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Pharmacognostic profile of B*urkea africana* hook (Fabaceae) leaves

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Abstract

Background: *Burkea africana* Hook (Fabaceae) is commonly used in traditional medicine to treat infections and other health issues in various cultures. This study aimed to perform an extensive pharmacognostic evaluation of *Burkea africana* (BA) leaves of the crude extract.

Methods: The macroscopic characteristics were analyzed to identify the color, odour, and shape of the leaves. Microscopic assessments, physiochemical and phytochemical tests were carried out using standard procedures.

Results: The macroscopic analysis showed that the leaves were light green and pinnately arranged. The microscopic examination showed polygonal epidermal cells featuring anomocytic stomata. The total ash percentage, acid-insoluble ash, water-soluble ash, and moisture content were measured at 2.23%, 0.28%, 1.22%, and 9.36%, respectively. Phytochemical analysis indicated the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and steroids.

Conclusion: This study provides essential information for the quality control and standardization of *B. africana* leaves.

Keywords: *Burkea africana*; Pharmacognostic study; Physicochemical evaluation; Phytochemical test

1. Introduction

Traditional medicine plays a crucial role in healthcare across Nigeria, with a significant portion of the population relying on plant-based remedies for various ailments (Degu et al., 2024). Ethnobotanical surveys have revealed the widespread use of medicinal plants in Nigeria, either as a primary therapeutic choice or in conjunction with conventional medicines (Clement et al., 2020). The knowledge of these plants is often passed down through generations, highlighting the rich experiences of traditional healers. To fully harness the therapeutic potential of these plants, rigorous scientific investigation, including pharmacognostic studies, is essential. *Burkea africana* Hook is a tree species native to tropical Africa, including Nigeria, and commonly known as wild syringa. It is locally called Osisi Oka, Bakin Makarho, and Apasa among the Igbos, Hausas, and Yoruba, respectively (Kaboré et al., 2024). Traditionally, different parts of *Burkea africana*, including the leaves, bark, and roots, are used to prepare remedies for ailments in Nigeria (Godam et al., 2021). Bark decoctions or infusions are used to treat fever, cough, catarrh, pneumonia, menorrhea, headache, inflammation of the tongue and gums, poisoning, and skin diseases. Powdered leaf is applied to ulcers and wounds, and to treat scabies (Ezenyi et al., 2021). Root decoctions or infusions are used to treat stomach-ache, abscesses, oedema, epilepsy, bloody diarrhea, gonorrhea, and syphilis. In addition, *B. africana* leaf was reported to possess antibacterial activity (Zengeni et al., 2021). Despite its widespread ethnomedicinal use in Southeast Nigeria, there is a lack of comprehensive scientific studies validating its pharmacognostic properties and establishing quality control parameters of either the stem or the leaf. Include the scientifically proven activities of any part of the plant. Due to a lack of these standard parameters,

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proper identification and ascertaining quality and purity in the event of adulteration have been thwarted (Namadina et al., 2020). The objective of this study was to establish the pharmacognostic profiles of *Burkea africana* leaf with the hope of assisting in its standardization for quality and purity.

2. Methodology

2.1. Collection and identification of plant materials.

The plant material utilized in this study was collected in November 2023 from Nsukka (lat. 6.85667 °N;long 7.39583 °E), located in Enugu State, Nigeria. Fresh leaves of *Burkea africana*, weighing 1 kg, were collected and subsequently identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, by Mr. Felix, a botanist and senior technologist. The plant specimen was mounted on a herbarium sheet, and a voucher number (ESUT/PCG/0106) was issued.

2.2. Preparation and extraction of plant material.

The leaves were air-dried under the shade at 30˚C for 14 days, ground into powder using an industrial milling machine. The extraction process was carried out using the cold maceration method described by Feenna et al., (Feenna, Estella, Chioma Obianuju, et al., 2020). The powdered leaf (500 g) was dissolved in 1.5 L of ethanol for 72 hours, with intermittent vigorous shaking. On the third day, the resulting mixture was filtered first using a white handkerchief. The filtrate was then subsequently filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator and dried in a desiccator until a constant weight was obtained.

2.3. Pharmacognostic evaluation

Macroscopic test: The macroscopic characteristics of the fresh leaves studied were leaf size, shape, type of venation, colour, margin, leaf base, leaf apex, odor, and taste of the leaf(Okonta et al., 2021).

2.4. Quantitative Microscopic Evaluation

The quantitative leaf microscopy was performed on epidermal strips using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA), the slides were magnified 40, 100, and 400 times. The photomicrographs were taken using the Moticam 2.0 image system with software (Motic Carlsbad, CA, USA) connected to the microscope. The following parameters were measured; the palisade ratio, stomata number, stomata index, vein islet number, and veinlet termination number (Okoh et al., 2024).

2.5. Chemomicroscopic Examination

The powder leaves sample was analyzed using standard methods to check for protein, cellulose, fatty oil, crystals of calcium oxalate, lignified walls, and starch grains (Trease & Evans, n.d.). The microscopy was done using a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA), the slides were magnified 40, 100, and 400 times.

2.6. Physicochemical Test

Determination of extractive yields: The alcohol and water extractive yields were determined using the method described by(Desai & Chanda, 2014).

2.7. Alcohol extractive value

Two grams of the powdered sample was macerated in 100 mL of absolute ethanol for 24 hours and filtered. The filtrate was dried in the oven at a temperature of 105 ^{0}C to obtain a constant weight dry extract, which was cooled in the desiccator. The weight of the dried extract was determined and recorded.

2.8. Water extractive value

The powdered sample (2 g) was macerated in 50 mL of chloroform water in the ratio 1:400 for 24 hours and filtered. The filtrate was dried in the oven at a temperature of 105 ^{0}C to obtain a constant weight dry extract, which was cooled in the desiccators. The weight of the dried extract was determined and recorded.

2.9. Determination of moisture content

Three (n=3) preheated porcelain crucibles were weighed (W_1), and 2 g of the powdered material was measured into each of the crucibles and reweighed (W_2). The sample was gradually heated in the oven up to a temperature of 105 ^oC for about 4 hours until a constant weight was obtained. The heated sample was cooled in the desiccators and weighed (W_3), and the average moisture content was calculated.

$$\% \text{ Moisture content} = \frac{\text{Weight of sample in crucible (W2)} - \text{Weight of constant weight sample in crucible (W3) X 100}}{\text{Weight of sample in crucible (W2)} - \text{Weight of crucible (W1)}}$$

2.10. Determination of ash values

2.10.1. Total ash values

Three (n=3) porcelain crucibles were placed in a muffle furnace for about 15 minutes at 350℃, cooled in a desiccator for about one hour, and the crucibles were weighed (W_1). 2 g of the sample was accurately weighed into each of the preheated porcelain crucibles and reweighed (W_2). The samples were ashed in a muffle furnace at 650 °C for about 6 hours until the samples turned grey (white ash). The crucibles were removed with a crucible tong, cooled in a desiccator, and reweighed (W_3). The percentage ash content is determined using the below;

Total ash (TA) = W3-W1

$$\%\text{Total Ash} = \frac{W3 - W1}{W2 - W1} \text{x } 100$$

2.11. Water soluble ash value

Total ash (TA) was obtained using the method stated above. About 5 mL of water was added to the TA and boiled for 5 minutes, filtered with an ashless filter paper, and the filter paper containing the residue was dried in the oven. The filter paper containing the residue was compressed into the crucible and subjected to heat until the ashless filter paper was eliminated, and the crucible was reweighed (W4).

$$\% \text{ Water soluble Ash (W)} = \frac{\text{TA} - (\text{W4} - \text{W1})}{\text{W2} - \text{W1}} \text{x } 100$$

2.12. Acid-insoluble ash value

The total ash obtained from incinerating the powdered leaf at 450 °C was transferred into a beaker containing 25 ml of dilute hydrochloric acid, boiled on a water bath for about 5 minutes, and filtered with an ashless filter paper. The beaker and crucible were washed repeatedly through the filter paper with hot water until they were free from acid (i.e., neutral to litmus paper).

The insoluble matter and the ashless filter paper were dried in the oven, ignited in the muffle furnace at 450 °C to a constant weight, and the amount of acid-insoluble ash per gram of the powdered drug was calculated.

$$\% \text{ weight of acid} - \text{insoluble ash} = \frac{\text{Weight of crucible and ash} - \text{weight of crucible}}{\text{Weight of sample}} \text{x } 100$$

2.13. Qualitative phytochemical test:

Phytochemical analysis was carried out on the crude extract of *BA* using standard methods (Ebele et al., 2021) as stated below.

- Test for Alkaloid: 2 g of powdered crude sample was heated in 10 ml 2% HCl in the hot water bath for about 2 minutes. The mixture was allowed to cool and filtered using filter paper. Drops of Dragendorff's reagent were added to about 2ml of the filtrate and shaken. The formation of a reddish-brown color precipitate indicated the presence of alkaloids.
- Test for Flavonoids (Sodium hydroxide solution test): 2 g powdered plant sample was dissolved in 20 ml of ethyl acetate and heated in the water bath for 2 min. The mixture was cooled and filtered. About 2 ml of 10% sodium hydroxide was added to 5 ml of the filtrate. Color changes in the lower alkaline layer indicate the presence of flavonoids.

- Test for Glycoside (Fehling's solution test): 2 g powdered sample was dissolved with 1 % HCl and heated in the hot water bath for 2 minutes. The mixture was filtered and the filtrate was made distinctly alkaline by adding 2 to 5 drops of 20% NaOH. 1ml of equal volumes of Fehlings A and B solution was added to the filtrate and heated in a hot water bath for 2 minutes. The formation of reddish-brown precipitate indicates the presence of glycoside.
- Test for Saponin (Frothing and emulsion test): 10 ml of distilled water was added to 2 g of the powdered plant sample and boiled in a hot water bath for 2 minutes. It was filtered and the filtrate was allowed to cool. 5ml of the filtrate was diluted with 5ml of distilled water and shaken vigorously. The formation of stable froth indicates the presence of saponin.

Two (2) drops of olive oil were added to the frothing solution. The formation of emulsion indicates the presence of saponins.

Test for tannins: An aqueous solution containing 5 g of the powdered drug with 10 ml of distilled water was prepared and boiled for two minutes. It was allowed to cool and was filtered. 2 ml of the extract was weighed into a test tube, and 2 ml of distilled water was followed by 3 drops of 1% ferric chloride solution. The formation of blue-black or dirty green coloration indicates the presence of tannins.

Test for phenols: 3 ml of the extract was measured into a test tube and 3 drops of 1% lead acetate solution was added into it. The formation of heavy precipitates indicates the presence of polyphenolics.

Test for carbohydrate (Molish's): A solution containing 2 g of powdered sample in 5 ml of distilled water in a test tube. A few drops of Molish reagent (alpha naphthol solution in ethanol) were added to the solution and the solution was shaken gently. Concentrated sulphuric acid was poured gently down the side of the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrates.

Terpenoids (Salkowski test): An extract was obtained by dissolving 2 g of the powdered plant sample in 5 ml of chloroform. The mixture was filtered. A few drops of concentrated sulphuric acid were added to the filtrate. The formation of a reddish-brown color at the interface indicates the presence of terpenoids.

3. Results

3.1. Macroscopic test

The leaf is pinnately compound with oval leaflets that are pubescent and have a characteristic smell, as shown in Figure 1 and Table 1.

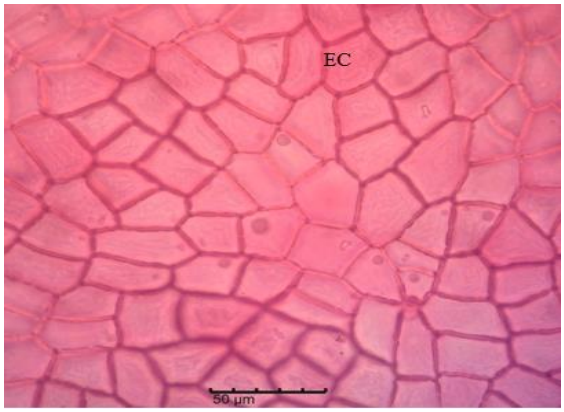
Figure 1 Live picture of BA showing the leaves' arrangement and the whole plant.

Table 1 Macroscopic characteristics of B*urkea africana* leaf.

Parameters	Types
Leaf Shape	Oval
Leaf Type	Pinnately compound
Leaf Texture	Pubescent
Colour	Light green
Odor	Characteristic smell

3.2. Microscopic test

The leaf has a polygonally shaped epidermal surface with straight anticlinal cell walls, and the adaxial surface lacks stomata (Figure 2). However, the abaxial surface of the leaf has numerous anomocytic-type stomata and unicellular covering trichomes (Figures 3 and 4). Prism-shaped calcium oxalate crystals were observed (Figure 5). The transverse section of the leaf and petiole showed trichomes, xylem, phloem, etc (Figures 6 and 7). The stomata number is 15.74±0.48, and the stomata size is 348.75 ± 10.63 (Table 2).

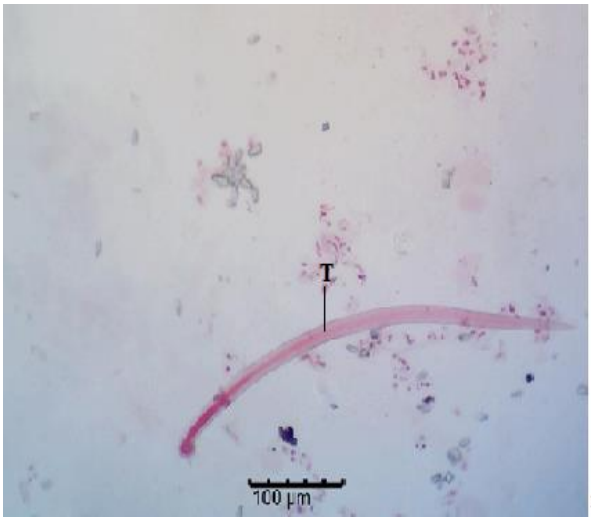


x400

Figure 2 Adaxial surface of the leaf showing epidermal cells (EC)

x400

Figure 3 Abaxial surface of the leaf showing stomata cell (SC), subsidiary cell (SB), and epidermal cell (EC)



x400

Figure 4 Fragment of trichome from the leaf. T=trichome.

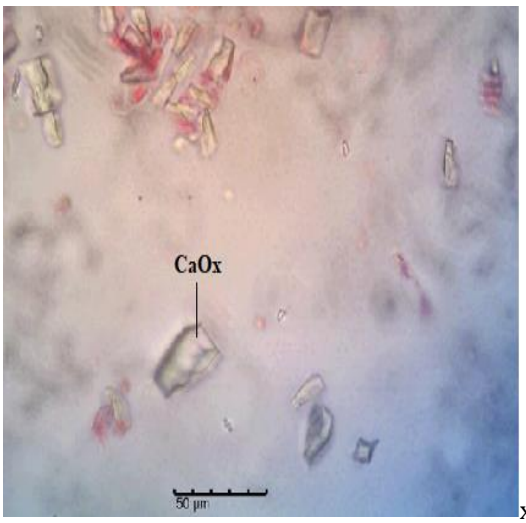


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Figure 5 Prism-shaped calcium oxalate crystals. Ca0x=calcium oxalate crystal

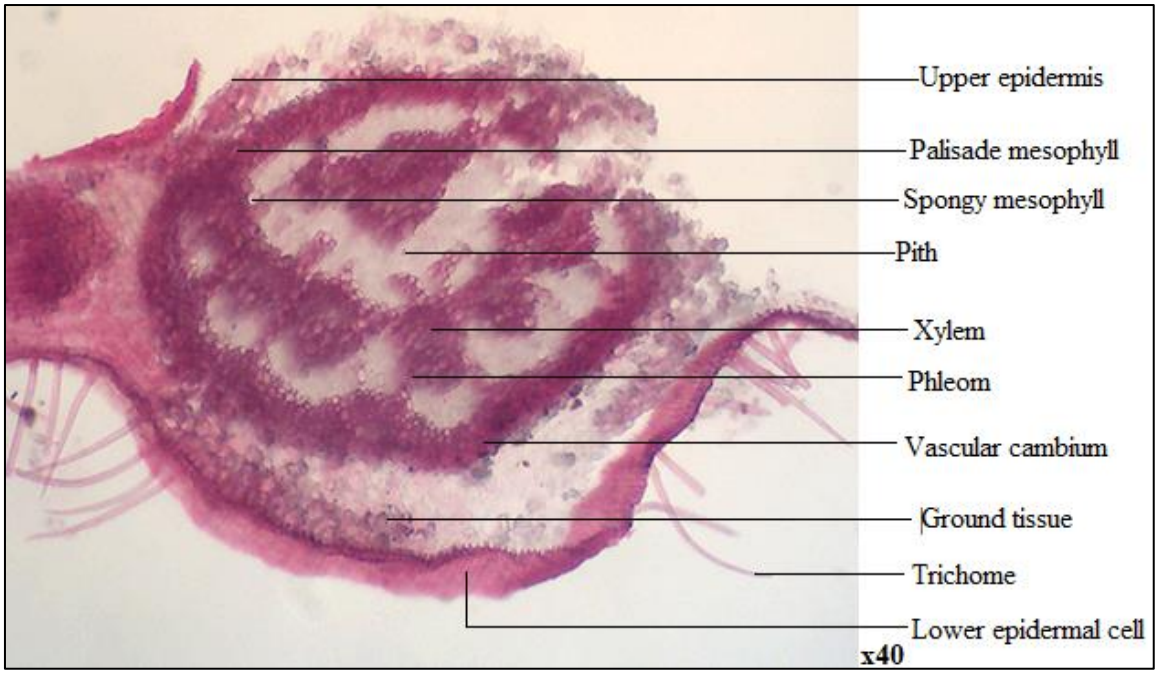

Figure 6 Transverse section of BA leaf

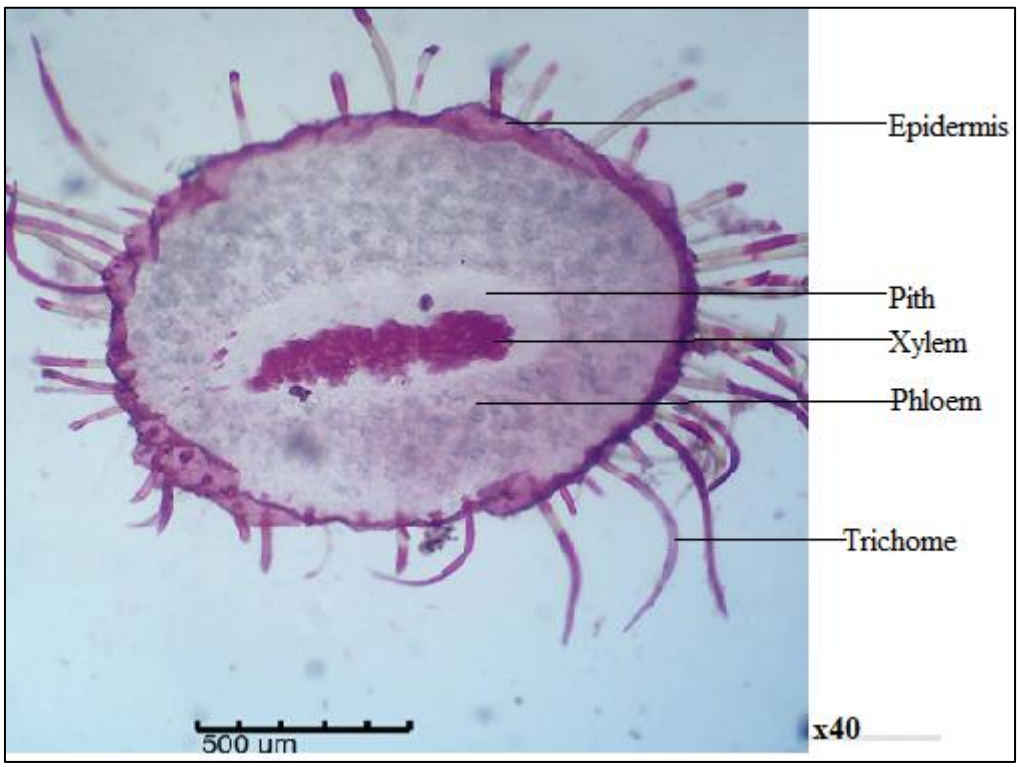

Figure 7 Transverse section of BA petiole

Table 2 Summary of the microscopic study of *Burkea africana*

Epidermal cell	Epidermal cells are polygonally shaped with straight anticlinal cell walls on both the upper and lower surfaces
Stomata type	The leaf is hypostomatic (stomata occur only on the lower/abaxial surface) with anomocytic type of stomata
Trichome	Present - unicellular covering trichomes
Stomata number (p.f.v.)	15.74 ± 0.48
Stomata density (mm^{-2})	92.65 ± 2.82
Stomata length (µm)	22.54 ± 0.17
Stomata width (µm)	15.47 ± 0.45
Stomata size ($µm^2$)	348.75 ± 10.63
Stomata index (%)	22.41 ± 0.35
Vein islet number	16.25 ± 0.45
Veinlet termination number	22.30 ± 0.52

3.3. Chemomicroscopic test

The presence of starch grains, lignified tissue, calcium oxalates, tannins, cellulose, protein, and the absence of mucilage were indicated (Table 3).

Table 3 Chemomicroscopic evaluation of leaf

Parameter	**Reagent(s)**	**Result**
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism-shaped
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Nihydrin	Present
Oil	Sudan III reagent	Present

3.4. Physicochemical test

The percentage total ash, water-soluble ash, and acid-insoluble ash were recorded as 2.23%, 1.22%, and 0.28%, respectively. The moisture content was indicated to be 9.36%. The water extractive, ethanol extractive, and n-hexane extractive values were 18.1%, 14.25%, and 1.98%, respectively. The total yield of the ethanol crude extract was 54.8 g, which gave a percentage yield of 10.96% (Table 4).

Table 4 Proximate analysis of the powdered sample of the leaf

Parameters	**Values (%)**
Total ash	2.23±1.44
Water-soluble ash	1.22±0.48
Acid insoluble ash	0.28±0.53

Moisture content	9.36±0.06
Water extractive	18.10±0.42
Ethanol extractive	14.25±0.67
N-hexane extractive	1.98±0.05
Percentage yield	10.96 (54.8 g)

3.5. Qualitative phytochemical test

The presence of alkaloids, flavonoids, phenolics, tannins, and saponins was indicated, whereas glycosides were recorded to be absent (Table 5).

Table 5 Qualitative phytochemical screening of the crude extract

S/N	Phytochemical compounds	Qualitative test
1	Alkaloids	+
2	Flavonoids	+
3	Phenolics	+
4	Tannins	+
5	Saponins	+
6	Terpenoids	+
7	Anthocyanins	+
8	Carbohydrates	+
9	Glycosides	-

4. Discussion

Establishing the accurate identity of a crude drug is pivotal for its standardization, particularly before its inclusion in an herbal pharmacopeia. Comprehensive definitions of pharmacognostic parameters and standards are essential for the effective integration of herbal drugs(Desai & Chanda, 2014). In this context, BA leaf is recognized for its diverse pharmacological activities; however, it currently lacks a detailed pharmacognostic profile, which hinders its identification and increases the risk of adulteration(Onugwu et al., 2025). A thorough gross macroscopical examination serves as a cornerstone for precise identification (Begum et al., 2020). In the case of powdered drugs, the significance of microscopic structures cannot be overstated, as these are largely dependent on the shape, presence or absence of specific cell types, and cell inclusions (Okoh et al., 2024). Critical elements in the identification process include the type of trichomes, shape of the epidermal cell walls, stomata types, and particular cell inclusions, including starch grains and prism-shaped calcium oxalate crystals, which are particularly noteworthy (Ogbue et al., 2023).

Physicochemical analysis plays an indispensable role in determining the identity, purity, and potential adulterants of crude drugs (Obinna et al., 2023). The present study revealed that the average moisture content, calculated via the loss on drying method, is 9.36%. This result signifies a reduced risk of microbial degradation during storage, given that the ideal moisture content for crude drugs should not surpass 14% (Ebele et al., 2021). Therefore, the moisture level documented in this research is within an acceptable range. Managing moisture content is critical to preventing degradation during storage, where lower moisture levels are associated with enhanced product stability (Okonta et al., 2021).

The extractive value is ascertained through the extraction of a specified quantity of plant material with a chosen solvent, resulting in a solution that encompasses various constituents(Orumwensodia & Uadia, 2022). The interaction between the properties of both the crude drug and the solvent considerably influences the composition of the phyto-constituents present (Anibogwu et al., 2021). This extraction process is additionally pivotal in evaluating any adverse effects associated with the crude drug (Olanlokun et al., 2017). The study revealed that water yielded a higher extractive value

of 18.10% w/w, compared to 14.25% w/w obtained from ethanol. However, ethanol was utilized for extraction in this study, as water can facilitate the growth of microbial organisms during the drying process. Preliminary phytochemical screening of the leaf extracts of *Burkea africana* indicated the presence of several bioactive constituents such as Alkaloids, terpenoids, tannins, phenolics, flavonoids, and steroids. However, glycosides were recorded to be absent. This was also in line with the result reported by Zengeni et al on the ethanolic leaf extract of the plant (Zengeni et al., 2021). The limitations of this study are the lack of a toxicity profile and pharmacological evaluation of *B. africana* leaf extract to confirm its safety and efficacy. However, the study on its toxicity, antinociception, and anti-pyretic effect is still ongoing and will soon be published.

5. Conclusion

The pharmacognostic standards established for the powdered leaves of *Burkea africana* can act as a diagnostic resource for the standardization and identification of this medicinal plant, ensuring its quality and purity, while also aiding in the creation of a detailed monograph on *Burkea africana*. However, the efficacy and safety of *B. africana* is still on going.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

Animals or human was not used in the study, hence, no ethical approval was obtained.

Authors contribution

Okoh Ginikachukwu Maryrose: project design, execution and writing

Dr. Onugwu Sabastin Obinna: Editing and corrections

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