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Evaluation of the pharmacognostic profile of the leaves of *Dialium guineese*Willd (Fabaceae) collected from Ukehe in Enugu state, Nigeria

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Abstract

Dialium guineese is a plant traditionally used as anticancer, antioxidant, antiulcer, antidiarrhea and heart diseases. However, the pharmacognosy of this plant could be more explored. Therefore, we have conducted this study to assess the distinctive qualities of the *D. guineese*. The leaves were extracted using ethanol, the crude ethanol extract was then fractionated using solvents of increasing polarity (n-Hexane, ethyl acetate, butanol and aqueous) fraction. The qualitative and quantitative phytochemical constituents, microscopy, macroscopy, and physicochemical properties were performed based on standard procedures by World Health Organization (WHO).

Macro and microscopical studies showed the presence of an entire margin with an obovate shape, acuminate apex, rounded base, and calcium oxalate crystals. Stomata arrangement was paracytic with numerous trichomes on both surfaces while phytochemical evaluation revealed the presence of alkaloids, tannins, steroids and saponins. The investigations also included numerical and quantitative leaf microscopy.

In light of these discoveries, future research on the species will be able to accurately identify and authenticate it.

Keywords: *Dialium guineense*; Phytochemical evaluation; Pharmacognostical studies and microscopic studies

1 Introduction

In many developing countries, herbs are used to treat various medical conditions. This awareness has been accepted and approved from one age bracket to another in rural communities [1]. In field of innovative allopathy drug research and development, a lot of bioactive components of plants was investigate through combinations with synthetic and chemical chemistry. Moreover, compared to synthetic counterparts, plant-based medications are generally safe, inexpensive and may have therapeutic benefits [2]. However, such medicines are limited in developed countries due to a lack of documented evidence regarding quality control and evaluation methods. Hence, its standardization through assessing pharmacognostic, physicochemical and phytochemicalparameters is crucial. This will further ascertain the reproducibility, safety and effectiveness of herbal medicines[3].

Dialium guineense Willd (Fabaceae) is a tree 30 m high, with a densely leafy crown, but often shrubby, bole without buttresses, the bark is smooth, grey, slash reddish, yielding a little red gum. It grows in dense savannah forests, shadowy canyons and gallery forests. *D. guineense* commonly known as black velvet and as Kedebe, Mako, Meko, mekohi (Fulani),

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Icheku (Igbo), and Awin (Yoruba) is a multipurpose tree in the West African region. The leaves and fruits are consumed during the dry season when farm produces are unavailable; they are sold in the market as refreshing drinks when mixed with water [4]. In ethno medicine, various parts of the plant have been used in the management of fever, diarrhea, palpation and as anti-bacterial. In some regions of Nigeria, traditional healers use *Dialium guineense* as a vitamin suppliment and antiulcer supplement [5] as well as a cure for heart problems, which may be related to the plant's tannin content. It is used as a chewing stick, and those who use it typically have healthy, robust, white teeth that are free of dental plaque [6].The plant of interest, *D. guineense*, has been shown to have anti-diarrhea, anti-ulcer, and anti-malarial properties and is used to treat jaundice, severe cough, bronchitis, wounds, stomach aches, and hemorrhoids. These curative properties were discovered to be present in *Dialium guineense* leaves and stem bark [4]. Despite being of enormous medical significance, little is known about the standardization parameters of *D. guineese*. Pharmacognostic standards were established using techniques like as microscopy and macroscopy, physicochemical characteristics, and extractive values. These factors, in turn, can help determine the drug's quality and aid in the gathering of pertinent monographs for its correct identification and potential inclusion in the African Pharmacopoeia.

2 Material and methods

2.1 Plant collection and identification

The leaves of *Dialium guineense* were collected at Ukehe Town, Enugu State, which is in the southeast of Nigeria. Validated at the International Centre for Ethnomedicine and Drug Development (INTERCEDD), Nsukka, Enugu State, Nigeria, by Mr. Alfred Ozioko, a certified taxonomist. For future use, voucher specimens (PCG 1194/A/003) were placed in the Nnamdi Azikiwe University Awka herbarium of the Department of Pharmaceutical knowledge and Traditional Medicine.

2.2 Extraction and Fractionation Procedures

The fresh leaves of were washed in a running tap to remove dust and other debris, and air dried for two weeks. Dried part of the plant were pulverized with grinding machine and kept in clean air tight amber bottle. The powdered material (100 g) was cold macerated in ethanol for 48 hours. The filtrate was recovered and concentrated to dryness using water bath at 40 ˚C The extract was stored in a refrigerator before use. The crude ethanol extract of *D. guineese* (15 g) was fractionated by adsorbing the crude extract on silica gel 60 g. Organic solvents of increasing polarity such as n-Hexane, ethyl acetate and butanol were used as the mobile phase, to obtain the different fractions [7].

2.3 Macroscopic evaluation

The organoleptic parameters, viz. texture, shape, size, colour etc of the plant material were noted by naked eye observation with a simple microscope.

2.4 Microscopic evaluation

2.4.1 Microscopic examination

Microscopic studies were carried out by preparing thin sections of leaf. The thin sections were further washed with water, staining was done by clearing in chloral hydrate solution then heat fixed and allowed to cool, then mounted using glycerine. The specimen was gently covered with a cover slip and placed on the stage of the microscope for observation (40x) [8] .

2.4.2 Chemomicroscopic examination

Examination of the powder for lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil and protein were carried out using standard techniques [9].

2.5 Physicochemical analysis

Analytical standards and physicochemical constants of the leaf were determined to evaluate the quality and purity of the drug .The parameters which were studied are moisture, ash values and extractive values [9,10].

2.6 Determination of moisture content

A preheated, tarred porcelain crucible was weighed and its weight with lid recorded (W1). A spatula full of the dried sample was introduced into the crucible and was reweighed, (W2). The sample was heated in an oven at the temperature

of 65 ˚C for 12 hours, at intervals of 6, 3, 2, 1, hours until a constant weight, followed by cooling in a desiccator before reweighing. The constant weight, W3 was noted [9]. The percentage moisture was calculated from the relationship:

$$\% \text{ moisture} = \frac{\text{Weight of sample in crucible (W2) – Constant weight (W3)}}{\text{Weight of sample in crucible (W2)–weight of crucible (W1)}} \text{x}100$$

Where
W2 –W1 = weight of sample
W2-W3 = weight of moisture

2.7 Phytochemical analysis

The phytochemical screening was carried out on the crude extract and fractions of *D. guineese* leaves according to standard methods to identify the classes of bioactive compounds present.[10,11]

3 Results

3.1 Extraction and Fractionation

The extraction process yielded 500 g of the crude extract (CE), and the yield of the fractionation is presented in Table 1.

Table 1 Yields obtained from the crude extract and fractions of *D. guineese* leaves

Extract/Fractions	Yield (g)
Ethanol Crude extract	500 g (32 % w/w)
N- Hexane fraction	0.54 (0.31 % w/w)
Ethyl acetate fraction	6.01 (5.59 % w/w)
Butanol fraction	12.33 (12% w/w)

3.2 Macroscopy result

Macroscopic evaluation of the powdered leaf of *D. guineese* (Table 1) revealed useful diagnostic characteristics. The plant powders are seen to be generally fleshy in texture. , dark green in colour, bitter in taste with a characteristic odour.

Table 2 Macroscopic Description of the Leaf of *D. guineese*

S/n	Macroscopic features	Description
1	Odour	Characteristic odour
2	Colour	Green
3	Taste	Bitter
4	Texture	Fleshy but leathery when dry
5	Apex	Acuminate
6	Margin	Entire
7	Venation	Net
8	Base	Rounded
9	Surface	Cuneate at the base
10	Size	7-13 cm long, 7 cm wide
11	Lamina composition	Simple
12	Shape of lamina	Linear
13	Shape	Obvate

3.3 Microscopic examination of the leaf *D. guineese*

3.3.1 Qualitative and quantitative Leaf microscopy

The microscopic examination of *D. guineese* revealed the presence of stomata, trichome, calcium oxalate and different quantitative microscopic characteristics of the plant such as stomatal number, stomatal index, palisade ratio, vein islet and vein termination (Table 2).

Table 3 Result of qualitative and quantitative Leaf microscopy of *D. guineese*leave

Parameter	***D. guineese***
Epidermal cell type	They are irregular in shape with undulated/wavy anticlinal cell walls on the upper surface but polygonal with straight anticlinal cell walls on the lower surface
Leaf type	The leaf is hypostomatic (stomata only occur on the lower surface)
Stomata type	Paracytic (two subsidiary cells lie parallel to the guard cells) type of stomata.
Trichome	Covering unicellular trichomes are abundantly present.
Stomata number (pfv)	18.25 ± 0.38
Stomata density (mm^{-2})	135.29 ± 2.40
Stomata index (%)	20.62 ± 0.21
Stomata length (µm)	18.41 ± 0.54
Stomata width (µm)	8.66 ± 0.21
Stomata size (μm^2)	159.35 ± 5.96
Vein islet number	6.15 ± 0.34
Veinlet termination number	9.60 ± 0.14
Palisade ratio	11.40 ± 0.64

Values are given as mean ± standard error of mean; n = 4

3.3.2 Results of Chemomicroscopic examination of D. guineese

Table 4 Powder microscopy of the leaves of *D. guineese*

Test Reagents	**Observation**	**Inference**
Iodinated zinc chloride solution	Yellow colouration observed in the xylem vessels	Lignin (+)
Iodinated zinc chloride solution	Blue black colouration observed on few grains in the parenchyma cells	Starch(+)
80% H_2SO4	Crystals of calcium oxalate dissolved	Calcium oxalate (+)
Iodinated zinc chloride solution	Blue colours observed on epidermal cells	Cellulose (+)
Ferric chloride solution	No greenish colour in some parenchyma cells	Tannins (+)
Sudan IV reagent	Pink colouration	Oil globules (+)
Ruthenium red	No colour change	Gum/Mucilage (-)
Biuret reagent;Nihydrin	Absence of yellow substances	Protein (+)

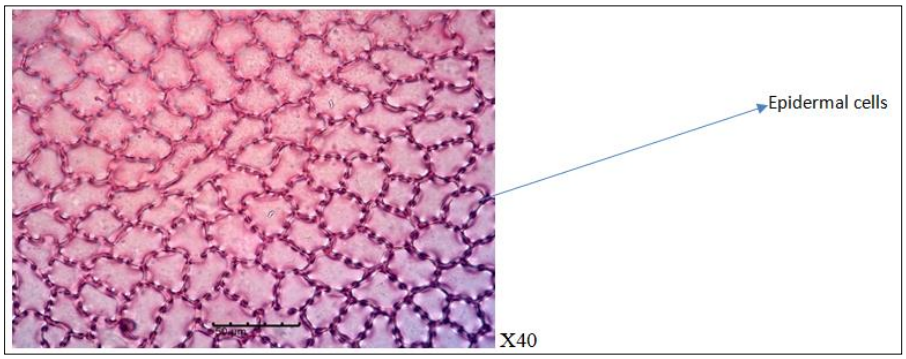

Figure 1 Upper epidermal surface of the leaf of *D. guineense* showing irregularly shaped and undulated epidermal cells. Stomata are lacking

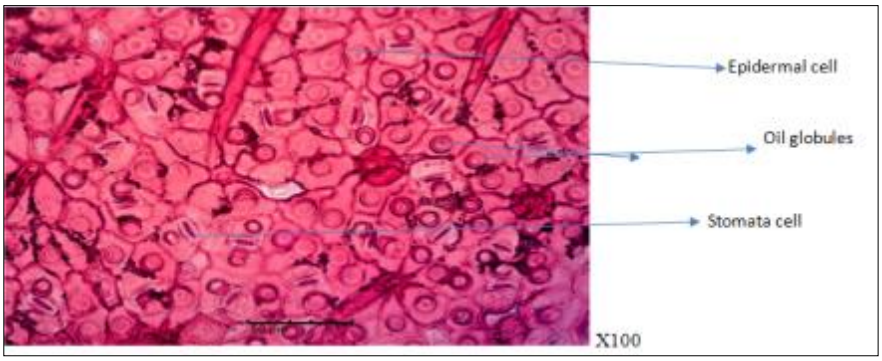

Figure 2 Lower epidermal surface of the leaf of *D. guineense* showing polygonal epidermal cells and paracytic type of stomata. Oil cells abundantly present

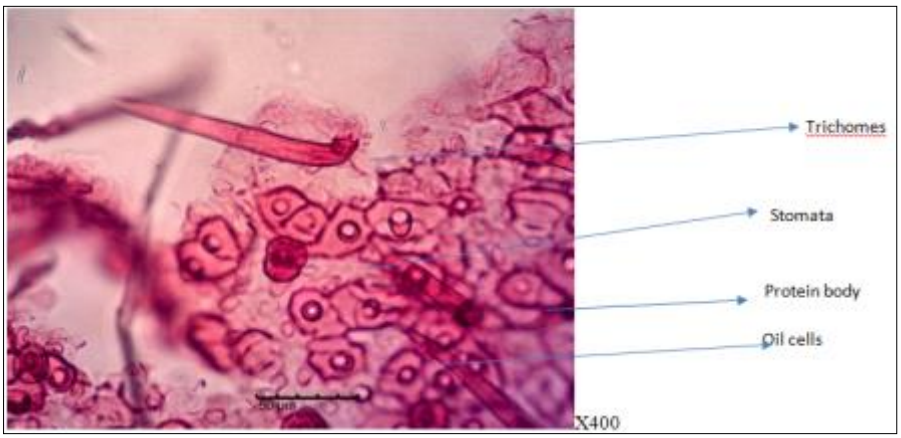

Figure 3 Fragment of the leaf of *D. guineense* showing oil cells, protein bodies and strands of unicellular covering trichome

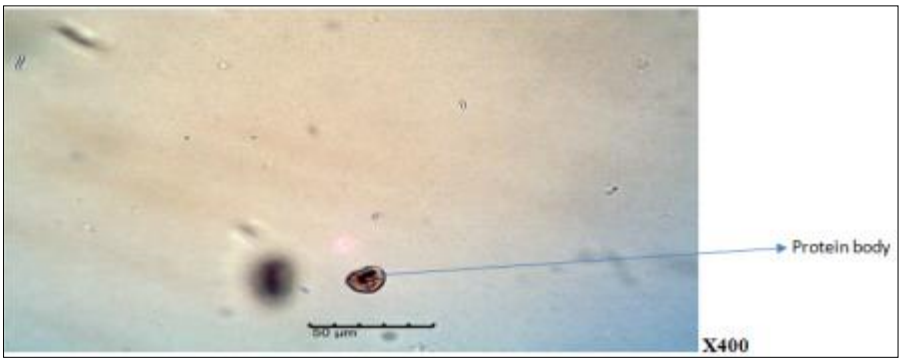

Figure 4 Chemomicrograph of the leaf powder showing a protein body

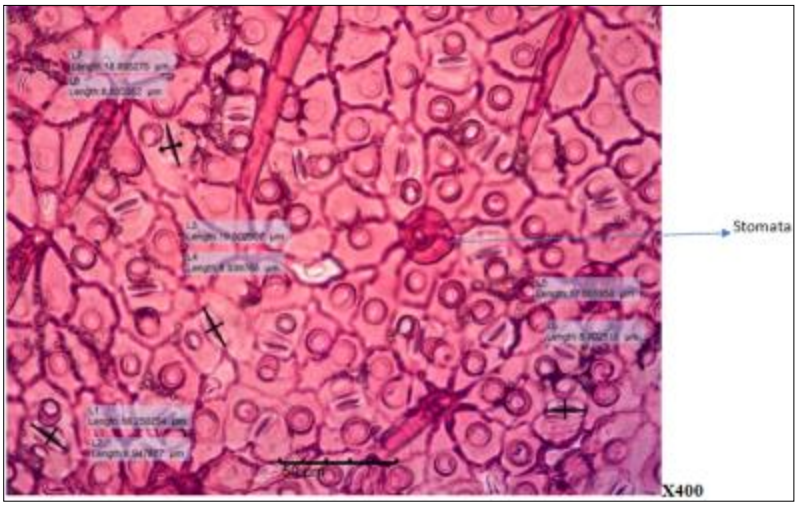

Figure 5 Stomata measurement

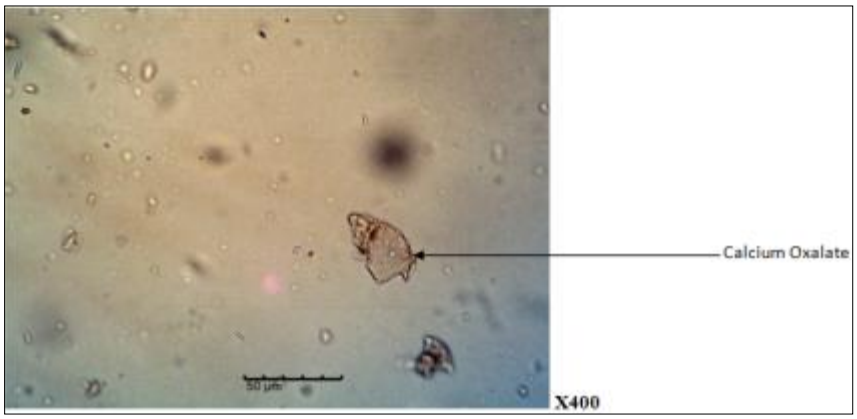

Figure 6 Chemomicrograph of the leaf powder showing a crystal of calcium oxalate

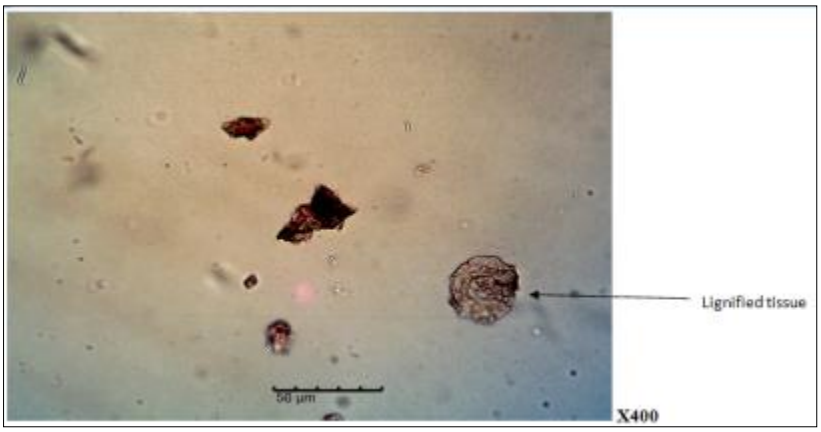

Figure 7 Chemomicrograph of the leaf powder showing lignified tissues

Figure 8 Chemomicrograph of the leaf powder showing a fragmented fibre element

3.4 Results of Physiochemical parameters and of *D. guineese*leaves

The Physiochemical parameters of *D. guineese* leaves showed the percentage composition of the total ash, water soluble ash, acid-insoluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content as presented in Table 4 below. The total ash obtained was 8.4 % and it's used as a measure of purity. Moisture content was 7.35 % and it's used as a measure of stability.

Table 5 Results of physiochemical Parameters

Parameters	% Composition
Total ash	8.4± 0.36
Water – soluble ash	2.05 ± 0.56
Acid – insoluble ash	1.69 ± 0.24
Water – soluble extractive	9.09 ± 0.30
Alcohol – soluble extractive	7.99 ± 0.05
Moisture content	7.35±0.01

3.5 Phytochemical analysis of *D. guineese* leaf

Phytochemical screening reported presence of glycosides, flavonoids, alkaloids, tannins, steroidsetc as shown in Table 5 and 6 below.

Table 6 Qualitative phytochemical analysis of *D. guineese* leaf

Phyto-constituents	EC	HF	EF	BF
Alkaloids	+	-	+	-
Saponin	+ +	-	+	+
Tannin	+ ++	+	+	+
Flavonoids	+ +	+	+	+
Steroids	+++	+	+	+
Terpenoids	+	_	+	+
Glycoside	-	_	_	-
Protein	+	_	+	_
Phenols	+	_	_	+
Reducingsugar	+	_	_	+

(-)=> NotPresent,(+)=> Presentinsmallconcentration,(++) => Presentinmoderatelyhighconcentration,(+++) =>Present in high concentration;EC= Ethanol crude extract, HF= n-Hexane fraction , EF= Ethylacetate fraction, BF= Butanol fraction

Table 7 Resultof quantitative phytochemical analysis of *D. guineese* leaf

Phytoconstituents	Composition (%)
Alkaloids	3.21
Saponins	8.6
Flavonoids	5.8
Tannins	9.8

Values are expressed as percentage(%)

4 Discussion

Evaluation of medicinal plants must consider many important elements, including accurate identification, quality control, and the establishment of pharmacognostic standards. The World Health Organization (WHO) states that before conducting any tests, the macroscopical and microscopic examination of a medicinal plant is the first stage in determining the identity and level of purity of such material [12]. Macroscopic analysis of *D. guineese*'s complete leaves revealed that the leaves have an entire border, net veination, an acuminate apex, etc., while organoleptic analysis reveals that the leaf's frontal surface is green in color and has a bitter taste.

For proper identification, macroscopic analysis of plants should be done to determine their identity and purity in order to ensure appropriate identification [14].

Several anatomical aspects of the plant were discovered by microscopic analysis of the leaf powder. This microscopic analysis will help in determining the extent of any adulteration with related species as well as in differentiating it from other plants. The types of stomata and trichomes that were found as well as the presence of calcium oxalate, epidermal cells, oil globules, and lignified tissues were all revealed. Only the lower epidermis was found to have paracytic type stomata, which were between 17.0 and 19.5 millimeters long and 8.0 to 8.9 millimeters wide. The presence of a specific type of trichomes is typically used in the taxonomy of various families, genera, and species, therefore the unicellular trichomes seen from the microscopy of the powdered leaf could serve as a diagnostic trait [13]. The epidermal cells

have wavy cell walls on the upper surface and polygonal, straight anticlinal cell walls on the lower side, giving them an uneven shape.The detection of source materials depends on this evaluation. For separating species, genera, and even families, anatomical characteristics are used as a criterion [12].

The standardization and quality control of herbal medications depend heavily on physicochemical characteristics. A popular test method for determining the amount of moisture in a powdered sample is loss on drying. Drugs should have a very low moisture content to prevent the formation of yeast, bacteria, or fungi while being stored. To evaluate the quality and purity of crude drugs, ash values are used. The presence of different contaminants, such as carbonate, oxalate, and silicate, is indicated. To determine the amount of inorganic components contained in medicines, water-soluble ash is utilized. The primarily silica-containing acid-insoluble ash is a sign of contamination with earthy material [15].

The amount of ash obtained in total, 8.4 %, represents the total amount of ash still present after ignition. This was done in order to eliminate all traces of organic stuff from the ash sample because their presence could interfere with the analysis. Crude extract on incineration normally leaves an ash consisting of carbonates, silicates and phosphates of sodium, calcium, potassium and the total ash of a crude drug determines how much care is required in its preparation. Acid insoluble ash test was thus performed to know if the plant extract has silica or calcium oxalate. The outcome showed an acid insoluble ash content of about 1.69 %, and water soluble ash of 9.09 %. This implies that a good portion of total ash constituents were soluble in water. The constituents of *D. guineese* leaves were soluble in both alcohol (7.99 %) and water (9.09 %) with the highest solubility being in water. This implies that there were presence of water soluble constituents like phenols, tannins, carbohydrates and saponins compared to alcohol-soluble constituents such as steroids, alkaloids, flavonoids [16].The moisture content value (7.35 %) shows minimal possible enzymatic hydrolysis and degradation of the active components on exposure to air. High moisture content is indicative of easy degradation by fungal or bacterial growth since degradation of the plant material depends on the amount of water present.[17].

Alkaloids, saponin, tannin, and flavonoids were among the phytoconstituents found in this study's crude extract and other fractions, whereas glycosides were absent, as shown in table 5. A study on D. guinese leaves undertaken by Fidelia, *et al.* [18] provides support for these conclusions. Because they are affordable, simple, and demand little in the way of resources, conventional phytochemical tests continue to be the best option for preliminary screening[19]. The healing abilities of medicinal plants may be due to secondary metabolites [20].Any crude medication can be extracted using a specific solvent to produce a solution that contains several phytoconstituents.

Depending on the drug's composition and the solvent employed, these chemical components have different compositions. Additionally, it shows whether or not the crude medication has been exhausted [21]. Extractive values can be calculated to help determine whether certain compounds are soluble in a given solvent and to assess the chemical composition of crude drugs. All medicinal plants have therapeutic potential because of their chemical components, or phytochemicals, whose physiological effects are well-known [22].

5 Conclusion

According to the study's findings, pharmacognostic analysis of *D. guineese* leaves provides a vital diagnostic tool for identification, authentication and development of quality parameters of this plant part. The discovery of its bioactivity, toxicity profile and the proof of its safety and effectiveness in clinical tests will be made easier by subsequent research made possible by this fundamental information as the data obtained in the present study may be considered to be the standard for future studies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The research work is in compliance with ethical standards.

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