

FOLIAR MICRO-MORPHOLOGY AND PHYTOCHEMICAL STATUS OF *LEEA GUINEENSIS* G. DON LEAVES

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Abstract: *Leea guineensis* G. Don leaves are known to contain some active compounds that certify its usage as a medicinal plant. The establishment of a comprehensive pharmacognostic profile of *L. guineensis* leaves will help in the standardization of quality and proper identification. Evaluation of the fresh and powdered leaves was carried out using standard methods to determine the macro-morphological, micro-morphological (both the qualitative and quantitative), chemo-microscopic and phytochemical profiles. The result obtained shows that macroscopically, the leaf was simple, opposite and entire in shape, having a cylindrical and undulating edge with a hard and smooth texture. The internodes are short with a spot of pink colour at the interval nodes (nodules). It has a simple trunk without thorns with a granular fracture surface. The colour is pale green when young and deep green at maturity. Microscopically, the stomata were paracytic on the abaxial and absent on the adaxial. The epidermal cells are irregular and rectangular, the epidermis possesses straight anticlinal walls and is slightly undulating both on the abaxial and adaxial epidermis. Trichomes are not present on both epidermises, while crystals are present on the abaxial epidermis. The stomata index (18.87%) was calculated for abaxial, the mean cell length (43.46 and 43.89) and width (30.50 and 31.61) was comparably similar for both abaxial and adaxial epidermis respectively. The number of stomata detected on the abaxial was 20. Chemo-microscopic characters present include Starch, Calcium carbonate crystals while lignin, fat, and mucilage were absent while phytochemical screening revealed that alkaloid, saponin, flavonoids, tannin, phenolics and anthraquinone were present. The foliar micro-morphological findings are of great importance in the proper and correct identification, standardization, and authentication of medicinal plants.

Keywords: chemo-microscopic, *Leea guineensis*, macroscopic, micromorphology, pharmacognostic.

Introduction

The foliar epidermal profile is an important taxonomic character that assists in understudying the leaf epidermis. The epidermal cells, trichomes, stomata, stomata frequency, cell length, cell sizes, distribution, and orientation are important in taxonomy [ALBERT & SHARMA, 2013]. Medicinal plants produce some secondary metabolites that are capable of being stored in different plant organs including leaves, stems, and roots. The outer cell layer of the plants also contains hairs that are functionally classified as glandular or non-glandular trichomes. *Leea guineensis* is a small tree or a shrub that belongs to the *Leeaceae* family. It grows up to 450-610 cm high [MUHAMMAD & AJIBOYE, 2010]. It originates from tropical Africa, distributed throughout Northern and Eastern Australia, New Guinea, South, and Southeast Asia and parts of Africa. It is mainly propagated through stem cutting or by seed. It germinates in 14-21 days at 21°C. In Yoruba language, it is called Alugbokita, while in Twi language, it is called Okatakya [MUHAMMAD & AJIBOYE, 2010]. It grows in the humid places found in the forest region, moister woodlands and forest area of tropical Africa. Traditionally, it is used in the treatment of diverse ailments. *L.*

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guineensis leaves are usually effective in treating toothache, skin ulcers, paralysis, vertigo, rheumatism and epileptic fits [MSHANA & al. 2000]. Also, they are used in pregnancy detection, purgative, general weakness, skin rash, gonorrhoea, convulsions, stomach troubles, boils and swollen spleen in children [HASSAN & ABD EL-RAZEK, 2011; BURKILL, 1985; MSHANA & al. 2000; MOLINA, 2009]. The micro-morphological feature gives detailed information to assist in a proper investigation of varieties of species, solving problems in evolutionary relationships [SEGARRA & MATEU, 2002]. Micro-morphological characteristics of plants are usually expressed in leaves, stems, roots, and bark of plants and it is always important in plant taxonomy avoiding taxonomic conflict in different species of the plant [SONIBARE & al. 2014]. Hence, the dire need to explore the proper identification, pharmacognostic and phytochemical status of *Leea guineensis*.

Materials and methods

Plant collection

Fresh healthy leaves of *Leea guineensis* were collected from the arboretum of the Forestry Research Institute of Nigeria, Ibadan, Oyo State. The samples were identified at the taxonomy unit of the institute and a voucher specimen (FHI 112460) was deposited at the Forest Herbarium Ibadan. The leaves sample were air-dried, powdered and stored in an appropriate container until required for use.

Variable assessed

The fresh leaves were used for qualitative and quantitative micro-morphology evaluation using a light microscope [EVANS, 2005; BRAIN & TURNER, 1975], macroscopic features [BRAIN & TURNER, 1975], chemo-microscopic examination [EVANS, 1996] and phytochemical status [BOYE & al. 2012; OMORUYI & al. 2012] of *Leea guineensis*.

Microscopic evaluation

This involves the description of the different microscopic characters of the plant such as leaf content, cell length, and width, stomata, trichomes, etc. [RADFORD & al. 1974; KHATIJAH & ZAHARINA, 1998; ADEDEJI, 2004; METCALFE & CHALK, 2004].

Epidermal section (ES) preparations using a light microscope

The leaf samples were soaked in concentrated (HNO_3) after being cut into reasonable portions. They are soaked in well-covered Petri dishes for about two hours depending on the leaf, in order to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were carefully peeled off with forceps and a fine camel hairbrush. The peeled layers were put into a clean Petri dish containing distilled water and later put into another Petri dish containing 2 ml of ethanol for 1-2 minutes to allow the hardening of cells. Afterwards, the tissues were stained with safranin (red stain), then removed and dipped into another Petri dish containing distilled water to remove excess staining. The tissue is then mounted on a microscopic slide, after that, a drop of glycerol was dropped on the tissue, then it was covered with coverslips, nail varnish was used as a sealant to protect the edges from dehydration and damage. The microscopic slides were labeled appropriately and then viewed under a light microscope. [RADFORD & al. 1974; KHATIJAH & ZAHARINA, 1998; ADEDEJI, 2004; METCALFE & CHALK, 2004; EVANS, 2005; BRAIN & TURNER, 1975].

The stomata index was analyzed using the formula below:

$$I = \frac{S}{E+S} \times 100$$

Where I – Stomata Index

S – No of Stomata per unit area

E – No of epidermal cells in the same unit area [SALISBURY, 1927].

Transverse section (TS) preparations

Anatomical sections of the fresh leaf, stem, and root were prepared using standard laboratory techniques. The transverse sections were cut with the aid of a sledge micrometer. The leaf blade that was cut, was then stained in a staining jar for 5 minutes. Distilled water was used to rinse the outer cell layer removed, followed by ethanol and it was stained again, finally being washed with absolute ethanol. It was put into a container containing 50/50 alcohol/xylene and rinsed vigorously until it was clear. The sections were cleared with chloral hydrate solution and the tissue was mounted on a slide with a drop of dilute glycerin. [EVANS, 2005; BRAIN & TURNER, 1975].

Macroscopic evaluation

The macroscopic features of the leaf and organoleptic properties like taste, odour, and colour were described according to the standard botanical method of BRAIN & TURNER (1975).

Chemo microscopic evaluation

The powdered leaf sample was cleared in a solution of chloral hydrate to remove chlorophyll; then, the cleared powdered leaf sample was mounted on the microscopic slides and observed under a compound microscope to reveal chemical substances like lignin, starch, calcium oxalate, calcium carbonate, fats and oil and mucilage [EVANS, 1996]

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 shows that starch is present.

Calcium oxalate crystals test

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

Calcium carbonate test

The powdered plant containing the chloral hydrate solution was mounted on a microscopic slide, after which 1-2 drops of the acetic acid solution were added. The evolution of gas reveals 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 placed on the slide and Ruthenium red (a drop) was added, a pink coloration shows the presence of mucilage.

Phytochemical screening

The phytochemical screening of *Leea guineensis* leaves was carried out as described by standard analytical methods of OMORUYI & al. (2012), BOYE & al. (2012). The phytochemicals to be detected include alkaloids, phenolics, saponin, flavonoid, cardiac glycosides, tannin, and anthraquinone.

Results

Microscopic examination

The results of the microscopic examination of *Leea guineensis* are expressed in Table 1. They show that the epidermal leaf shape is irregular, rectangular to polygonal and slightly undulating on both the lower and upper epidermis. It has a straight anticlinal wall. There is a presence of paracytic stomata on the lower epidermis and absent on the upper epidermis (Figure 2E). Trichomes are absent on both epidermises. Meanwhile, the cell length for the lower and upper epidermis is (43.46 and 43.89) respectively, while the cell width for the lower and upper epidermis is (30.50 and 31.61) respectively. The cell density for both epidermises is (86.0 and 64.0). Stomata length and width were calculated only for the lower epidermis alone, while the mean stomata number is 20. The stomata index is 18.87%, and it is calculated for the abaxial epidermis.

Table 1. Epidermal characters and their qualitative and quantitative descriptions

Epidermal features	Characters	
	Lower epidermis (abaxial)	Upper epidermis (adaxial)
Cells		
Shape	Rectangular to polygonal and slightly undulating	Rectangular to polygonal and slightly undulating
Anticlinal walls	Straight and irregular	Irregular
Cuticle	Present	Present
Mean length (µm)	43.46	43.89
Mean width (µm)	30.50	31.61
Density (µm)	86.0	64.0
Stomata		
Type	Paracytic	Absent
Frequency	Numerous	Absent
Stomata length (µm)	16.06	Absent
Stomata width (µm)	7.0	Absent
Mean stomata	20	Absent
Stomata index (%)	18.87%	Absent
Trichomes		
Type	Absent	Absent

Macroscopic examination

The macroscopic and organoleptic features of *Leea guineensis* fresh leaves are expressed in Table 2. The result shows that the leaf has a light green color when young and

deep green at maturity. It has a faint odor and a bitter taste. The leaf is small, cylindrical and has undulating edges. The arrangement of the leaf is in opposition. The fractured surface is non-glandular and the petiole is short. The leaf (Figure 1) is simple, opposite and entire. The venation is pinnate and its margins are smooth, apex of the leaf is acute, while the base is equal. It has a slightly hard and smooth texture with a smooth surface and spot of pink nodules. The internodes are short.



Figure 1. *Leea guineensis* leaves

Table 2. Macroscopic and organoleptic characters of the leaf of *Leea guineensis*

Features	Descriptions
<i>Leaf shape</i>	Small, cylindrical and undulating edges
<i>Arrangement</i>	Opposite
<i>Fractured surface</i>	Granular
<i>Petiole</i>	Short
<i>Lamina</i>	
Composition	Simple, opposite and entire
Venation	Pinnate
Margin	Smooth
Apex	Acute
Base	Equal
Texture	Slightly hard and smooth
Surface	Smooth with spot of pink nodules
Internode	Short
<i>Organoleptic properties</i>	
Color	Light green
Odor	Faint
Taste	Bitter

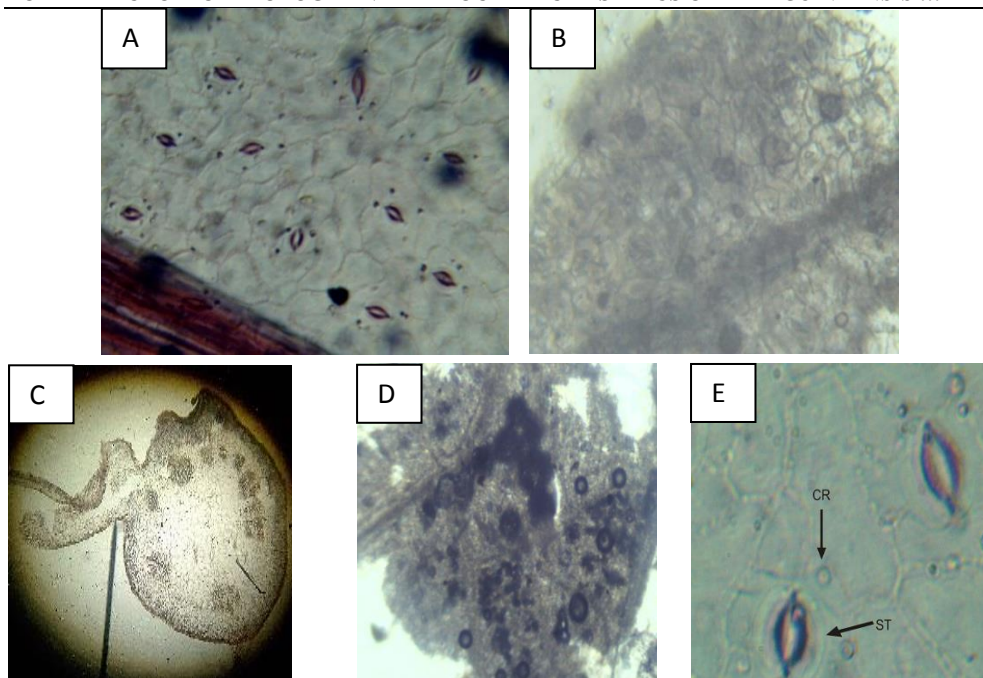


Figure 2. Leaf Micrograph of the leaves of *Leea guineensis*. (A) Leaf clearing showing secretory cavities and epidermal cells of the abaxial surface. (B) Stained Leaf clearing showing epidermal cells and starch grains. (C) Anatomical overview of the leaf blade. (D) Stained powdered leaf showing starch grains (sg). (E) Leaf clearing showing paracytic stomata and calcium carbonate crystals (cr) on the lower epidermis

Chemo-microscopic evaluation

The chemo-microscopic evaluation of the powdered leaves sample of *Leea guineensis* in Table 3. Shows that lignin, fats, mucilage and calcium oxalates are absent in the epidermal layer of the plant while starch, calcium carbonate and crystals are present.

Table 3. Chemo microscopic evaluation of the powdered leaves sample

Parameter	Observation	Result
Lignin	No red colouration observed	-
Starch grains	A blue colouration	+
Fats	No pink colouration	-
Calcium oxalate crystals	No effervescence	-
Calcium carbonate	Effervescence	+
Mucilage	No pink colouration	-
Crystals	A blue colouration observed	+

Phytochemical screening

Table 4 presents the results of the preliminary phytochemical screening of *L. guineensis* leaves. The results reveal that all the phytochemicals examined which are alkaloids, saponin, flavonoid, tannin, phenolics, anthraquinone and cardiac glycosides are present except steroids and

phlobatanins. The phytochemicals present in *L. guineensis* leaves suggests that the plant is of high medicinal value and could be used in the management and treatment of various diseases.

Table 4. Phytochemical screening of *Leea guineensis* leaves

Phytochemicals	<i>Leea guineensis</i>	Remarks
Alkaloids	+	Present
Saponin	+	Present
Flavonoid	+	Present
Tannin	+	Present
Phenolics	+	Present
Anthraquinone	+	Present
Cardiac glycosides	+	Present
Steroids	-	Absent
Phlobatanins	-	Absent

Discussions

Wrong identification of medicinal plants usually creates a big obstacle in the use and the misuse of herbal drugs and medicines. It is a fact that medicinal plants' therapeutic potency depends on its mode of identification. However, complete acceptance of herbal alternative medicines is still facing some obstacles as a result of the dearth of proper documentation as well as appropriate standardization and quality control processes. Thus, proper identification of the medicinal plant which is used for various medical implications is very imperative to ensure its chemical components and its pharmacognostic details.

Foliar microscopic description

The qualitative and quantitative leaf micro-morphological characteristics of the epidermal cells of *Leea guineensis* are summarized in Table 1. The photomicrographs of the light microscope of *Leea guineensis* leaf surfaces revealed a paracytic type of stomata. In paracytic (parallel-celled) type of stomata, the secretory cavity is always accompanied by one or more guard cells on both sides. They are widely distributed on the abaxial epidermis and are absent on the adaxial epidermis. The result obtained for stomata index on the abaxial is 18.87%. The stomata are arranged randomly on the lower epidermis and are very obvious. The stomata length and width could not be calculated because the lower epidermis are tightly closed and appear very small at magnification x10. Stomata are secretory cavities usually located on the lower epidermis which allow the exchange of water vapour, oxygen and carbon dioxide. The stomata opening increases rate of transpiration which increases water absorption and nutrient, mostly at night, the stomata are closed and often transpiration rate drops, consequently plant nutrient and water intake reduces. The stomata are surrounded by guard cells with some intercellular spaces. The stomata exist only on the lower epidermis. The outer layer cells are medium sized having an irregular cell on both surface of the plant epidermis. The leaf possesses straight anticlinal walls and are slightly undulating on both the epidermis. Trichomes are not evident both on the abaxial and adaxial epidermis of *Leea guineensis*. Micro-crystals are present on the lower epidermis and absent on the upper epidermis. This aligns with the result reported by METCALFE & CHALK (2004). Further epidermal research findings reveal that the mean average number of cells ranged from 64.0 μm on the adaxial epidermis to 86.0 μm on the abaxial epidermis. The stomata length and width are (16.06 μm and 7.0 μm) respectively. While the cell length (43.46 μm) for abaxial have almost the same value with the cell length (43.89 μm) on the adaxial and also the cell width (30.50

µm) on the abaxial epidermis is a bit lower than the cell width (31.61 µm) of the adaxial epidermis. The stomata number recorded for abaxial is 20.

Macroscopic evaluation

The macroscopic features of *Leea guineensis* showed that the leaves are opposite, simple and entire. The color is pale green when young and deep green at maturity with an odorless smell and bitter taste. The internodes are short, smooth with spot of pink at each node. The bark is rough and the texture is hard and smooth. The shape is cylindrical and are undulating at the edges. The fractured surface is granular, evident, trunk hard and brittle when dry.

Chemo microscopic evaluation

Crystal deposits were detected on the surface of the leaves Figure 1E. The crystals deposit on the leaf usually gives it anti-herbivory features, meanwhile, the crystals act as a defence mechanism on the epidermal surface of the leaves of many plant species [ASHAFA & al. 2008]. Crystals play important role in the cellular ion balance and the rigidity of the tissue. Also it helps in the detoxification of dangerous metals [OTANG & al. 2014].

Phytochemical composition

Medicinal plants always contain some active substances that have been widely reported to contribute their metabolic, physiologic and protective effects to humans [EDEOGA & ERIATA, 2001]. The result obtained in this study corroborates the one reported for *Leea guineensis* leaves by AWOTEDU & al. (2019), except for the absence of steroids and phlobatanins. It is also in consonance with the one reported for other plants [OMOTAYO & BOROKINI, 2012; OYEYEMI & al. 2014]. Saponin produced by plant always fight infections produced by parasites. When saponin is taken by human beings, it helps the body system to fight against viruses and bacteria. However, the occurrence of saponin in this study suggest it for use in fighting against infections and recommending it for soap making properties because of its foamy abilities. Alkaloids have been known to have antihypertensive, anti-inflammatory, antifungal, antifibrogenic and microbiocidal effect [GHOSHAL & al. 1996]. Alkaloid present in this study also aligns with the work reported by AWOYINKA & al. (2007) who also reveals that alkaloid is present in *Cnidocolus aconitifolius*. Alkaloids are beneficial chemicals to plants, serving as repellent to predators and parasites. Tannins is reported to serve as antidotes for many poisons [NORTON, 2000], antibacterial [AKIYAMA & al. 2001], anti-parasitic [KOLODYIEJ & KIDERLEN, 2005] and also it can help in the protection of the kidney. Hence, the presence of tannin in this study makes the plant a useful source of antidotes for poisons and serve as immediate relief for people with sore throat, diarrhea and dysentery and wounds [OKWU, 2004]. In this study, flavonoid is present in the leaves of *Leea guineensis*, this agrees with that reported by AKUBUGWO & al. (2007) for *A. hybridus*. Presence of flavonoids in plants generally serve as flavouring agents [KUJUMGIEV & al. 1999]. Availability of all the secondary metabolites suggest that *Leea guineensis* is an important herbal drug that can be used in folkloric medicine.

Conclusion

The information obtained from this study can serve as a proper guide for the exact identification and authentication. Besides identification and authentication, micro-morphological and macro-morphological evaluation of the leaves could provide a rich information about certain plants physiological performances. The quantitative determination

of some diagnostic features is useful for setting proper standard in comparing and differentiating closely related plant species.

Acknowledgement

Special thanks to Mr. Adeniyi of Forest Product and Utilization department for his great and unquantifiable contribution he made towards the success of this work.

Notes on Contributors

Olamilekan Lanre AWOTEDU is a plant physiologist and biochemist, a PhD student and a senior research fellow with special interest in plant physiology, biochemistry, phytochemistry and ethnobotany.

Paul Oluwatimilehin OGUNBAMOWO is an environmental chemist and a biochemist, a PhD student and a senior research fellow with special interest in phytochemistry and analytical chemistry.

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How to cite this article:

AWOTEDU O. L. & OGUNBAMOWO P. O. 2019. Foliar micro-morphology and phytochemical status of *Leea guineensis* G. Don leaves. *J. Plant Develop.* **26**: 13-22. <https://doi.org/10.33628/jpd.2019.26.1.13>
