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Phytopharmacognostic Evaluation of the Leaves OF *Gnetum africanum* Welw (Gnetaceae)

Romanus A. Umoh[1*], Uwemedimo F. Umoh[1], Imoh I. Johnny[1], Omodot T. Umoh[2], Victor U. Anah[3], Anwanabasi E. Udoh[4], Akwaowoh A.Elijah[5], Moses A. Adefabi[1] and Etido A. Matthew[1]

[1]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.
[2]Department of Botany and Ecological Studies, Faculty of Sciences, University of Uyo, Akwa Ibom State, Nigeria.
[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.
[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria.
[5]Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RAU and IIJ designed the study, performed the experimental procedures, statistical analysis, author RAU wrote the first draft of the manuscript. Authors RAU and IIJ supervised lab experiments. Authors UFU, OTU, VUA, AEU, AAE, MAA and EAM organized data, managed the literature searches, assisted in plant material preparation. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Gnetum africanum* Welw (Gnetaceae) also called African salad and Afang in Ibibio language is an evergreen, perennial, shade-tolerant vine with woody stems which can climb up to 12m or more from a tuberous root-stock. It has culinary and medicinal importance.

**Corresponding author: E-mail: romaumoh2008@yahoo.com;*

Objective: The aim of this study was to evaluate pharmacognostic parameters of *Gnetum africanum*.
Methods: The leaves were identified, collected, air-dried, pulverized, weighed and subjected to the evaluation of its microscopy, micromeritics, chemomicroscopy, fluorescence, soluble extractive values, moisture content and ash values using standard procedures.
Results: The results obtained from microscopy revealed that the leaf has brachyparacytic, stomata, 3-5 armed and stellate trichromes on the abaxial surface. The epidermal cell wall pattern was undulate on the abaxial surface and sinuous on the adaxial surface. Stomatal number was found to be 3.1 ± 0.25 on the abaxial surface and Stomatal index was found to be 16.8% on the abaxial surface. The micromeritics analysis of the leaf powder revealed passable flow with the angle of repose of 42^0. The result of chemomicroscopy of the leaf revealed the presence of mucilage, lignin, calcium oxalate crystals, starch and oil. For water-soluble extractive value, the result was 13.25%$^w/_w$, methanol-soluble extractive value 4.25%$^w/_w$, ethanol-soluble extractive value 4%$^w/_w$, moisture content 10.5%$^w/_w$, total ash value was 5%$^w/_w$, acid-insoluble ash value 1%$^w/_w$, water-soluble ash value 2%$^w/_w$ and sulfated- ash value 6% $^w/_w$.
Conclusion: The results obtained from the pharmacognostic study provides information for the identity, quality and purity of *Gnetum africanum.*

Keywords: Chemomicroscopy; fluorescence; Gnetum africanum; micromeritics; microscopy; phytopharmacognostic.

1. INTRODUCTION

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours, fragrances and medicines [1]. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The global market value of medicinal plant products exceeds $10 billion per annum [2]. The use of medicinal plant has attained a commanding role in health care system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions [3].

Traditional medicine continues to play an important role in improving and maintaining health in developing countries [4]. WHO defines traditional medicine as diverse health practices, approaches, knowledge and beliefs incorporating plants, animals, and/or minerals based medicines, spiritual therapies, manual techniques and exercises applied similarly or in combination to maintain well-being as well as to treat, diagnose or prevent illness [5]. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Eventually information regarding medicinal plants was recorded in herbal pharmacopoeias [6].

Gnetum africanum Welw. is traditionally a wild vine and is considered to be a wild vegetable [7]. It is a perennial plant that grows approximately 12 meters long with thick-papery like leaves growing in groups of three. The leaves grow to approximately 8cm long and at maturity the vine will produce small core-like reproductive structures. The seeds of the vine resembled a fleshy fruit, sized 10-15mm x 4-8mm and are red-orange in colour when fully ripe [8]. It is a plant of humid, lowland rainforests, being found at elevations from sea level to 1,200 meters. It grows best in areas where the annual rainfall is around 3,000mm, succeeds in dappled shade in the woodland. Plants growing in full sun produce thinner leaves which are not so well liked by consumers. Cultivated plants need to be given some support, such as a tree, to grow on. *Gnetum africanum* leaves are decussately opposite, sometimes whorls of 3, simple, stipules absent, petiole up to 1cm long, canaliculated above, blade ovate-oblong to elliptical-oblong, rarely lanceolate, 5-14cm x2-5cm, base attenuate, apex abruptly acuminate, entire margin, thick-papery, glabrous, pale green above, paler beneath, with 3-6 pairs of strongly curved lateral veins looped near the margin. Inflorescence is an unbranched catkin, axillary or terminal on a short branch, solitary but male inflorescences occur at apex of branches often in groups of 3 up to 8cm long, jointed, peduncle 1-1.5m long, with a pair of scale-like triangular bracts male inflorescence with slender internodes and whorls of flowers at nodes. Flowers small, 2mm long, with moniliform hairs at base and an envelope, male flowers with a tubular envelope and exerted stamina column

bearing 2 anthers, female flowers with cupilar envelope and naked, sessile ovule.

Some studies have shown that the leaf extract is high in antimicrobial activity justifying its use as an antimicrobial agent [8]. Report was published on the lowest minimum inhibitory concentration (MIC) of (62.5mg/mL) was found in aqueous extract against *Staphylococcus aureus*. (125mg/mL) was recorded against *Escherichia coli* exhibited by methanol extract and also (125mg/mL) was recorded against *Penicillium chrysogenum* exhibited by ethanol extract. The efficacy of the extracts is probably due to the identified secondary metabolites such as tannins, alkaloids, saponins, glycosides and steroids. Moreover, the plant can be used to source for oral antibacterial drugs that can treat infection caused by susceptible gram-negative and gram-positive bacteria. The data obtained from a study [9] indicated that the plant possessed antifungal potential and could be used as natural fungicides. Leaves-raw or cooked wherever it occurs in Africa is valued as a tasty vegetable usually eaten finely shredded for addition to soup, stew or made up into condiments, or even eaten raw as local salad. To soften this tough vegetable, people often mix it with *Talinum triangulare (*Jacq*.)* Willd. Shredded leaves can be dried and preserved for later use. It is also a good source of protein and vitamins [9, 10, 11].

It is a very common practice that herbal products are used more in recent times due to their affordability, accessibility and acceptability, there is need for proper identification of these crude drugs. Thus, the investigation of *G. africanum* is necessary to establish proper identity of the plant which would be useful in the preparation of its monograph.

1.1 Phylogeny of *Gnetum africanum* (Scientific Classification) According to Angiosperm Phylogeny Group (APG) System [12]

Kingdom - Plantae
Clade - Tracheophytes
Clade - Gymnosperm
Order - Gnetales
Family - Gnetaceae
Genus - *Gnetum*
Species - *G. africanum* Welw
Common name - African salad
Local name - Ibibio/Efik-Afang, Igbo-Ukazi

2. MATERIALS AND METHODS

2.1 Identification and Collection of Plant

The leaves of this plant were collected from Obio Offot off Abak Road, Uyo, Akwa Ibom State in June 2019. The plant was identified in the faculty of Pharmacy, University of Uyo herbarium and deposited with Herbarium number – UUPH 32(a). The fresh plant material was air-dried, pulverized and packed in a dry container, well labeled and used when needed.

3. ANATOMICAL STUDIES

3.1 Microscopic Evaluation of Leaf

Matured fresh leaves of the plant were cut at the petiole about 1mm from the node. Microscopical examinations of the Epidermis of both adaxial and abaxial surfaces were made by placing the leaf on a glass slide. The sample was irrigated with water and scraped gently with a sharp razor blade till loose cells from the epidermis were washed away with water and the desired epidermis was reached. The epidermal peels were further cleared with absolute sodium hypochlorite and rinsed gently with water. The epidermal peels were stained with aqueous solution of Safranin-O for (five) 5 minutes. The stained samples were mounted on 10% glycerol to obtain the prepared slides on a binocular microscope. Photographs of the microscopic features such as stomatal morphology, epidermal cell wall pattern and calcium oxalate crystals of the prepared slides were taken with an Amscope MD500 mounted on Olympus CX21 microscope. Also, the transverse section and powder microscopy of the plant were observed and photographs taken [13, 14].

3.2 Quantitative Microscopy of the Leaf

Quantitative microscopic parameters such as leaf constant studies which include stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number and thickness of anticlinal epidermal cell wall were obtained using standard procedures [13].

All measurements were made using a calibrated ocular micrometer and ten (10) microscopic fields chosen at random were used and data presented as mean ± SEM (Standard Error of Mean).

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Fig. 1A and B. *Gnetum africanum*

The Stomatal Index (S.I) was determined according to Metcalfe and Chalk [14, 15] using the formula:

Stomatal Index (SI) = (S/E +S) x 100

Where: S = number of stomata per unit area
E = number of epidermal cells in the same area.

3.3 Micromeritics

The flow property was determined using standard methods [16] which constitutes:

3.4 Bulk Density and Tapped Density

The weight of 10 g of dried powdered leaf was weighed into 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density is calculated using the formula below;

$$B\rho = \frac{M}{Vb}$$

Where;

$$T\rho = \frac{M}{Vt}$$

Where $B\rho$ = Bulk density
M = Mass of powder
Vb = Bulk volume of powder
$T\rho$ = Tapped density
Vt = tapped volume

Interparticulate porosity is calculated using the formula below;

IP = $\frac{\rho T - \rho B}{\rho T * \rho B}$

3.5 Hausner's Ratio and Carr's Index

Hausner's ratio a function of interparticle friction is calculated using the formula

$$Hausner's\ ratio = \frac{T\rho}{B\rho}$$

While Carr's Index is measured as

$$Carr's\ index = \frac{T\rho - B\rho}{T\rho} \times 100$$

Where; $T\rho$ = Tapped density
$B\rho$ = Bulk density.

3.6 Angle of Repose

$$\theta = Tan^{-1}(\frac{Heap\ height\ of\ powder}{Radius\ of\ heap\ base})$$

3.7 pH

A pH meter (Jenway, Stafford Shire, UK) was used to determine the pH of both hot and cold extract of the leaf.

3.8 Chemomicroscopic Analysis of Leaf Powder

The leaf powder was examined for its chemomicroscopic properties such as mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard methods [17].

3.9 Fluorescence Analysis of Leaf Powder

Fluorescent analysis of dried leaf powder was carried out using standard procedures [18].

3.10 Physico-chemical Evaluation of Leaf

Physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash, water-soluble ash and sulfated ash values), soluble extractive values which include ethanol-soluble, methanol-soluble and water-soluble extractive values were performed according to the official method prescribed and the WHO guidelines on quality control methods for medicinal plant materials [19, 20, 21].

4. RESULTS

The results of the epidermal and stomatal characteristics of *G. africanum* are summarized in Table 1, Figs. 2 and 3.

The micromeritics evaluation for the leaves of *G. africanum* are summarized in Table 2. The leaf powder had Hausner's ratio of 3.20±0.00, compressibility index of 38.5±1.06 and angle of repose 42^0. The pH of 7.8 when cold and 7.7 when hot

4.1 Chemomicroscopic Evaluation

The chemomicroscopic evaluation of the powdered leaf revealed the presence of Lignin, Starch, oils, Calcium oxalate crystals and mucilage while Calcium Carbonate was absent as summarized in Table 3.

4.2 Fluorescence Analysis

The results of the fluorescence analysis exhibited a wide range of fluorescence colours under the ultraviolet and visible light as summarized in Table 4.

Table 1. Results of Microscopy of *G. africanum* leaf

Leaf surface	Abaxial surface	Adaxial surface
Epidermal anticlinal cell walls pattern	Undulate	Sinuous
Distribution of stomata	Hypostomatic	Nil
Stomatal length (µm)	276.49(325.74) ±7.82)349.85	Nil
Stomata width (µm)	150.29(173.9) ±4.72)189.56	Nil
Stomatal types	Brachyparacytic	Nil
Stomatal Index (%)	16.8	Nil
Stomatal number	2(3.1±0.25)4	Nil
Epidermal cell number	12(15.4±0.80)21	23(30±2.22)47
Epidermal thickness (µm)	10.17(12.6±2.87)34.33	6.77(9±0.62)10.93
Type of trichome	2-5 armed and stellate trichromes.	Nil
Length of trichome (µm)	37.5(502±52)662.10	Nil
Width of trichome (µm)	54.79(66.9±19)102.21	Nil
Length of epidermal layer (µm)	411.40(576±40.21)781.62	427.48(512±21)602.31
Width of epidermal layer (µm)	183.41(208±16.7)311	138.28(173±16)236.80

Results presented as Mean ± SEM of Ten (10) Replicates

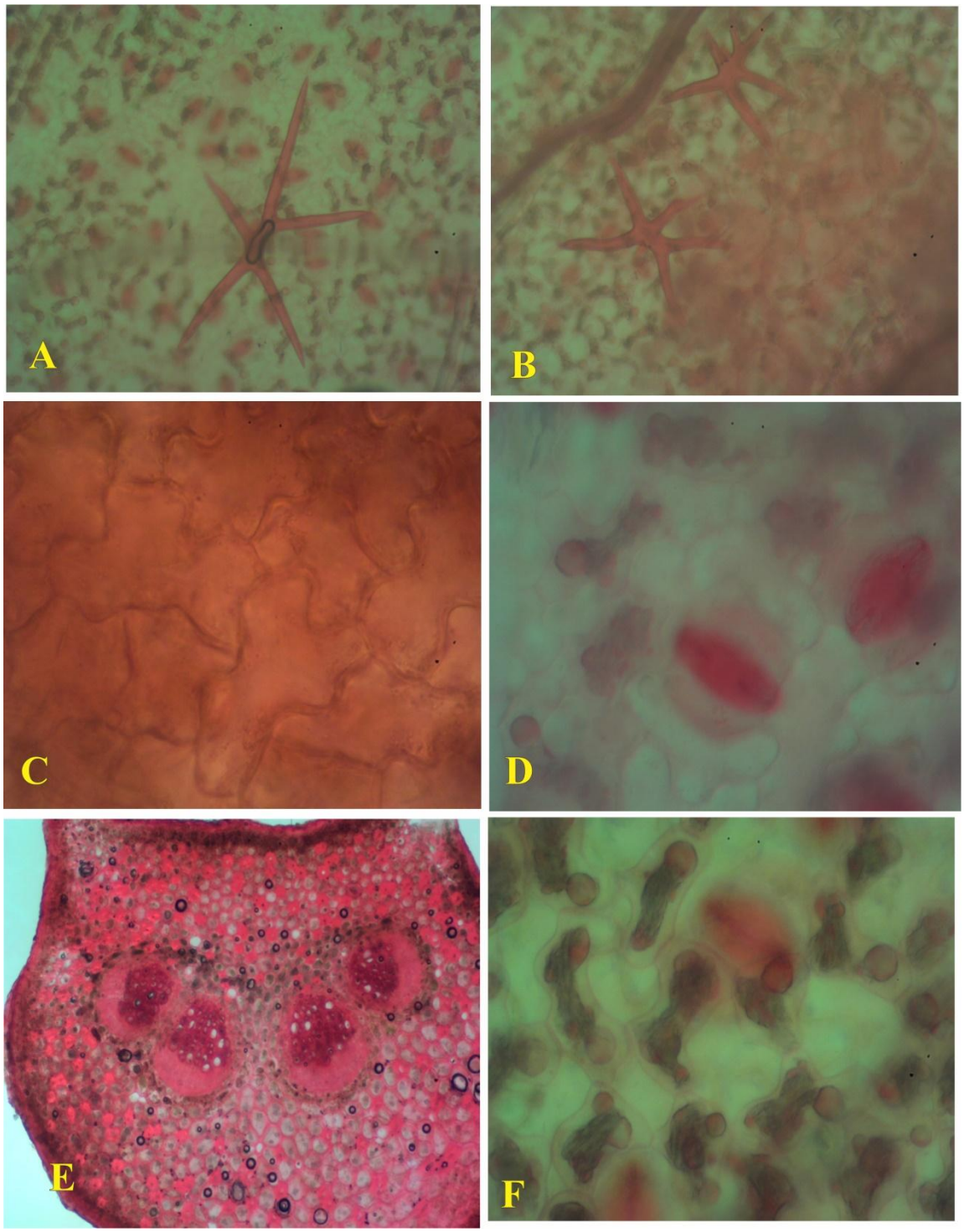

Fig. 2. Microscopy of *Gnetum africanum*

A. Abaxial surface : 2-5 armed Trichome × 400
B. Abaxial surface :Double armed and stellate trichomes × 400
C.Abaxial surface: Irregular epidermal cell shape and undulate anticlinal cell pattern × 400
D. Abaxial surface: Brachyparacytic stomata
E. Xylem and phloem tissues of petiole × 400
F. Abaxial surface: Chloroplast in the epidermal cell wall

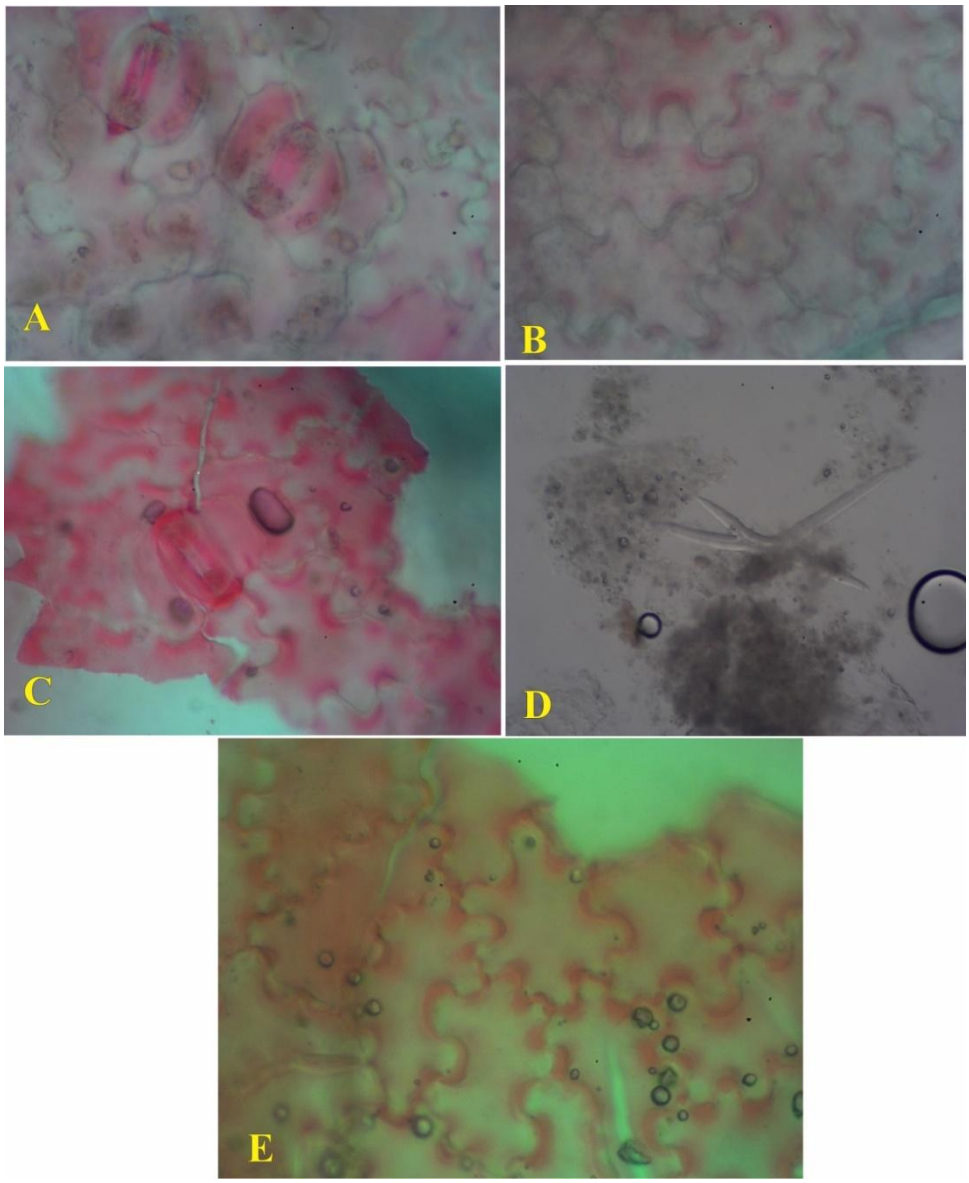

Fig. 3. A. Abaxial surface showing T-Pieces brachyparacytic stomata (×400); B. Adaxial surface showing sinuous epidermal cell wall pattern; C. Powdered microscopy Showing Brachyparacytic stomata (×400); D. Powdered microscopy Showing 2 - 4 armed trichome; E. Powdered microscopy showing undulate U- shaped Anticlinal cell wall pattern (× 400)

4.3 Physicochemical Constants of Leaf of *G. africanum*

The results of the physicochemical evaluation of *G. africanum* leaf powder is as summarized in Table 5.

5. DISCUSSION

Long before the advent of conventional modern medicine, Africans had developed their own effective way of dealing with diseases through the use of plants. Ethnomedicinally, the leaves of *Gnetum africanum* has been used in the treatment of various health conditions. The result obtained from microscopy of *G. africanum* leaf in Table 1 was found to be hypostomatic (stomata on the abaxial surface only) with brachyparacytic stomata, 3-5 armed trichome (stellate trichromes) was found on the abaxial surface. The epidermal cell pattern was undulate on the abaxial surface and sinuous on the adaxial surface. Stomatal index was found to be 16.8% on abaxial. Stomatal index is relatively constant and is not

Table 2. Micromeritic Properties of *G. africanum* powdered leaf

Parameters	Results
Bulk Volume (µm)	81±1.41
Tapped Volume (µm)	43.33±0.40
Bulk Density (g/mL)	0.123±0.00
Tapped Density (g/mL)	0.2±2.40
Hausner's ratio	3.20±0.00
Diameter (cm)	6.98±0.17
Angle of repose (^{0})	42
Carr's Index	38.5±1.06
pH	Hot 7.7
	Cold 7.8
Height of heap (cm)	3.16±0.11
Flow time (s)	152±25
Flow rate (g/secs)	0.07

Results presented as Mean±SEM of Three (3) Replicates

Table 3. Showing chemomicroscopic evaluation of leaf of *G. africanum* powder

Parameters	Leaf
Lignin	+
Starch	+
Oils	+
Calcium Carbonate	-
Calcium Oxalate crystal	+
Mucilage	+

+ = Present, - = Absent

Table 4. Fluorescence analysis of *G. africanum* powdered leaf

Extract	Ordinary light	UV - 365 nm	UV-253.7nm
Water	Green	Orange	Grey
Methanol	Green	Orange	Grey
Ethanol	Green	Brown	Grey
Dichloromethane	Green	Orange	Black
N-Hexane	Yellow	Orange	Black
Ethyl acetate	Green	Orange	Grey

Table 5. Result for physicochemical constants of *G. africanum* powdered leaf

Parameter	Weight (g)	Percentage (% $^w/_w$)
Moisture Content	0.21±0.00	10.50
Total Ash value	0.10±0.00	5.00
Acid-insoluble ash value	0.02±0.00	1.00
Water-soluble ash value	0.04±0.00	2.00
Sulfated ash value	0.12±0.00	6.00
Water-soluble extraction value	0.53±0.18	13.25
Methanol-soluble extraction value	0.17±0.00	4.25
Ethanol-soluble extraction value	0.16±0.00	4.00

Results presented as Mean±SEM of Three (3) Replicates

much affected by factors such as age of plant, size of leaf, environmental conditions etc. It is more significant in the evaluation of plant's leaves. The microscopic study also showed the presence of brachyparacytic type of stomata on the abaxial surface. This is similar to the report of Aworinde [22] reported same for *C. millenni* and *C. hispida* with the absence of stomata on their adaxial surfaces but present on their abaxial surfaces. The results of microscopic evaluation

of *G. africanum* leaf could be used as diagnostic features for judging the authenticity, quality, purity of the drug from its closely related species and for detection of adulterant.

The micromeritic properties like bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index indicated the flow properties as well as interparticulate resistance in the powder. This information predicts the stability and solubility of crude drug. Angle of repose in Table 2 was 42^0 which indicates a passable flow which means flow may hang up if not aided as compared to that reported by Umoh *et al* [23] on *Culcasia scandens* P. Beauv. with the angle of repose of 38^0 which had a fair flow property meaning such flow does not require any aid as preformulation characteristics related to interpaticulate friction. The micromeritic properties help to characterize and standardized the preformulation properties of herbal drug powder, in order to determine its suitability for formulation into solid dosage forms.

Chemomicroscopy analysis in Table 3 recorded the presence of mucilage, lignin, calcium oxalate crystals, starch and oil which are important and useful constituents of the plant. This confirms the pharmacological potency of this plant.

The fluorescence analysis of the powdered drug treated with water, methanol, ethanol, ethylacetate, n-hexane, dichloromethane observed under ordinary light, short wavelength of UV light (253.7nm) and long wavelength of UV light (365nm) showed different colours at different wavelengths. The colours were distinctive and reproducible revealing the solvent properties to the phytoconstituents as shown in Table 4. From the result in Table 5, water-soluble extractive value showed the highest value, which was found to be 13.25% $^{w}/_{w}$ compared to the ethanol-soluble and methanol-soluble extractive values. This may be due to the presence of high amount of water soluble compounds in the leaf of *G. africanum*.

The moisture content of *G. africanum* powdered leaf was 10.5%$^{w}/_{w}$ which is within the recommended range of 8-14%$^{w}/_{w}$ for vegetable drug according to African Pharmacopoeia [21]. This is an indication that the plant can be stored for a long period of time with less probability of microbial attack. Ash value gives estimation about purity, quality of drug and also gives information relative to its adulteration/contamination with inorganic matter. Total ash value of *G. africanum* leaf was 5%$^{w}/_{w}$ which is within the limit indicated in European Pharmacopoeia [24]. The European pharmacopoeial limit of total ash value for crude vegetable drug range should not exceed 14% $^{w}/_{w.}$ Total ash content which is the total amount of material remaining after incineration.

Acid-insoluble ash gives more consistent value than total ash value. Acid-insoluble ash for leaf of *G. africanum* was 1% $^{w}/_{w}$ which is within the normal range according to European Pharmacopoeia [24] (not to exceed 2% $^{w}/_{w}$).

Water-soluble ash value represents the water-soluble portion of total ash and was 2% $^{w}/_{w,}$ Sulfated ash was 6% $^{w}/_{w}$ which indicated that the residual substance not volatilized when the sample was incinerated with concentrated sulphuric acid. Determination of sulfated ash is a method intended for determining the amount of inorganic substances contained as impurities in an organic substance, but occasionally for determining the amount of inorganic substance contained as component of an organic substance.

6. CONCLUSION

The result obtained from the pharmacognostic study provides information for the identity, quality and purity of *G. africanum* and provide useful information for further studies on this plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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