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PHARMACOGNOSTIC STANDARDIZATION AND PHYSICOCHEMICAL ANALYSIS OF THE STEM BARK OF BARTERIA NIGRITIANA HOOK F. (PASSIFLORACEAE)

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ABSTRACT

Barteria nigritiana plays a significant role in Africa in the prevention, management and treatment of ailments such as wounds, microbial infections, pains, and inflammatory disorders. Despite the therapeutic uses, the pharmacognostic features are not on record. The study was designed to standardize the pharmacognostic features of *B. nigritiana* stem bark. The fresh *B. nigritiana* stem bark was collected, air-dried under shade for 14 days and examined macroscopically using botanical and organoleptic evaluation. Microscopic, proximate, phytochemical, chemomicroscopic and fluorescence analyses were carried out using standard techniques. The powdered stem bark was extracted exhaustively with 95% methanol by cold maceration. Extraction of powdered stem bark yielded 18 %w/w of dry crude extract respectively. The macroscopic studies were rough dark grey outer surfaces and multiple trunks without thorns. Microscopic studies showed the presence of diamond-shaped calcium oxalate, fibres and phloem parenchyma cells. Chemomicroscopic studies showed the presence of cellulose, tannins, and calcium oxalate. Fluorescence analysis indicated the presence of dark green colour at 254 and deep brown colour at 365 nm. Physicochemical parameters (mg/100g) showed total ash (7.17 ± 0.03), sulphated ash (3.82 ± 0.04), acid insoluble ash (2.25 ± 0.02), alcohol soluble extractive (4.10 ± 0.01) and moisture content (9.0 ± 0.01). The pharmacognostic standards observed in this study will support the quality assurance of *B. nigritiana* stem bark.

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INTRODUCTION

Standardization is a secret code that ensures an accurate or acceptable range of drug materials in required quantities for a desired curative effect [1]. Pharmacognostic standardization of herbal drugs describes the steps involved in the preparation of herbal products from the time of identification and collection of the herbal raw materials to their packaging

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and use as medicine [2]. The pharmacognostic standards such as correct identification, macroscopic, microscopic analysis, physicochemical analysis, qualitative and quantitative studies, and fluorescence and chemomicroscopic analysis confer identity, authenticity and purity of herbal drugs [3]. According to the World Health Organization, improper and misidentification during plant collection of raw material could introduce unwanted plant species in drug preparation and to surmount this problem proper identification is essential [4].

Barteria nigritiana is a tree, 10-20 feet high, with attractive flowers enough to be a focal point in the tropics and sub-tropics owing to its white sweet scent and light-green form, alternate simple leaves and ovoid yellowish fruit, with compressed pitted seeds [5]. It is native to many African countries, Cameroon, Spanish, and Guinea and occurs wild, often found in the coastal districts. It is commonly known as ukwoifia (Igbo), perata (Ijaw), oko (Yoruba), ogeyben (Edo), in varied African languages and regions [6]. In Nigeria, B. nigritiana fruits in March and grow more than 10 m tall. The seed is dispersed by birds and its seedlings occur in heavy shade and produce hollow branches when it grows taller than 1.15 m tall. B. nigritiana is colonised by small ants while B. fistuola are colonised by big ants. B. nigritiana grows 50-100 cm per year and may die after 25-30 years of reproductive season [5].

The plant B. nigritiana has numerous ethnomedicinal uses; the stem bark decoction is used to treat psychotic and venereal diseases. The powdered leaf possesses anti-haemorrhagic activity. In many formulations, the bark is used to treat ulcerous sores, snake bites, scabies and itch. After washing with the decoction, the affected area is dusted with the powder bark. In Nigeria, the stem bark is used to treat sores, inflammation, infectious diseases and wounds African study Monography [7]. The diverse ethnomedicinal properties of B. nigritiana have placed the plant on the map of important ethnomedicine and there is an urgent need to standardize the plant for possible inclusion in the official books. The study, therefore, evaluated the physicochemical, fluorescence, chemomicroscopic and phytochemical properties of B. nigritiana.

MATERIALS AND METHODS

Chemical, reagent and equipment

Methanol, ethanol, lead subacetate, HCl, H_2SO_4, NH_4OH, $CHCl_3$, NaOH, CCl_4, and $AlCl_3$ were procured from Merk, Germany. Fehling I and II, Drangendorff, Wagner, Mayer, Hager, and Chloral hydrate solution were obtained from Apotex Chem, Canada. Phloroglucinol (SR Chem Tech, china), iodine (BDH), α- naphthol (Buyers Guide chem, India), glacial acetic acid (M and B), ethyl acetate (Visker Lab, England). The desiccator, stage micrometre, electron microscope, electronic balance, oven (Gallenkamp, England), sledge microtome, motic image plus 2.0 digital camera, muffle furnace, rotary evaporator (R-114 Buchi, Germany).

Collection and authentication of plant material

The bark of *B. nigritiana* was collected in August 2016 from Lejja (6º 51' 10" N, 7º 22' 40" E), Enugu, Nigeria. The plant material was authenticated by a taxonomist, Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The voucher (PCG/UNN/0328) of the plant material was deposited at the herbarium of the Department for future reference. The plant name and relevant botanical information were further confirmed at *http://www.theplantlist.org* during the period of collection.

Extraction of the plant material

The fresh stem bark (Figure 1) was chopped into smaller bits, rinsed in clean water and allowed to dry at 25 °C. The dried sample was reduced to 1 mm mesh size with a grinder (G×160 Delmer 5.5 HP). One kg of the powdered stem bark was extracted with 5 L of methanol (95 %v/v) using the cold maceration method. The extract was completely dried *in-vacuo* using a rotary evaporator set at 40 ºC and further exposed at 15-25 °C for 72 h and kept in air-tight glass vials in desiccators until needed for use.

Phytochemical analysis

The phytochemical tests were based on methods outlined previously [8, 9]. The tests were carried out on the crude extract and solvent fractions of the extract.

Macroscopic examination of powdered stem bark

The stem bark was visually examined. The macroscopic characters of the stem bark namely shape, surface, internode, trunk, and fracture were recorded. The organoleptic characteristics of the samples, including colour, taste, and odour, were also noted.

Transverse section of stem bark

A sledged microtome was used to cut a thin transverse piece of the stem bark, which was then cleaned in a chloral hydrate solution. Phloroglucinol in 1% HCl was used to stain the section, which was then coated with glycerine and a cover slip before being examined under a microscope. On a second slide, the thin portion that had been cleared was stained with iodine, coated with glycerine, and placed under a microscope. The tissue distribution and the structures of individual cells were photographed with motic image plus 2.0 digital cameras [10]

Quantitative phytochemical analysis

Evaluation of alkaloids

200 mL of 10% HOAc in EtOH was added to a 250 mL beaker containing 5 g of the plant material. After letting the mixture stand for 2 h, the extract was filtered, and the volume was lowered in a water bath to a quarter of its initial size. Step by step, concentrated NH_4OH was added till the precipitation

was finished. After allowing the entire solution to settle, the precipitate was gathered on filter paper and weighed. After drying and weighing the residue, the alkaloid concentration was determined using the following formula in Equation 1 [8,11]

$$Alkaloids\ (\%) = \frac{w2 - w1}{w3}\ x\ 100$$ ..... Equation 1

Evaluation of flavonoids

5 g of the sample and 100 mL of 80% aqueous MeOH were combined in a 250 mL beaker, and the mixture was allowed to stand at 25 °C for 24 h. The residue was extracted three times using 250 mL of ethanol after the supernatant was disposed of. The sample filtrate was then placed in a crucible and evaporated over a water bath to dry it [8, 10, 12]. The contents were cooled in a desiccator and then weighed until they reached a constant weight. Thereafter, the flavonoid percentage was determined using Equation 2.

$$Flavonoids\ (\%) = \frac{w2 - w1}{w3}\ x\ 100$$ ..... Equation 2

Evaluation of tannins

About 500 mg of plant material and 50 mL of water were combined in a 50 mL flask, and the mixture was spun for an hour. After the substance was filtered into a 50 mL volumetric flask, the volume was changed. After that, 5 mL of the filtrate sample was pipetted into a test tube, and 2 mL of 0.10 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide were added [8, 10,13]. The absorbance at a wavelength of 395 nm was measured within 10 min using a spectrophotometer. The same wavelength was used to measure the blank sample. The tannin content was obtained from the formula in Equation 3.

$$Tanin\ content = \frac{Absorbance\ of\ samle}{\text{Absorbance of the standard x standard concentration}}$$

..... Equation 3

Estimation of phenolic content

A 2 g powdered sample was defatted for 2 h in 100 mL of EtOAc using a Soxhlet device. The defatted sample (0.50 g) was boiled for 15 min in 50 mL of ether to extract the phenolic components. Furthermore, 5 mL of the extract was combined with 5 mL of concentrated amyl alcohol, 2 mL of 0.1 N NH_4OH solution, and 10 mL of distilled water. To produce colour, the mixture was then left to react for half an h [8, 11]. The optical density was measured at 505 nm. Tannic acid (0.20 g) was dissolved in distilled water and diluted to the 200 mL mark (1 mg/mL) to get ready for the phenol standard curve. Five different test tubes with different concentrations (0.2–1.0 mg/mL) of the standard tannic acid solution were pipetted with two millilitres of NH_4OH, five millilitres of amyl alcohol, and ten millilitres of water. The solution was combined to a 100 mL volume and left to react for 30 minutes to create the colour.

Estimation of saponin content

Five grams of the powder sample and roughly 100 mL of 20% aqueous ethanol were mixed in a hot water bath at 55 °Celsius for 4 h while being continuously agitated. After filtration, the mixture was extracted again using 100 mL of 20% aqueous ethanol and heated for 4 h at 55 °C while being continuously stirred [8, 11,14]. The extract was evaporated to 40 mL in a water bath that was heated at 90 °C. In a 250 mL separator funnel, 20 mL of diethyl ether was put into the concentration, exposing the aqueous layer and discarding the ether layer. Using 60 millilitres of n-butanol and two extractions using 10 mL of 5% NaCl, the purification procedure was carried out twice. Following the removal of the sodium chloride layer, the leftover solution was dried in a crucible under a water bath for 30 minutes. Percentages were used to represent the saponin contents shown in Equation 4.

$$\text{Saponins}\ (\%) = \frac{\text{Weight of saponins}}{\text{Weight of sample}}\ \text{x}\ 100$$

..... Equation 4

Estimation of cardiac glycoside content

In a 250 mL round-bottom flask, 1 g of the powdered sample was mixed with around 200 mL of distilled water, and the combination was let to stand for 2 h to enable autolysis. Full distillation was carried out in a 250 mL conical flask with 20 mL of 2.5% NaOH after the sample had been treated with tannic acid. The distillate or distillates were mixed with 100 mL of cardiac glycoside, 8 mL of 6 M NH_4OH, and 2 mL of 5% KI, and then titrated with 0.02 M $AgNO_3$ against a black background. The sample's cardiac glycoside content was determined using Equation 5 [8, 11,15].

$$\text{Cardiac glycoside}\ (\%) = \frac{\text{Titre value} \times 1.8 \times \text{extract volume}}{\text{Aliquot volume} \times \text{sample weight}}\ x\ 100$$ ..... Equation 5

Microscopic examination of powdered stem bark

Qualitative microscopic analysis

A tiny amount of the powdered bark sample was taken with a needle and placed on a clean glass slide. To moisten the powder and act as a clarifying agent, two drops of chloral hydrate solution were added. Until bubbles appeared, the slide was carefully moved across a Bunsen burner flame at an appropriate distance from the burner. After cooling, the slide was covered with glycerine and a cover slip before being examined under a microscope. Critical observations of the microscopic features were made, as previously described. [8, 16].

Determination of physicochemical constant

Determination of total ash values

After being heated to 575 °C for 15 minutes in a silencer furnace and chilled for approximately an hour in a desiccator, a tarred nickel crucible was weighed. The nickel crucible was filled with 3 g of the powdered material, which was then gradually heated until dried and the sample was fully burned. The sample turned grey (white ash) when increasingly heated until the carbon had evaporated and the residue was carbon-free at 650 °C. Using crucible tongs, the crucible was taken out, desiccator-cooled, and then weighed again. The amount of ash was determined using the formula in Equation 6 [8,17,18].

$$\text{Ash (\%)} = \frac{\text{Weight of the ash}}{\text{Sample weight}} x\ 100 \quad \text{..... Equation 6}$$

Determination of water-soluble ash value

After being fired to a constant weight at 450 °C, a nickel crucible was cooled and weighed. After spreading 3 g of the substance throughout the crucible's bottom, the weight was measured again. By progressively raising the temperature until it was carbon-free, the plant material was burned to 450 °C. After cooling in desiccators, the crucible was weighed again. The contents of the crucible were transferred to a beaker, and five millilitres of water were added. The mixture was then heated to a boil for five minutes. The mixture was filtered through ash-free filter paper, and the residue and filter paper were dried in an oven. The residue-containing ash-less filter paper was squeezed into the crucible and heated until the ash-less paper was removed [8,18]. After reweighing the crucible, the water-soluble ash value was determined using the formula in Equation 7.

$$\text{Water soluble ash (\%)} = \frac{\text{Sample weight} - \text{Initial weight}}{\text{Final weight of the crucible}} x\ 100$$

....Equation 7

Then, Water soluble ash (%) = total ash (%) - water insoluble ash (%)

Determination of acid-insoluble ash value

The aforementioned ash was put into a beaker with 25 millilitres of diluted HCl, and it was cooked for 5 min. An ashless filter paper and a sintered crucible were used to capture the insoluble material. Until there was no more acid, the crucible and beaker were repeatedly cleaned with hot water and filter paper. At roughly 500 °C, it was ignited to constant weight and the acid-insoluble was calculated using equation 8 [8,18].

$$\text{Acid insoluble ash (\%)} = \frac{\text{Samle weight} - \text{Initial weight}}{\text{Final weight of the crucible}} x\ 100$$

....Equation 8

Then, Acid soluble ash (%) = % Total ash (%) - Water insoluble ash (%)

Determination of extractive yield

Determination of alcohol soluble extractive value

After weighing 5 g of the substance, it was put in a conical flask with a stoppered top. After adding 100 millilitres of 90% alcohol, the conical flask's cap was securely put back in place. After being mechanically shaken for roughly 6 h, the flask and its contents were left to macerate for a further 18 h before being filtered. The residue was dried at 105 °C after the filtrate was gathered and dried by evaporation [8,18].

Determination of water-soluble extractive value

After precisely weighing 5 g of the substance, it was put in a conical flask with a stoppered top. After adding 100 mL of $CHCl_3$ water, the conical flask's stopper was securely put back in place. After 6 h of mechanical shaking, the flask and its contents were left to macerate for a further 18 h before being filtered. The residue was dried at 105 °C after the filtrate was gathered and dried by evaporation [8, 18].

Determination of moisture content

A porcelain crucible that had been heated and covered with tar was weighed and its weight was noted. After being reweighed, a spatula full of the dry sample was added to the crucible. For 12 h, the sample was heated to 65 °C in an oven at intervals of 8, 6, 4, and 2 h, until its weight remained constant [8]. It was then allowed to cool in desiccators before being weighed again. The moisture content was calculated from Equation 9.

$$\text{Moisture content (\%)} = \frac{\text{Samle weight in crucible} - \text{Constant weight}}{\text{Weight of sample}} x\ 100$$

.....Equation 9

Chemo-microscopic analysis

To find out whether different chemical constituents such as starch grains, cellulose, calcium oxalate crystals, lignin, tannins, lipids, and oil were present or absent, chemomicroscopic examination was used [19]. Two drops of iodinated $ZnCl_2$ solution (for lignin, starch grains, and cellulose), Sudan III solution of picric acid (for fibres), 80% H_2SO_4 (for calcium oxalate crystals), and $FeCl_3$ solution (for tannins) were added to each slide containing a small amount of *B. nigritiana* stem bark powder. To observe the individual changes, the slide was covered and examined using a light microscope (Olympic monocular microscope, Tokyo, magnification × 40).

Fluorescence analysis

One or two drops of freshly made solution were added to a small amount of dried powdered stem bark sample on a grease-free microscopic slide. The slide was gently tilted to mix the mixture, and the mixture was allowed to stand for 1-2

minutes. The slide was then seen in daylight, 254 nm, and 365 nm UV radiations in a UV viewer chamber. The colours seen when various reagents were applied in varied radiation levels were noted [9]

RESULTS

Extraction of stem bark

After *B. nigritiana*'s stem bark was thoroughly extracted using the cold maceration process, the total yield of the dried powdered sample was obtained. The results indicated that the extraction yielded 18 %w/w.

Qualitative phytochemical analysis

A qualitative phytochemical study was carried out on *B. nigritiana* extract to ascertain the distribution of secondary metabolites in the plant. The result (Table 1) showed that saponins were not detected.

Quantitative phytochemical analysis

The phytochemical constituents of the extract detected were further quantified. The results (Figure 2) showed that phenols (103.84 mg/100g), and tannins (80.03 mg/100g) maintained high concentration. Alkaloids (3.18 mg/100g), terpenoids (16.15 mg/100g) and flavonoids (19.30 mg/100g) were present in moderate concentration while saponins (0.07 mg/100g)) and steroids (0.39 mg/100g) were detected in minute concentration.

The macroscopic properties showed a characteristic choking odour and bitter taste with multiple trunks and a short internode (Table 2).

Microscopic properties of *B. nigritiana*

Transverse section of stem *B. nigritiana*

The external wall of *B. nigritiana* reveals a thick layer of cells termed the periderm (replacing the epidermis at maturity). The periderm is made up of the phellogen (a lateral meristem), phellen (true cork) and phelloderm (secondary cortex). Below this is a layer of polygonal-shaped cells known as the cortex, composed of collenchyma and parenchyma (Figure 3). The vascular bundles are continuous, collateral and arranged in a ring leaving a parenchymatous pith at the centre. Between the xylem and the phloem is a thin layer of actively dividing cells called the cambium. The xylem vessel was diffuse-porous in aggregates with banded axial parenchyma as shown in Figure 3A.

Microscopic examination (Figure 4) showed the presence of bundles of sclereids (Figure 4g - i) which maintains plant structure and function, calcium oxalate (Figures 4d - f) preserves the plant health, structure and function, cork cell (Figures 4m and n) that play a role in tissue toughness and cell signalling, as well as fibre (Figures 4j - l) for elasticity, conductivity and resistance to stress.

Analytical standards

The analytical standard of stem bark of *B. nigritiana* shows the percentage composition of moisture content, total ash, water-soluble extractive, acid-insoluble ash, water-soluble ash, and alcohol-soluble extractive. The results (Figure 5) show that moisture and total ash are predominantly high with 9 and 7.2 % respectively.

Chemomicroscopic analyses

Chemo microscopic analysis was conducted to examine qualitatively the presence of cellulose, tannins, calcium oxalate and other parameters. The results showed the presence of all the chemicals tested (Table 3).

Fluorescence analysis

The fluorescence analysis (Table 4) showed the various colour changes that were observed such colours as green, dark brown, black, purple, and deep brown.

DISCUSSION

For an herbal drug to be fit for consumption, pharmacognostic standards such as purity, safety, efficacy and quality control of the herbal raw materials are fundamental [19]. The first step in drug standardization is the establishment of correct identification and authentication of the plant material. According to the World Health Organization, identification, histology, morphology and purity of herbal raw material are given utmost precedence before further tests can be done [2].

Phytochemical constituents tested positive for alkaloids, tannins, terpenoids, flavonoids, and cardiac glycosides. The sample plant tested negative for the presence of saponins and anthraquinones. These findings correlate with those of the previous studies and attest to the presence and absence of some phytochemicals in the methanol extract of *B. nigritiana* bark [4,11]. In other words, Etuk and Akpan confirmed the absence of saponins in water extract

Figure 1. Images of *B. nigritiana* stem bark

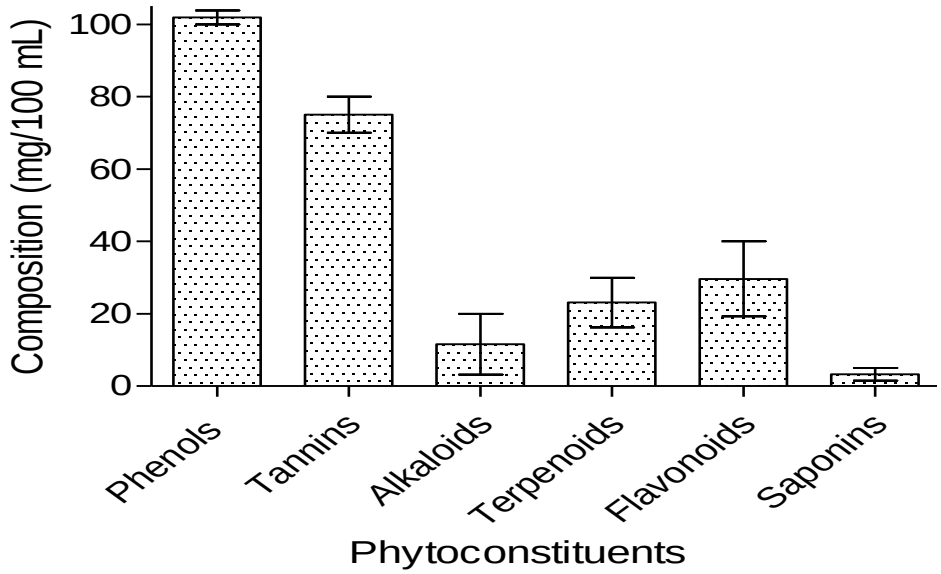

Figure 2: Quantitative phytochemical constituents of *B. nigritiana* stem bark

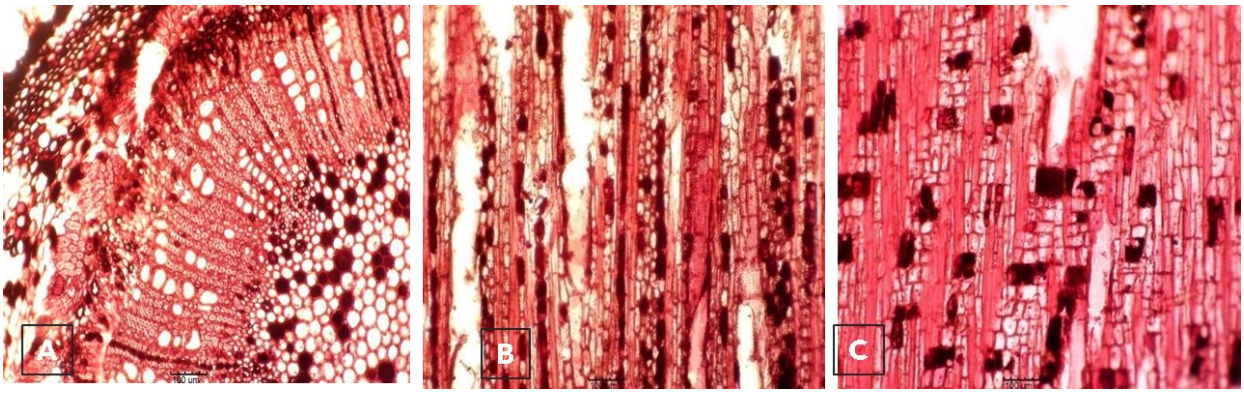

Figure 3: Sections of the stem of *Barteria nigritiana (*A = transverse, B = tangential longitudinal and C = radial longitudinal. The tangential (**Figure 3B)** and radial longitudinal sections (**Figure 3C**) are shown respectively. The TLS shows the presence of multiseriate rays and RLS shows both upright and procumbent rays (heterocellular). Vessels and fibres were also present.

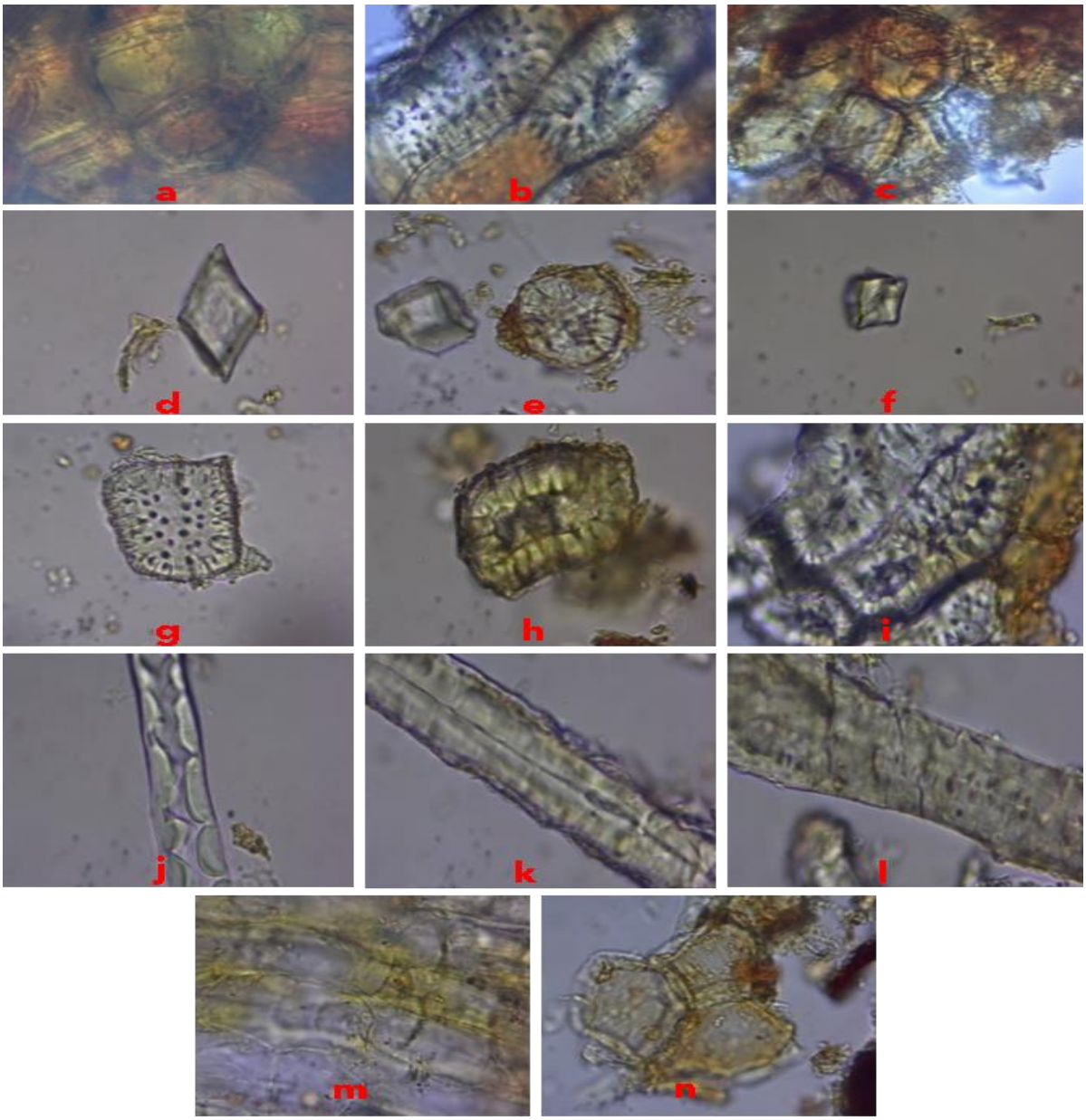

Figure 4: Powder microscopy of the stem bark of *B. nigritiana* (a = cork cells, b = underlying stone cell and c = lignified tissues., d = crystals of prisms, e = diamond and f = rectangular calcium oxalates with stone cell., g = group, h = triangular and i = rectangular sclereids., j = fibre with serrated cell wall, k = elongated fibre with narrow and l = large pitted tube., m = phloem parenchymal cell and n = long fragment of cork cell

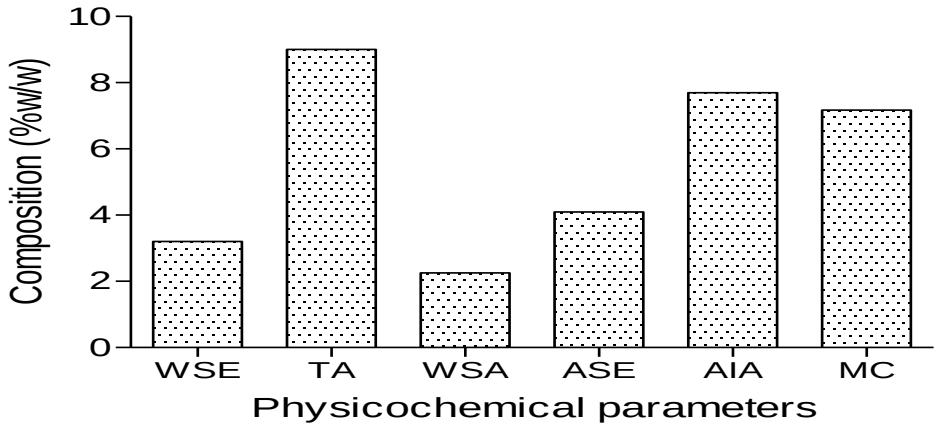

Figure 5: Physicochemical composition of *B. nigritiana* (water-soluble extractive =WSE, total ash = TA, water-soluble ash = WSA, alcohol soluble extractive = ASE, alcohol insoluble ash = AIA, moisture content = MC)

Table 1: Phytochemical constituents of stem bark

Phytochemical	Test	Remarks
Alkaloids	Dragendorff	+
Glycosides	Fehling solution I and II	+
Phenols	General test	+
Terpenoids	General test	+
Flavonoids	1 % NH_4Cl solution tests	+
Saponins	Frothing, emulsion and Fehling's tests	-
Tannins	$FeCl_3$, $Pb(OAc)_2$ tests	+
Resins	Precipitation and colour tests	+
Fats and oil	General filter paper test	+

***Key**: (-) not present, (+) present*

Table 2: Macroscopic properties of the stem bark of *B. nigritiana*

Parameters	Properties
Shape	Cylindrical when fresh but curved when dry
Odour	Choking characteristic odour
Colour	Dark brown
Taste	choking bitter
Trunk	Multiple trunks without thorns but inhabited by ants
Internode	Internode remain short
Consistency	Rough bark
Branches	10-20 cm long, hollow and slightly swollen

Table 3. Chemo microscopic analysis of an extract

Chemicals	Reagents	Observation	Inference
Cellulose	0. 002 N I_2 + 80% H_2SO_4	Prominent blue colour	Present
Tannins	70% MeOH + dilute $FeCl_3$	Blue-black colour	Present
Calcium carbonate	Acetic acid +50% H_2SO_4	Effervescence, needle-shaped crystals separated	Present
Fats and fatty oils	Sudan IV+ heat	Brick red substances	Present
Proteins	Few drops of Ninhydrin + gentle warming for 5 min	Yellow colour and scattered	Present
Lignans	Few drops of phloroglucinol + stand for 2-3mins + drop of conc. HCl	Very bright red colour	Present
Starches	A few drops of 0.02 N iodine	Slight olive-green colour	Present

Table 4: Results of fluorescence analysis

Detection Reagents	UV Wavelength (nm)	
	254	365
Petroleum ether	Dark green	Bright brown
Methanol	Dark green	Deep brown
50% Hydrochloric acid	Greenish black	Dark brown
50% sulphuric acid	Black	Purple
Ammonia	Greenish	Dark brown
Ethyl acetate	Dark green	Dark brown

with a high concentration of phenols in methanol extract [6]. The presence of these chemical constituents may be the reason for various biological activities.

The quantitative phytochemical studies show the concentrations of phenols and tannins in relatively high concentrations (103.84 and 80.03 mg/100g) respectively. Flavonoids, terpenoids and alkaloids were present in moderate to trace quantities of 40.30, 3.18 and 16.15 mg/100g. This is in line with the findings of previously reported high concentrations of phenols, flavonoids and alkaloids in the methanol extract of *B. nigritiana* [11].

Macroscopic and microscopic evaluations of crude drugs are carried out purposely for the identification of the correct variety and search for plant adulterants [20]. The stem bark of *B. nigritiana* is 10-20 cm long, has hollow and slightly swollen branches, rough bark, hard and scaly in appearance, outer bark is longitudinal, transverse cylindrical when fresh wrinkles are evident but curved when dry in shape. Multiple trunks without thorns but inhabited by ants. The internode remains short while the organoleptic characters show a dark brown colour, a rough texture with an astringent bitter choking odour. Crucial diagnostic traits for identifying both fresh and powdered crude pharmaceuticals as well as identifying adulterants in plant material are provided by anatomical features of the internal structure of plant drugs. [21].

When the plant material is employed in powdered form, the results of microscopic and microscopic examinations can be used as a diagnostic tool for accurate identification and standardization of *B. nigritiana* to avoid adulteration and substitution. Certain cell wall components and cell inclusions, including tannins, cellulose, calcium carbonate, fat, proteins, starch, lignans, and oils, were detected by chemomicroscopy of the powdered *B. nigritiana*; however, calcium oxalate was not present. These cell wall components can lessen the effects of reinforcing vascular plants, controlling development, and mechanically strengthening them [21]. To identify powdered medications based on the presence or absence of specific cell types, changes in colour, structure, and chemical reactions were crucial [22].

Utilizing the purity, stability, and phytochemical components of plant medications, physicochemical constants are crucial for standardization and quality control [23]. When compared to earlier research that reported a moisture content of 5.5%, the phytochemical parameter of the powdered stem bark of *B. nigritiana* revealed a percentage moisture content of 9.01% as evaluated by the drying or loss method [6]. The moisture content of the crude medication must generally not exceed 10 ± 2%, and the value found in this study fell within the permitted range [24]. Determining the moisture content stops product deterioration during storage; the lower the value, the

less likely the drug will degrade, which is a sign of improved stability of the product.

Ash values are one of the analytical tools for the determination of purity and quality control of crude drugs. Total ashes value shows inorganic residue left after incineration of plant drug. While the water-soluble ash is the total ash soluble in water, the acid-insoluble ash is the portion of the total ash that is soluble in diluted acid and exhibits silica [6]. In comparison to the limits of 1-3% and 2-5% for acid-insoluble and water-soluble ash, respectively, the total ash value in this study was found to be 7.17%, the acid-insoluble ash value to be 2.25%w/w, and the water-soluble ash value to be 1.5w/w. A trustworthy method for identifying drug adulteration is total value. To determine the purity state of phytoconstituents from the crude medication in a certain solvent, extractive values are one of the standardized criteria that are helpful to assess the chemical constituent predominate in the crude drug [20]. The findings of the alcohol and water extractive values *of B. nigritiana* powdered bark show that the alcohol has a higher extractive yield (4.1%w/w) than the water extractive (3.2%), indicating that more of the plant's phytoconstituents are soluble in alcohol than in water. To avoid substitution and adulteration, these extraction values can be used to identify medications that are already used and weakly effective [25]. The aforementioned pharmacognostic guidelines serve as a guide for identifying and ensuring the quality of the crude medicine produced by *B. nigritiana*.

The fluorescence analysis is one of the simplest among the parameters for pharmacognostic standardization and the essential steps in standardization with complete scientific validation of crude drugs [26]. The chemical reagents used in fluorescence analysis react with the numerous constituents in the plant, and samples and they fluorescence under light at different wavelengths short UV light at 254 nm and long UV light of 365 nm, which are distinctive for a particular drug and could be used in the findings of adulterants. Fluorescence identifies plant species, varieties or contaminants by detecting, unique fluorescence compounds, purifying plant extract, and detecting adulterants or impurities [27,28]. It creates a unique fluorescence profile for each plant, adding authentication and distinguishing between similar species. It aids in the standardization of medicinal plant extracts ensuring consistent levels of active compounds [29,30].

CONCLUSION

The study provided important phytochemical, and extractive values (ash, water and alcohol, chemo microscopic and fluorescence data for *B. nigritiana*. The pharmacognostic standards obtained in this study will support the quality assurance of *B. nigritiana* stem bark and provide important data necessary for the inclusion of *B. nigritiana* in the official compendia.

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AUTHORS' CONTRIBUTION

Conceptualization, Christopher Ezugwu and Uchenna Odoh; methodology, Charles Nnadi and Edith Diovu.; formal analysis, Charles Nnadi and Calister Ugwu.; investigation and resources, Edith Diovu; data curation, Edith Diovu; writing—original draft preparation, Edith Diovu and Calister Ugwu; writing—review and editing, Charles Nnadi, Christopher Ezugwu and Uchenna Odoh; supervision, Christopher Ezugwu and Uchenna Odoh. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors alone are responsible for the content of this research and report no conflict of interest.

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