Plants and Environment (2020) 2(2): 54-58
https://doi.org/10.22271/2582-3744.2020.jun.54

Research article

Comparative studies of two medicinal plants: *Petiveria alliacea* L. and *Hilleria latifolia* (Lam.) H. Walter (Petiveriaceae) based on foliar anatomy

S. A. Odewo[1], O. E. Nwankwo[2]*, I. M. Adeniyi[1] and E. C. Odozie[1]

[1]Forest Research Institute of Nigeria, Ibadan, Nigeria
[2]Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria

*Corresponding Author: ephraimnwankwo8@gmail.com

Abstract: Comparative studies of *Petiveria alliacea* and *Hilleria latifolia* of the family Petiveriaceae were carried out to provide diagnostic features of the taxa based on foliar anatomy. Conventional practical methods as reported by previous authors of related studies were employed to carry out the studies. There was presence of irregular cell shape, undulate and straight to curved anticlinal walls with paracytic stomata on abaxial and adaxial surfaces of *P. alliacea* and *H. latifolia*. The two taxa differ with the presence of starch grains on the anticlinal cell wall of *H. latifolia*. The foliar anatomy of the two species (*H. latifolia* and *P. alliacea*) shows consistent and uniform concentric vascular bundle, irregular parenchyma cells, large and single central vascular bundle. The midrib portion of *H. latifolia* shows single vascular bundle in concentric shape, irregularly arranged parenchyma cells are common. The pores are solitary, round and numerous with large vessels. Dorsiventral orientations of the leaf with 2 layered palisade tissues are observed in *H. latifolia* while the midrib portion of *P. alliacea* shows a single vascular bundle in concentric shape, irregularly arranged parenchyma cells; pores are solitary round and numerous with large vessel. Leaf orientation of *P. alliacea* is isobilateral containing spongy parenchyma cells.

Keywords: Foliar anatomy, Petiveriaceae, Anticlinal walls, Paracytic stomata.

INTRODUCTION

Medicinal plants are very useful in traditional medicine within Africa. Africans rely on the plants for treatment of diseases. However, the continuity of plant application for drug development in any country only relies on identification, classification and nomenclature for better understanding. A review of the literature showed that there are still seemingly insurmountable problems of misidentification in many medicinal plant groups. Therefore, studying various parts of the plants for their true diagnostic features is necessary (Brinckmann, 2011; Howard *et al*., 2012).

The studies focused on *Petiveria alliacea* L. and *Hilleria latifolia* (Lam.) H. Walter of the family Petiveriaceae. Leaves of *Petiveria alliacea* are simple, alternate, elliptic nearly glabrous, attenuate, acute or obtuse at base and acute to acuminate. It has determinate inflorescences. Moreover, *Hilleria latifolia* is a herb up to 2 m tall, with some weak bristly hairs on young branches. Leaves alternate, simple and entire; stipules absent; petiole blade ovate or elliptical to broadly lanceolate and often unequal, apex long-acuminate. The uses of these plants have been reviewed by so many researchers (Hernández *et al.*, 2014). They have been reported to be used to eliminate bacteria, fungi, candida, and viruses thereby enhancing the immune system, increasing urination, lowering the blood sugar levels, elimination of cancer cells, arthritis, allergies and jaundice.

Anatomical and micro-morphological characteristics of leaves have played an important role in plant taxonomy, especially of particular groups at generic and specific levels. Plant morphologists and systematics use data obtained from macro-characters and foliar anatomy to resolve taxonomic problems in various plant (Kahraman & Celep, 2010). Although many studies have been carried out on the two taxa, there is no convincing and satisfactory study on the macromorphology and foliar anatomy of the taxa under study. Therefore, the study aimed at determining the macro-morphological and foliar anatomical features that would aid the proper identification and distinguishing the two species from each other.

MATERIALS AND METHODS

Specimen collection

The two species (*Petiveria alliacea* and *Hilleria latifolia*) were collected at Odofin-Agbegi village in Ikire Osun State, Nigeria (Fig. 1). The specimens were identified and authenticated at the Forest Herbarium, Ibadan (FHI), and Nigeria.

Submitted: 02 December 2019 Accepted: 15 April 2020 Published Online: 30 June 2020

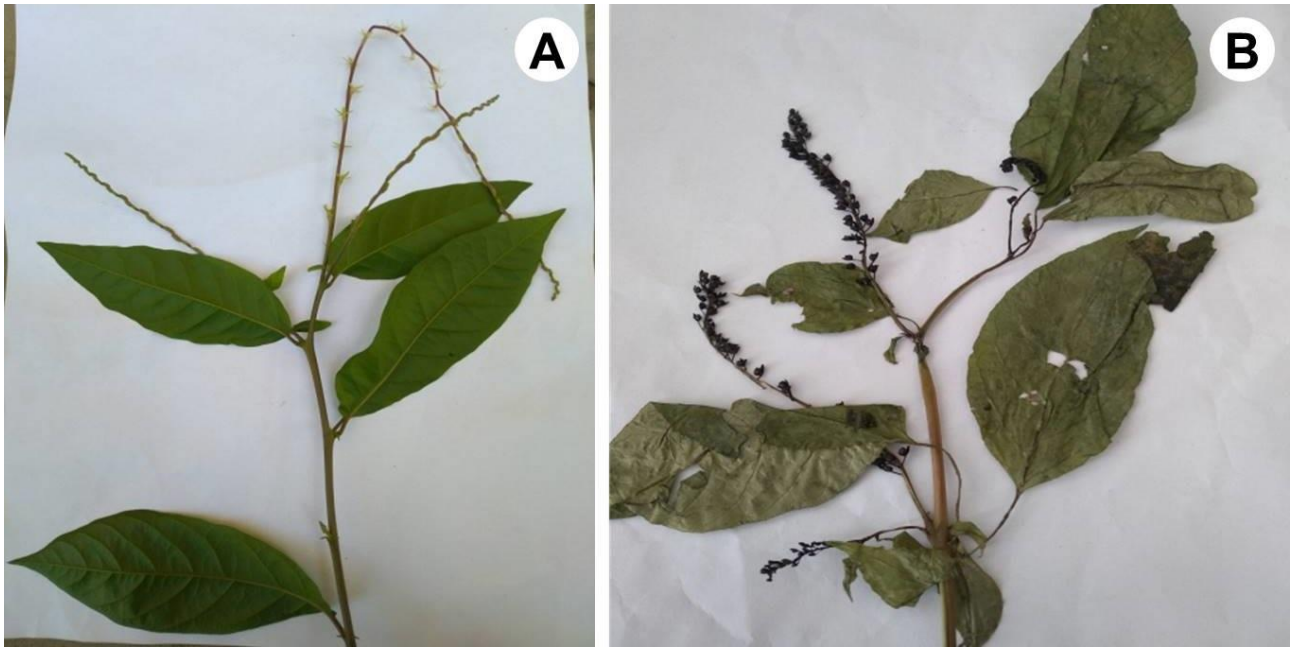

Figure 1. A, *Petiveria alliacea* L.; **B**, *Hilleria latifolia* (Lam.) H. Walter.

Surface tissue preparation

Epidermal preparations were obtained using the technique of Nwankwo & Ayodele (2017). Fresh plant specimens were used for the studies. Each sample was macerated in concentrated Trioxonitrate (V) acid for 2-4 hours. The sample was transferred to water in Petri-dish while adaxial and abaxial epidermises were carefully separated using forceps and dissecting needle. The inner parts (mesophyll tissue) of leaves were carefully cleared with a camel hair brush and the isolated epidermal layers were washed in several changes of water before transferring in 50% alcohol for 2 minutes to harden them. The tissue was then transferred to a clear glass microscopic slide and stained after draining off excess water, with safranin for less than 4 minutes and the excess stain was washed off using a dropping pipette to add and remove water from the tissue. They were later mounted in glycerin on a slide with the edge of the coverslip ringed with nail varnish to prevent dehydration and to seal the coverslips on the slides. The slides were labelled and viewed properly under light microscope. Photomicrographs were obtained at magnification of X400 using Olympus Biological microscope model ex31, fitted with Olympus E-330 digital SLR camera through E330- ADU 1.2 microscope adapter

Transverse section

Leaf samples of *Petiveria alliacea* and *Hilleria latifolia* were sectioned on a sliding microtome to about 20 micrometer thick. Leaf samples were embedded in paraffin wax before sectioning. Sections were washed with distilled water and covered with safranin stain for two minutes after which the sections were later washed with distilled water until the water became colourless. Dehydration was done by passing the sections through a series of the bath of increasing concentrations of ethanol which replaced water. The specimens were later covered with clove oil for 1 hour to drive off alcohol, and later placed on a clean slide, excess clove oil was drained off using filter paper; a slight amount of Canada balsam (a mounting medium and a synthetic substance) was added while the slide was cover with a cover glass and air bubbles were removed by applying heat gently. Measurement of vessels was done on the transverse sections using a stage micrometer and an eyepiece graticule.

RESULTS

The stomata characters of the selected species have been shown in table 1. The study revealed the absence of stomata on the adaxial surfaces of the two species studied. Qualitative features of the epidermal morphology and stomatal type of plants are recorded in table 2. The epidermal cells on the abaxial surface of *Petiveria alliacea* show irregular cell shape, undulate and curve anticlinal walls with paracytic stomata (Fig. 2), while on the abaxial surface of *Hilleria latifolia* shows irregular cell shape, straight to curved, undulate anticlinal walls with paracytic stomata (Fig. 3). Moreover, the adaxial surfaces of *Petiveria alliacea* indicates irregular cell shape, undulate and curve anticlinal walls while the adaxial surfaces of *Hilleria latifolia* show irregular cell shape, straight to curved anticlinal walls with starch grains. Stomata are absent in adaxial surfaces of the selected species (Table 2).

The photographs and photomicrographs of the plants and transverse section of leaf and mid-rib of *Hilleria latifolia* and *Petiveria alliacea* are shown in figures 4 & 5. The midrib portion of *Hilleria latifolia* shows single vascular bundle in concentric shape, irregularly arranged parenchyma cells are common. The pores are solitary, round and numerous

with large vessels. Dorsiventral orientations of the leaf with 2 layered palisade tissues are observed in *H. latifolia* while the midrib portion of *Petiveria alliacea* shows a single vascular bundle in concentric shape, irregularly arranged parenchyma cells; pores are solitary round and numerous with a large vessel. Leaf orientation of *P. alliacea* is isobilateral containing spongy parenchyma cells.

Table 1. Stomata characters of *Petiveria alliacea* L. and *Hilleria latifolia* (Lam.) H. Walter.

Species	Stomatal length (µm)		Stomatal width (µm)		Number of stomatal per view	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Petiveria alliacea	13.08 (3.22±1.02)	AB	8.61 (1.47±0.47)	AB	11.50 (2.69±0.85)	AB
Hilleria latifolia	15.02 (1.21±0.38)	AB	8.43 (6.22±1.97)	AB	11.7 (1.42±0.44)	AB

Table 2. Qualitative micro-characters and stomata type of *Petiveria alliacea* L. and *Hilleria latifolia* (Lam.) H. Walter.

Species	Stomatal type		Shape of epidermal cell		Anticlinal cell wall	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Petiveria alliacea	Paracytic	Absent	Irregular cell shape	Irregular cell shape	Undulate and curve anticlinal walls	Undulate and curve anticlinal walls
Hilleria latifolia	Paracytic	Absent	Irregular cell shape	Irregular cell shape	Straight to curved, undulate anticlinal walls	Straight to curved anticlinal walls with starch grains

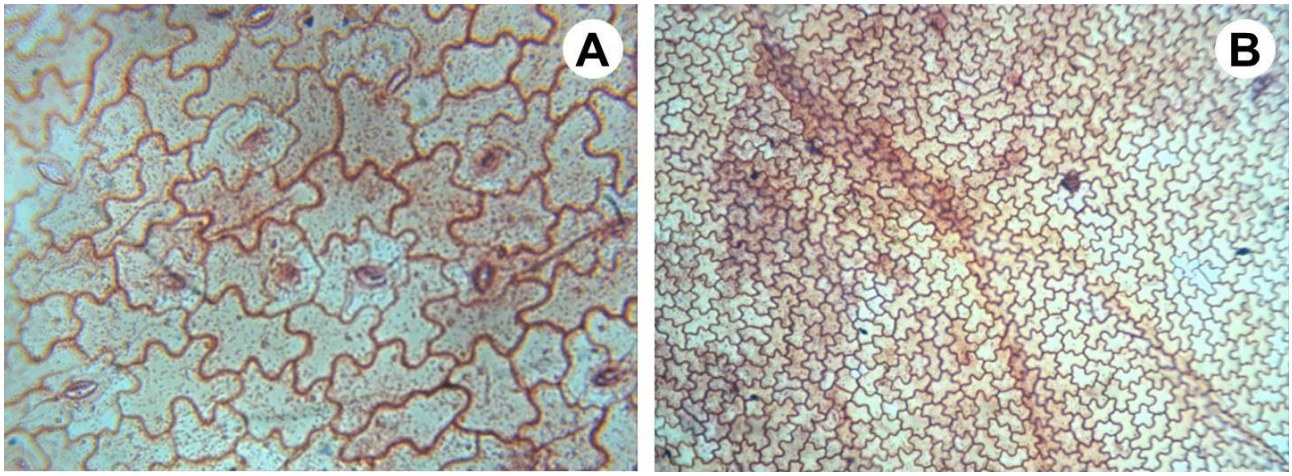

Figure 2. Photomicrograph of leaf surface of *Petiveria alliacea* L. (X400):**A**, Abaxial surface showing irregular cell shape, undulate and curve anticlinal walls and paracytic stomata; **B**, Adaxial surface showing irregular cell shape, undulate and curve anticlinal walls, absent stomata.

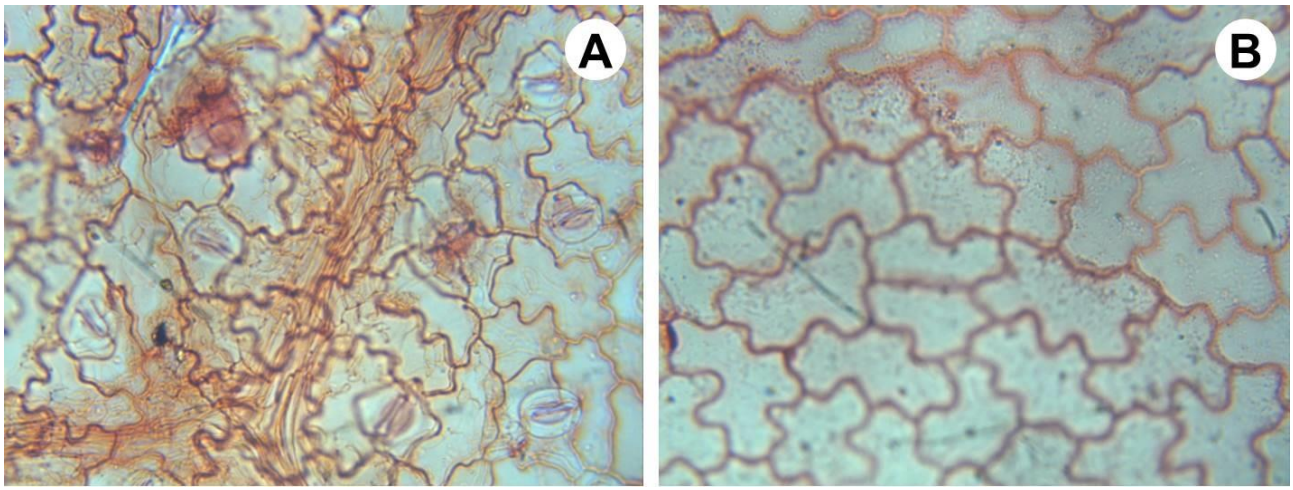

Figure 3. Photomicrograph of leaf surface of *Hilleria latifolia* (Lam.) H. Walter. (X 400): A, Abaxial surface showing irregular cell shape, straight to curved, undulate anticlinal walls and paracytic stomata; **B**, Adaxial surface *showing* irregular cell shape, straight to curved anticlinal walls with starch grains.

DISCUSSION

The presence of paracytic stomata and the absence of stomata on the adaxial surfaces of the two species and epidermal cell shape confirm the similarity occurring between the studied species. Besides, the stomata sizes of the two

species are relatively close. Furthermore, the anticlinal cell walls (straight to curve with starch grains) in *Hilleria latifolia*, undulate and curve in *Petiveria alliacea* revealed clear variation in both adaxial and abaxial surfaces, all of which could be employed in species identification. The leaf anatomy of the two species (*Hilleria latifolia* and *Petiveria alliacea*) show consistent and uniform concentric vascular bundle, irregular parenchyma cells, large and single central vascular bundle while the characteristics such as solitary vessels, dorsiventral orientation of the leaf (*Hilleria latifolia*), isobilateral orientation of the leaf delimit the taxa from each other. According to Ahmed (1976) and Stace (1965), size, distribution and frequency of stomata are important parameters in taxonomy and phylogeny of green plants.

Ayodele & Olowokudejo (1997) also reported that straight or curved walls are characteristic of species grow in drier conditions while undulate walls are found mostly in species growing in areas of high humidity. Ohewandamilo & Akinriulade (1991) also emphasized that the systematic value of epidermal characters vary from group of plants to another.

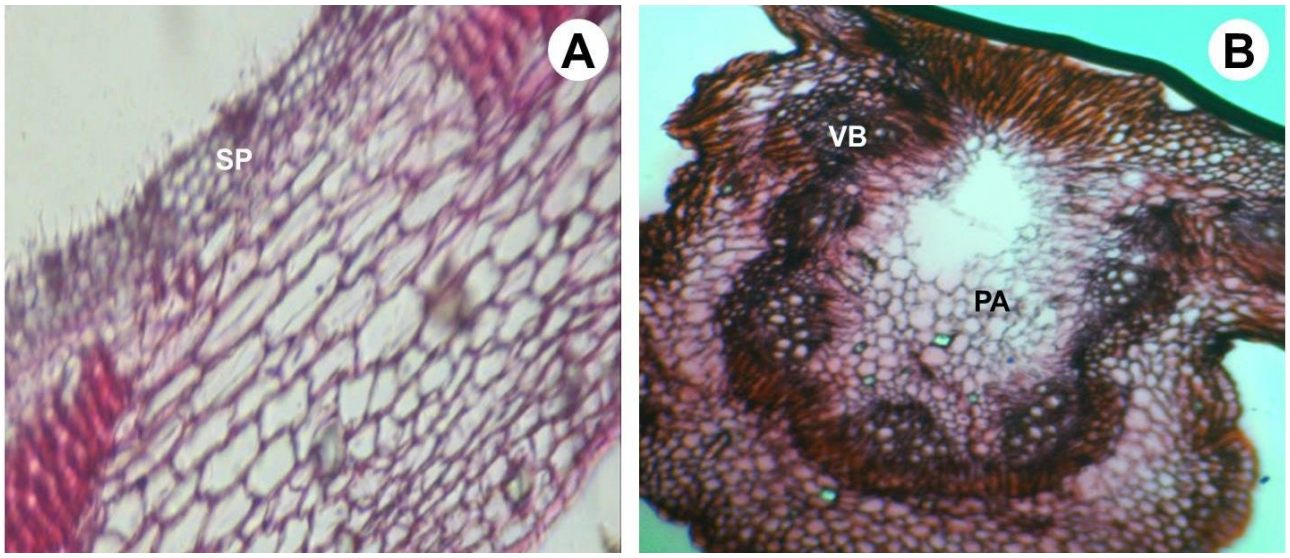

Figure 4. Photomicrograph of transverse section of leaf and mid-rib of *Petiveria alliacea* L. (X400): **A**, Transverse section of the leaf (SP- Spongy parenchyma cells); **B**, Transverse section of the mid-rib (VB- Vascular bundle; PA- Parenchyma cells).

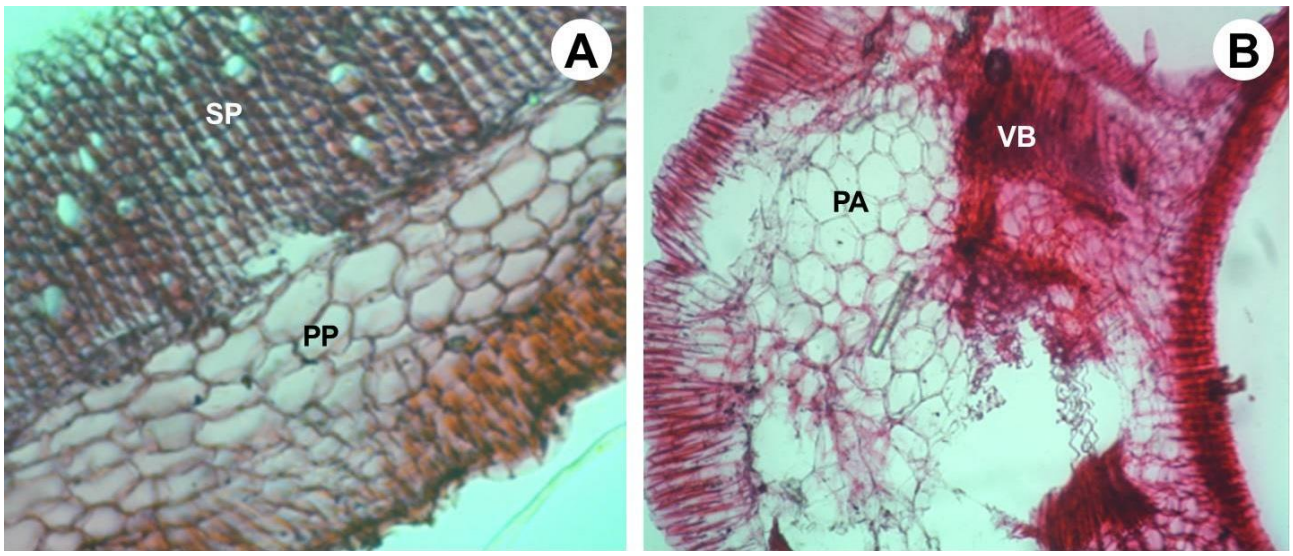

Figure 5. Photomicrograph of transverse section of leaf and mid-rib of *Hilleria latifolia* (Lam.) H. Walter. (X 400) **A**, Transverse section of the leaf (SP- Spongy parenchyma cells; PP- Palisade parenchyma cells); **B**, Transverse section of the mid-rib (VB- Vascular bundle; PA- Parenchyma cell).

CONCLUSION

However proper identification of medicinal plants is very crucial to have effective sustainability and utilization as well as for detecting adulteration of plant drugs. Thus, micro-morphological character variations observed in the study had provided the basis for the identification of these plants highlighting their taxonomic relationships. The morpho-anatomical features observed in the two medicinal plants are sufficiently distinctive and may be used at a specific level for the delimitation of plants. The different features peculiar to each of the medicinal plants provide the basis for its

detailed assessment and form part of the catalogue of characters that may be employed in the proper identification of the plants and as quality control standards.

There is, therefore, the need to use other taxonomic tools such as cytology, genetics, molecular and palynology to ascertain the authenticity of crude drug samples that would be generated from species of study.

ACKNOWLEDGEMENTS

I use this medium to acknowledge and appreciate my project colleagues, Nwankwo, O. E and Odozie, E. C.for their assistance in the collection and identification of the plant species. The effort of Adeniyi, I. M. in proofreading the article can never be forgotten. I am highly indebted to them.

REFERENCES

Ahmed K.A. (1976). Epidermal studies in some species of *Hygrophila* and *Dyschoriste* (Acanthaceae). *Journal of Indian Botanical Science*, 55: 48-52.

Ayodele A.E. & Olowokudejo J.D. (1997). Systematic importance of leaf epidermal characters in West Africa species of family Myrtaceae. *Boletim da Sociedade Broteriana (Portugal)*, 68: 35-728.

Brinckmann J. (2011). Reproducible efficacy and safety depend on reproducible quality: matching the various quality standards that have been established for botanical ingredients with their intended uses in cosmetics, dietary supplements, foods, and medicines. Herbal Gram, 91: 40-55.

Hernández J.F., Urueña C.P., Cifuentes M.C., Sandoval T.A., Pombo L.M., Castañeda D., Asea A. & Fiorentino S. (2014). A Petiveria alliaceae standardized fraction induces breast adenocarcinoma cell death by modulating glycolytic metabolism. Journal of Ethnopharmacology, 153(3): 641-649.

Howard C., Socratous E., Williams S., Graham E., Fowler M.R. & Scott N.W. (2012). Plant ID-DNA-based identification of multiple medicinal plants in complex mixtures. Chinese Medicine, 2: 7-18.

Kahraman A. & Celep F. (2010). Anatomical properties of *Colchicum kurdicum* (Bornm) Stef. (Colchicaceae). *Australian Journal Crop Science*, 4(5): 369-371.

Nwankwo O.E. & Ayodele A.E. (2017). Taxonomic Studies of the genus *Indigofera* Linn., in Nigeria, *International Digital Organisation Journal for Scientific Research*, 2(3): 10-26.

Ohewandamilo T. & Akinriulade O. (1991). Epidermal micromorphology of some species of *Albizia* Duraz (Mimosaceae). *Phytomorphology*, 48: 325-335.

Stace C.A. (1965). The taxonomy importance of leaf surface current concept of plant taxonomy. Bulletin of British Museun (Natural History) Botany, 4: 3-7.