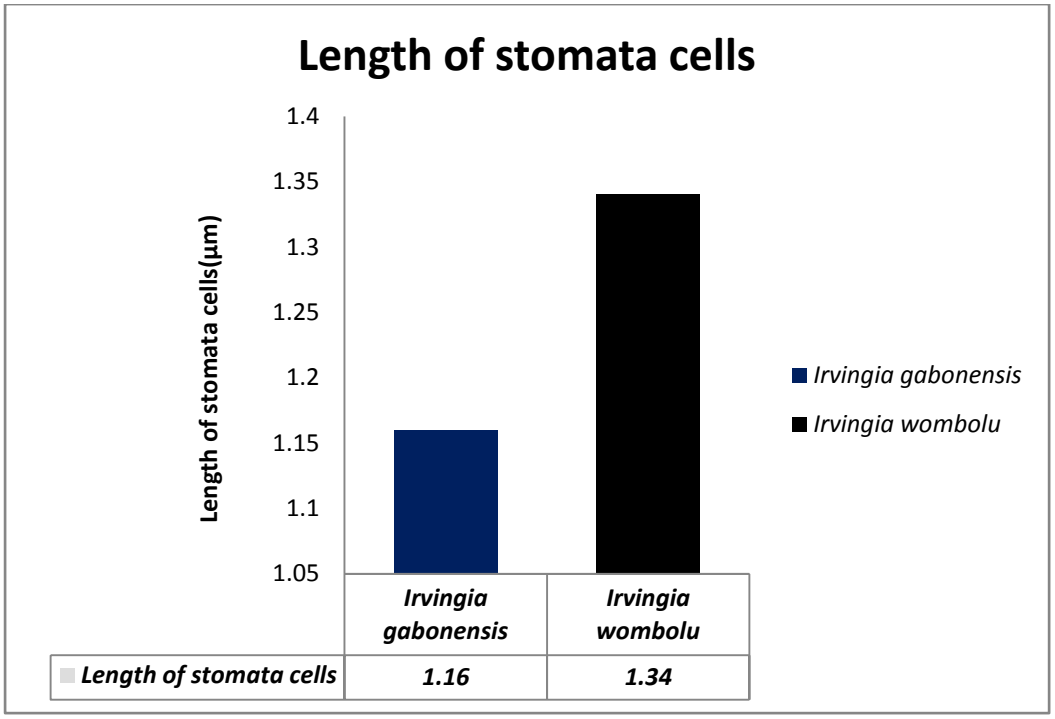

Figure 1.8: bar chart showing the relationship between the length of stomata cells of the abaxial of *Irvingia gabonensis* and *Irvingia wombolu*

The figure 1.9 shows the differences in the width of the stomata cells. The stomata cells of *Irvingia wombolu* (**1.43µm)** are seen to be wider than that of *Irvingia gabonensis* (**1.20µm).**

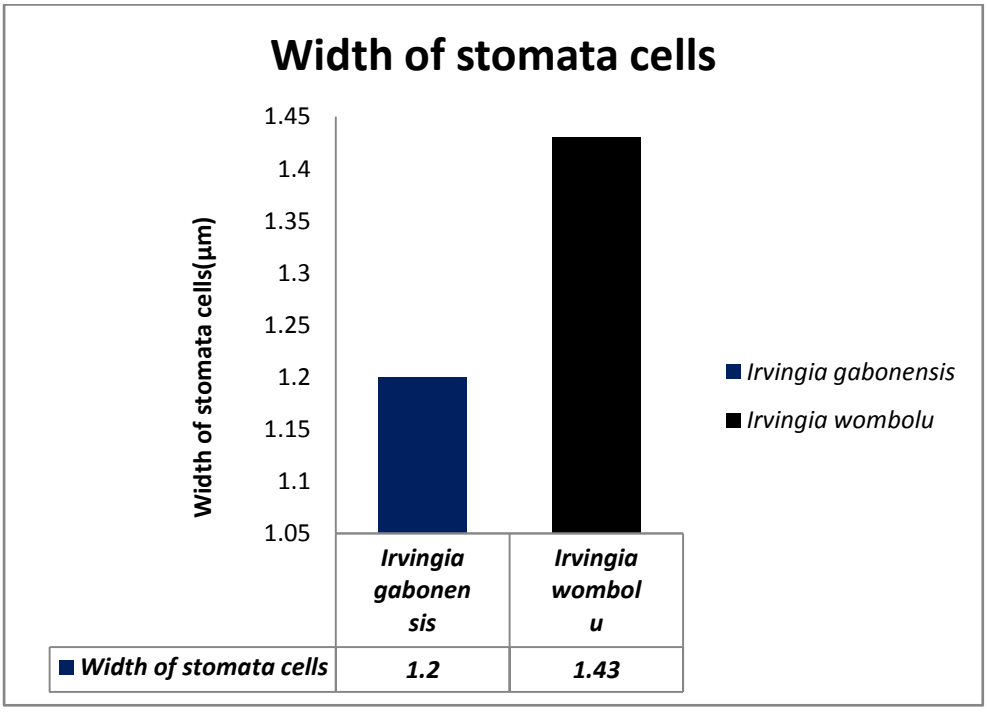

Figure1.9: bar chart showing the relationship between the width of stomata on the abaxial of *Irvingia gabonensis* and *Irvingia wombolu*

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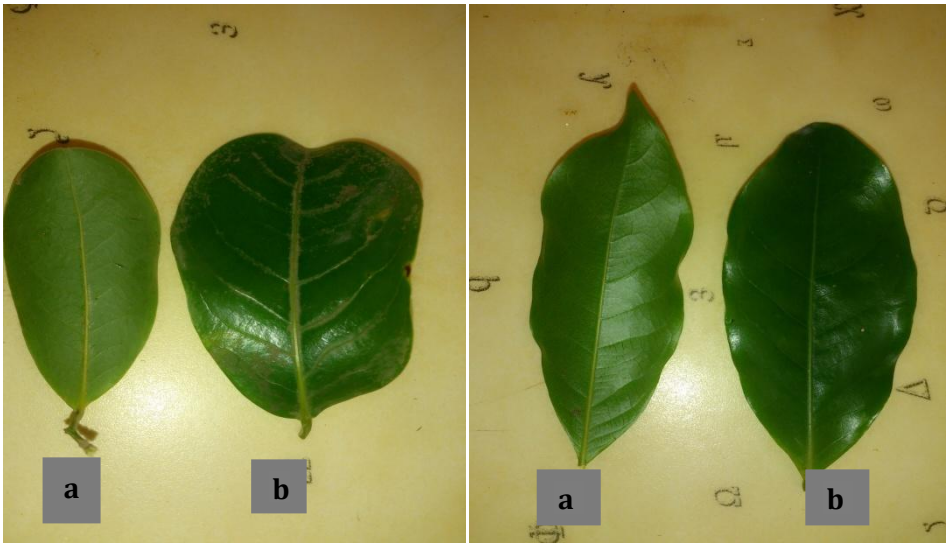

a = abaxial surface of *I. gabonensis*
b= adaxial surface of *I. gabonensis*

a = abaxial surface of *I. wombolu*
b= adaxial surface of *I. wombolu*

Plate 1.1: Images of Leaves of *Irvingia gabonensis* and *Irvingia wombolu*

Table 1.1 below shows a summary of the stomata and epidermal cell types observed on the abaxial and adaxial surfaces of the leaves of the studied plant species of *Irvingia gabonensis* and *Irvingia wombolu.* It is observed that the epidermal wall pattern and cell shape were similar on the abaxial with both species having irregular and curved epidermal cells. Difference was observed on the adaxial with *Irvingia gabonensis* having irregular and curved epidermal cells and *Irvingia wombolu* having polygonal and straight epidermal cells. The stomata in both Species studied were distributed only on the abaxial (Hypostomatic) and were Paracytic.

Table 1.1: Stomata and epidermal cell types Key: P-Polygonal, I- Irregular, S- Straight, C- Curve

Species	Surface	Epidermal Cells shape	Epidermal Wall Pattern	Stomata Types	Leaf Condition
Irvingia gabonensis	Abaxial	I	C	Paracytic	Hypostomatic
	Adaxial	P	S	Nil	
Irvingia wombolu	Abaxial	I	C	Paracytic	Hypostomatic
	Adaxial	I	C	Nil	

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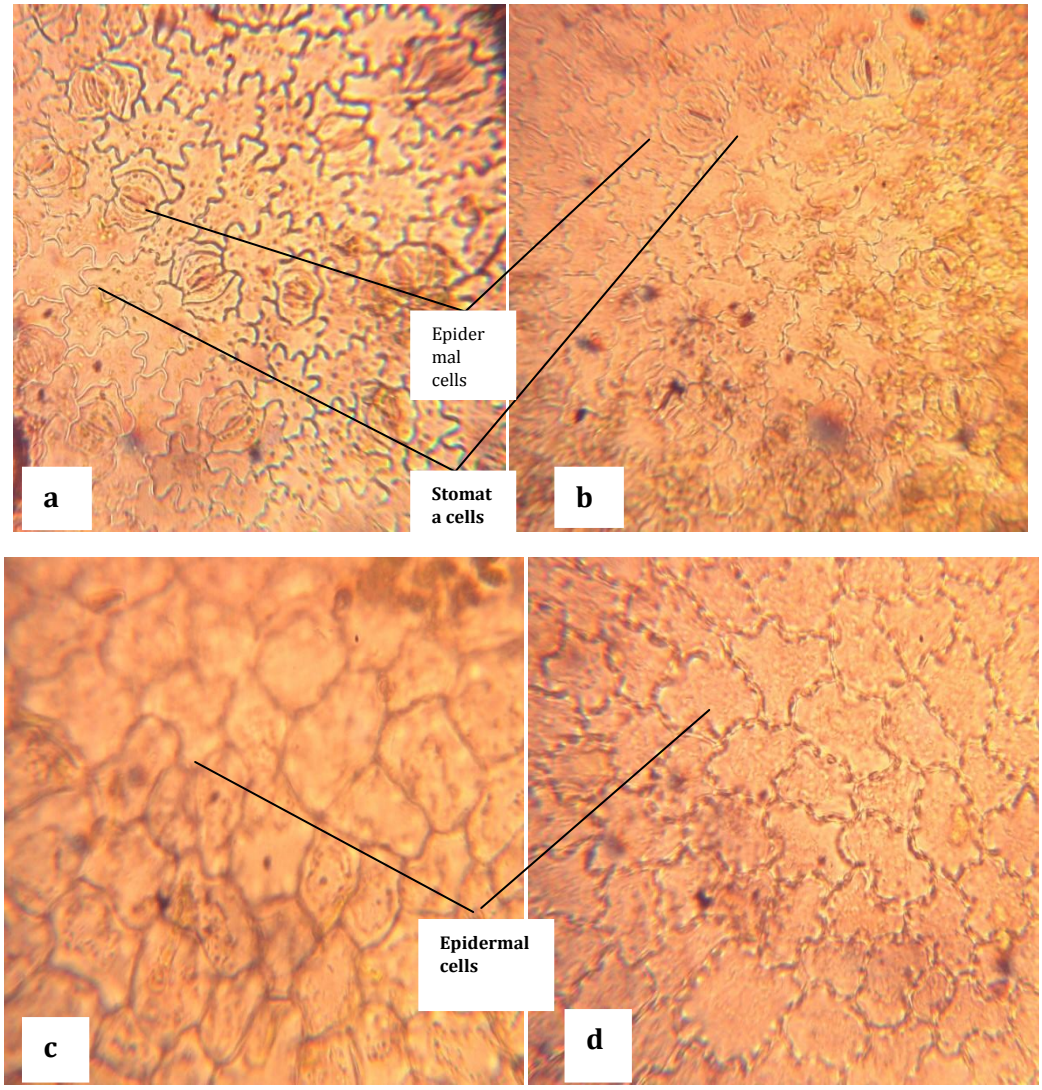

KEYS: a=*Irvingia wombolu* (Abaxial), b= *Irvingia gabonensis* (Abaxial)
c= *Irvingia gabonensis* (Adaxial), d= *Irvingia wombolu (*Adaxial)

Plate 1.2: Photomicrograph of the Abaxial and Adaxial leaf epidermal surfaces of the studied species

Phytochemical Composition

The results of the phytochemical compositions are shown in Table 1.2. Apart from Saponin, they were no significant differences (P> 0.05) in the quantity of the phytochemicals (Alkaloid, Flavonoids, Tannins and Phenol) present in the seeds of *I. gabonensis* and *I. wombolu.*

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Table 1.2: **Phytochemical Composition of *Irvingia gabonensis* and *Irvingia wombolu***

Species	Alkaloids	Flavonoids	Tannins	Saponins	Phenol	Sterol
I. gabonensis	**1.99±0.02^{c*}**	**1.83±0.01^{a}**	**1.25±0.01^{d}**	**0.09±0.01^{a}**	**0.05±0.03^{a}**	**0.21±0.02^{a}**
I. wombolu	**1.82±0.01^{c}**	**0.26±0.01^{a}**	**1.17±0.02^{d}**	**0.73±0.01^{b}**	**0.03±0.01^{a}**	**0.13±0.01^{b}**
SME	**1.91±0.09**	**1.05±0.79**	**1.21±0.04**	**0.41±0.32**	**0.04±0.01**	**0.17±0.04**
	NS	**NS**	**NS**	**S**	**NS**	**NS**

*Values followed by same superscript in a column are not significantly different at P>0.05 Keys: NS-No Significance, S- Significant

DISCUSSION

The problematic distinction between *Irvingia gabonensis* and *Irvingia wombolu* was extensively reported by Vihotogbe R. [1]. In this study, attempt was made to compare this species based on leaf epidermal study and seed phytochemical analysis. Result of the epidermal features of the studied species revealed some diagnostic characteristics that could be used for taxonomic decision. Features that separated the species and the accessions from one another are in line with the earlier works of [10, 11]. They both used leaf epidermal studies to compare different species and establish relationship among various taxa. The validity of leaf epidermal study as a taxonomic tool is also backed by the research of [12] that the leaves of vascular plants possess characters which are second to those of flowers and fruits in their value and use in taxonomic studies. The number, length, width and type of epidermal cells as well as the stomatal type, number, length and width revealed the differences and correlations among the species. Both species were Hypostomatic; possessing stomata only on the abaxial surface. They both possessed Paracytic stomata types with irregular and curved shaped epidermal cells on the abaxial surface, slight difference was observed on the adaxial surface; the epidermal cells of *Irvingia gabonensis* were polygonal and straight while that of *Irvingia wombolu* were irregular and curved. Slight difference was also observed in the distribution of epidermal cells and stomata cells with *Irvingia gabonensis* having higher number of stomata and epidermal cells than *Irvingia wombolu.* The limited number of visible distinctive characters on the epidermis of the leaves may be ascribed to the limited number of sample species evaluated and the plants exposure to different environmental conditions under domestication. This slight variation among the studied plant species can stand as an indication of genetic diversity of the two species. It can be implied that the relatively high stomata on the abaxial surfaces of both species may have taxonomic importance and ecological implication on the transpiration among members of this family, this is similar with the results from earlier works by Essiett and Akpabio [13] on comparative anatomy of *Talinum triangulare* and *Talinum portulacifolium* where they concluded that the role of stomata in plant species cannot be over-emphasized. It is also probable that the distribution of stomata only on the abaxial is an adaptation for survival in its habitat. The absence of stomata on the adaxial is a key taxonomic tool that may be used to delimit this plant species from other *Irvingia* species and shoot further research. The results from the phytochemical analysis indicated that *Irvingia gabonensis* seeds contained higher quantity of the studied phytochemicals, although the difference in concentration was slight compared to *Irvingia wombolu* it is probably one of the reasons for the increased draw ability of the seed. The variation in concentration of the phytochemicals support earlier conducted research by [14] that the degree of accumulation of secondary metabolites which occurred in different parts of these two plants varied and that these secondary metabolites occur naturally in plants and serve as natural defense mechanism against herbivory and pathogen attacks. They presence of this phytochemicals in the seeds of the studied plants is an indication of potential therapeutic values of the plants. This result supports the recent works by [1] in comparing the morphological attributes of the bitter and sweet *Irvingia* species and concluding that *Irvingia gabonensis* and *Irvingia wombolu* are indeed of different species and should be accorded different taxonomic descriptions.

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CONCLUSION

The two analyses undertaken in this research (leave epidermal study and phytochemical study) revealed the diversity patterns within two *Irvingia* species (*Irvingia gabonensis* and *Irvingia wombolu*) and establish the taxonomic characters that can be used to distinguish the two species as well as to assess the taxonomic level most suitable for their classification. Epidermal difference exists among the leaves of the two species as evidenced by the slight difference in the shape and arrangement of epidermal cells on the adaxial of the two leaves (polygonal and straight in *Irvingia gabonensis*, irregular and curved in *Ivingia wombolu)*, as well as distribution of stomata and epidermal cells on the abaxial, the higher number of stomata on the leaves of the *Irvingia gabonensis* can be attributed to the adaptation to survive in drier areas compared to *Irvingia wombolu* which has lesser stomata cells. This study has also established the presence of certain phytochemicals in the seeds of *Irvingia gabonensis* and *Irvingia wombolu* in varying concentrations.

RECOMMENDATIONS

In order to achieve maximum detail on the variation within *Irvingia* species, the comparative studies on *Irvingia* species in other fields of biology like palynology, molecular biology and genetics is necessary. Studies should also be carried out on all the species of *Irvingia* to determine the distribution and arrangement of the epidermal cells at different seasons of the year (Rainy and dry season).

CONFLICTS OF INTEREST

The authors have no conflicts of interest and are solely responsible for the content of this work.

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