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Original Research Article

Evaluation of the Phytochemical, Proximate and Mineral Constituents of *Breynia nivosa* Leaf

Abstract

Medicinal plants have played a key role in the world health care with about 80% of Africans depending on phytomedicine which has shown a wide range of uses in treatment of diseases. The usage of plants, plant extracts or plant derived pure chemical to treat diseases in Nigeria has become a therapeutic modality which has stood the test of time. *Breynia nivosa* which is a shrub seen around the neighbourhood has been reported to be effective in treating headache, toothaches and inflammatory diseases. This study evaluated the ethnomedicinal uses of *Breynia nivosa* by determining the phytochemical, proximate and mineral composition of the plant which could contribute to its effectiveness in the treatment of diseases. The leaf of the plant was evaluated using standard method as approved by AOAC. The result showed reasonable quantities of important chemicals such as alkaloid (3.29 ± 0.01), flavonoid (1.51 ± 0.00), oxalate (0.24± 0.02), saponin (0.79 ± 0.00) and cyanogenic glycosides (5.34 ± 0.02). The proximate values obtained include moisture content (9.31 ± 0.01), dry matter (90.65 ± 0.10), ash content (13.46 ± 0.01), crude protein (16.84 ± 0.00), and carbohydrates (47.79 ± 0.01) while the mineral content include calcium (28.42 ± 1.01), magnesium (12.52 ± 0.03) and sodium (13.68 ± 0.03). From the above result, the use of the plant for effective treatment of diseases should not be in question, since it contains appreciable phytochemicals and mineral constituents. It is therefore recommended for use in the formulation of drugs for treatments of diseases.

Key words: phytochemical, mineral, proximate composition, *Breynia nivosa*, leaf

Introduction

For many years, people depended exclusively on leaves, flowers and barks of plants for medicine. Although synthetic drugs have come into use, in many instances they are replicas or modifications of chemicals identified in plants [1]. *Breynia nivosa* is a tropical shrub, about 2m high and primarily used for its attractive foliage. It is found in gardens and publication. It has small mottled multi-coloured variegated leaves with white, green and red colouration [2]. Ethnomedically, *Breynia nivosa* is used in folklore for the treatment of headache, toothache and tooth infections [3] and in the treatment of fever and malaria by the Ibibios of Niger Delta region of Nigeria. The stem of *B. nivosa* is commonly used as chewing sticks in southeastern Nigeria [4], while the leaves are use as antimicrobials [3,5], analgesics, anti-inflammatory and antioxidants activities [3]. Amadi et al [5] reported the antibacterial activity of ethanol and hot water extracts of fresh leaves of *B. nivosa* against Streptococcus mutans isolated from dental caries patients. The results of their study confirmed the fact that herbal preparations of *B. nivosa*

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can be used for cleansing of oral cavity, maintenance of oral hygiene and prevention of dental caries. This study aimed at evaluating the phytochemical, proximate, mineral properties of *B. nivosa* leaf.

Figure 1: *Breynia nivosa* Leaves

Source: www.missouribotanicalgarden.org

Materials and Methods

Collection of Samples and Analysis: Fresh leaves of *Breynia nivosa* were harvested from old GRA (Government Reserved Area), Aba North LGA, Abia State. The plant was identified and authenticated by the taxonomist in the Department of Biology, Abia State Polytechnic, Aba. The harvested plant samples were air dried and grinded into powder. Following the procedure by Oyagade et al [6], the plant materials were air-dried in the laboratory for two days and then grounded into powder using a food blender. 50g of the powdered plant materials were soaked in 250 of 95% ethanol and allowed to stand for 48 hours for extraction of active ingredients. After 48 hours, the samples were double filtered using Whatman No 1 filter paper and porcelain cloth. The filtrates were concentrated at 40oC using rotary evaporator.

Phytochemical Screening of the Leaves of *B. Novisa* [7, 8, 9]

Test for Alkaloid: 1mL of the extract was shaken with 5mL of 2% HCl on steam bath and filtered. Into 1mL of the filtrate, Wagner's reagent was added. A reddish- brown precipitate confirmed the presence of alkaloids.

Test for Tannins: 5mL of the extract was added to 2mL of 1% HCl. Presence of red precipitates confirmed the presence of tannin.

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Test for Steroids: 1mL of the extract was dissolved in 2mL of chloroform in a test tube and then 1mL of concentrated H_2SO_4 was added. Formation of reddish brown colour at the interphase confirmed the presence of steroids.

Test for Phenols: 1mL of the extract was added into 10mL of 10% ferric chloride. The formation of a greenish-brown or black precipitate or colour is taken as presence of phenols.

Test for Flavonoids: 1mL of the extract was added in 1mL of diluted NaOH. Formation of precipitate shows the presence of flavonoids.

Test for Saponin: 10mL of the extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Cyanogenic Glycosides: The plant sample was placed in a test tube or small vial. One or two drops of water were added into the tube. Feigl-Anger strip was suspended over the material and corked. A colour change on the strip from pale blue-green to bright blue or purple showed the presence of cyanide gas.

Test for Oxalate: A part of the sample was heated with 2mL dilute sulphuric acid for 1 minute. Two drops of potassium permanganate solution was added and mix. The decolonization and evolution of bubbles confirmed the presence of oxalate.

Quantitative Determination of the Phytochemicals

Determination of Alkaloids: The gravimetric method of Harborne [7] was used in determining the alkaloids. About 5 g of the sample was dispersed in 50 mL of 10% acetic acid solution in ethanol. The mixture was shaken and allowed to stand for four hours before it was filtered. The filtrate was evaporated to quarter of its original volume. Concentrated NH4OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH4OH solution and weighed. The precipitate was dried at 60°C for 30 minutes and reweighed. By weight difference, the weight of alkaloid was determined.

Determination of Tannins: The methods of Van-Burden and Robinson [10] were employed for the determination of tannin. 0.5 g of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

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Determination of Steroids: The steroid content of B. nivosa leaves was determined using the method described by Harborne [7]. Five grams (5.0 g) of the powdered leaf sample was hydrolyzed by boiling in 50mL hydrochloricacid for 30 minutes and filtered using Whatman's filter paper (No 42). Equal volume of ethyl acetate was added. The content was properly mixed and allowed to separate into 2 layers. The ethyl acetate (extract) was recovered, while the aqueous layer was discarded. The extract was heated at 100°C for 5 minutes in a steam bath and later heated with concentrated amylalcohol to extract the steroids. The turbid mixture was filtered and dried. The dry extract was cooled and used to determine the steroids.

Determination of Phenols: This was determined using the method described by Oberlease [11]. The sample was first extracted with 0.2mL of NH_4Cl. 0.5mL of the extract solution was pipette into a test tube fitted with a ground glass stopper. 1mL of ferric solution was added and the tube heated in a boiling water bath for 30 minutes. After heating, the tube was cooled in ice water for15 minutes and allowed to adjust to room temperature. The tube was then centrifuged for 30 minutes at 300 rpm. 1mL of the supernatant was transferred to another tube and 1.5mL of 2, 2, bipyrindine solution was added and the absorbance measured at519nm against distilled water.

Determination of Flavonoids: Flavonoid in the sample was determined by the acid hydrolysis gravimetric method of Harborne [7]. Five grams of the sample was mixed with 1mL of diluted HCl in a ratio of 1:10w/v. The mixture was boiled for 30 minutes. The boiled extract was allowed to cool and filtered. 20 mL of the filtrate was treated with ethyl acetate to precipitate the flavonoids. The precipitate was measured and determined by weight difference.

Determination of Saponin: The method used was described by AOAC [12]. About 5g of dry sample was weighed inside extractor thimble and transferred into the soxhlex extractor chamber fitted with condenser and a round bottomed flask. Some quantity of acetone enough to cause a reflux was poured into the flask. The sample was exhaustively extracted of its lipid and interfering pigments for 3 hours by heating the flask on a hot plate and the solvent distilled off. This is the first extraction. For the second extraction, a pre weighed round bottomed flask was filled unto the soxhlex apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol should be enough to cause the reflux. The saponin is then exhaustively extracted for 3 hours by heating the flask on a hot plate after which the difference between the final and the initial weight of the flask represents the weight of saponin extracted.

Determination of Oxalate: Oxalate is determined by the method of Munro and Basir [13]. The oxalate was extracted with dilute HCl at 50^oC and treated with ammonium hydroxide and glacial acetic acid. Further treatment with $CaCl_2$ solution precipitated calcium oxalate, which was solubilized with hot dilute H_2SO_4 and titrated against $KMnO_4$ as equivalent to 2.2mg of oxalate.

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Determination of Hydrogen Cyanide: This was carried out according to AOAC [14]. 1g of the sample was soaked for 4 hours in distilled water. The suspension was steam-distilled into a dilute NaOH solution. The distillate was then treated with dilute KI and titrated against $AgNO_3$ to a faint and permanent turbidity. The hydrocyanate was calculated taking 1mL of 0.02 $AgNO_3$ as equivalent to 1.08mg HCN.

Determination of Proximate Composition

Determination of moisture Content: Moisture content was determined by the gravimetric method described by James [15]. Five grams (5.0 g) of the sample was weighed into weighed moisture can. The can and content were dried in the oven at 150°C for 3 hours. It was cooled in a desiccators and reweighed and the weight recorded. The sample was retained in the oven for further drying. The drying, cooling and weighing was continued repeatedly until a constant weight was obtained. The difference in weight after drying was used for the calculation of moisture content. The moisture content was calculated as shown below:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W1= weight of empty moisture can.

W2 = weight of can and sample before drying.

W3 = weight of can and sample after drying to constant weight.

% dry mass= 100- % moisture

Determination of Crude Fibre: This was determined by the Wende method [15]. Five grams of the sample was defatted and boiled in 200 mL of 1.25% H_2SO4 solution under reflux for 30 minutes. This was later washed with hot water using a two-fold muslin cloth to trap the sample particles. The sample was carefully transferred to a flask and 20 mL of 1.25% NaOH solution was added before boiling for 30 minutes and washed as before with hot water. The sample was very carefully transferred to a weighed porcelain crucible and dried in the oven at 150°C for 3 hours. After cooling in a desiccators, the sample was reweighed (W2) and then put in a muffle furnace and burned at 550°C for 2 hours until they turned ash. Again they were cooled in desiccators and reweighed. The crude fibre content was calculated gravimetrically as:

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Where W1 – weight of sample

W_2 = weight of crucible + sample after washing and drying in oven

W_3 = weight of crucible + sample as ash

Determination of Crude Protein: This was determined by kjeldahl digestion method [14]. About 0.5g of the sample was mixed with 10mL of concentrated sulphuric acid in a kjeldahl digestion flask. A tablet of selenium catalyst was added to it and the mixture was digested under a fume cupboard, until a clear solution was obtained. The mixture was carefully transferred to

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100mL flask and made up to a mark in the flask with distilled water. A 100mL of each digest was mixed with equal volume of 45% NaOH solution in kjeldahl distilling unit. The mixture was distilled and the distillate collected into 10mL of 4% boric acid solution containing 3 drops of mixed indicator, bromo cresol green and methyl red. A total of 50mL distillate was obtained and titrated against 0.02N H_2SO_4 solution. The end point is obtained from the initial green colour to a deep red point. The total nitrogen was determined and multiplied with the factor 6.25 to obtain the protein. The nitrogen content was calculated as shown below:

$$\text{Nitrogen} = \frac{100}{W} \times \frac{N \times 14}{100} \times \frac{Vf}{Va} T$$

Where, W= weight of analyzed sample

N = concentration of H_2SO_4 titrant

Vf= Total volume of digest

Va= volume of digest distilled

T= titre value- black

% crude protein = %N X 6.25

Determination of Fat Content: The method used was described by Pearson [16]. 2g of sample was wrapped in Whatman No 1 filter paper and put in a soxhlex reflux flask containing 200mL petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electro-thermal heater, it evaporates and condenses into the reflux flask. The wrapped sample was completely immersed into the solvent and remained in contact with it until the flask is filled up and siphoned over, thus carrying oil extract from the sample down to the boiling flask. The process was allowed on repeated for 4hours before the defatted sample was removed and reserved for crude fibre. The solvent was recovered and the extracting flask with its oil content dried in the oven at 60^oC for 3minutes to remove any residual solvent. After cooling in the desiccators, the flask was reweighed. By difference, the weight of fat (oil) extracted was determined and expressed as a percentage of the weighed sample.

$$\% \text{ Fat} = \frac{W_3 - W_1}{W_2} \times 100$$

Where W_1=weight of empty extraction flask

W_2= weight of sample

W_3= weight of flask and oil extract

Determination of Carbohydrates: The carbohydrate content was calculated by difference as the nitrogen free extraction (NFE), a method described by James [15]. The NFE was calculated by using the formula below:

% NFE= 100- (MC+ % ash + % CF + % EE + %CF)

Where MC= moisture content

CF= crude fibre

EE= ether extract

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CP= crude protein
Ash= % ash

Determination of Minerals: The sample for the determination of the elements was subjected to acid digestion using concentrated hydrochloric acid and subsequently, the different elements were determined using appropriate methods as described by James [15].

Statistical Analysis: The data obtained from this study were analyzed using descriptive statistics and presented as mean ± standard error of mean of three (3) determinants (mean± SEM) using the statistical software package (SPSS) for window version. Differences between means were separated using the analysis of variance (ANOVA) and multiple comparison tests. The proximate analysis was expressed in percentage (%) while the phytochemical and mineral contents were expressed in g/100 g.

Results

The result of the phytochemical composition of *B. nivosa* leaf extract is shown in Table 1. The result showed the presence of hydrogen cyanide (5.34± 0.02mg/100g), alkaloid (3.29± 0.01mg/100g), flavonoid (1.51± 0.00mg/100g), tannin (1.74±0.02mg/100g), saponin (0.79±0.00mg/100g), phenols (0.24± 0.01mg/100g), oxalate (0.24± 0.02mg/100) and steroids (0.16±0.01mg/100g).

Table 1: The Phytochemical Composition of *Breynia nivosa*

Phytochemical Composition	Quantity (mg/100g)
Alkaloid	3.29± 0.01
Flavonoid	1.51± 0.00
Oxalate	0.24± 0.02
Saponin	0.79± 0.00
Phenols	0.24± 0.01
Sterol	0.16± 0.10
Cyanogenic glycosides	5.34± 0.02
Tanin	1.74± 0.02

The quantitative proximate composition of leaf extract of *B. nivosa* is shown in Table 2. The results from this study showed *B. nivosa* had 47.79± 0.00% carbohydrate content, crude protein (16.84±0.01%), crude fiber (11.54± 0.00%), moisture (9.31±0.01%) and ash (13.46±0.01%), ether extract (1.06± 0.01%) and dry matter (90.65±0.01%).

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Table 2: Proximate Composition of *B. nivosa* Leaf Extract

Proximate Composition	Quantitative Composition (%)
Moisture content	9.31± 0.01
Dry matter	90.65± 0.01
Ash content	13.46± 0.01
Crude fibre	11.54± 0.00
Ether extract	1.06± 0.01
Crude protein	16.84± 0.01
Carbohydrate	47.79± 0.00

Table 3 shows the quantitative mineral composition of leaf extract of *B.nivosa*. Calcium was 28.42 ± 1.01 gm/100g, Sodium (13.68± 0.03mg/100g), iron (0.95 ± 0.01 mg/100 g), phosphorus (0.85 ± 0.02 mg/100 g), and magnesium (12.52± 0.03mg/100 g).

Table 3: Mineral Content of *B nivosa* Leaf Extract

Mineral Composition	Quantitative Composition (mg/100g)
Calcium	28.42± 1.01
Magnesium	12.52± 0.03
Iron	0.95± 0.01
Phosphorus	0.85± 0.02
Sodium	13.68± 0.03

Discussion

Plants have always been a component of man's health care system. This is either directly or indirectly. Directly, the plant parts or even whole plant are used in treatment of diseases. Indirectly, the plant forms a biochemical template for the eventual development of what is referred to as orthodox medicine. In this study, phytochemical, proximate and mineral composition of B. nivosa was investigated. The investigation revealed the presence of alkaloids, flavonoids, oxalate, saponin, phenols, cyanogenic glycosides, steroids and tannin in B. nivosa leaf extract. This is in line with the findings of Ezekwesili and Ogbunugafor [17]; Onyebule et al [3] and Ezemokwe et al [18]. These phytochemical compounds have been implicated in antimicrobial activity and the treatment of other human diseases [3, 19, 20]. The secondary metabolites (phytochemicals) obtained from this study were in quantities that are not necessarily toxic (Munron and Basir, [13]. According to Sodipo et al [21] most phytochemicals serve as natural antibiotics, which assist the body in fighting microbial invasion and infections. Extraction of phyto-compounds from this plant could serve as a cheap and steady means of providing this secondary metabolite in industries where they are greatly needed.

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The quantitative proximate composition also showed the leaf of *B. nivosa* is rich in carbohydrate (47.79± 0.00mg/100g), crude protein (16.84± 0.01mg/100g), ash content (13.46± 0.01) and crude fibre (11.54± 0.00).The plant was also shown to possess major mineral elements like calcium, sodium, magnesium, iron and phosphorus. The rich calcium content could be the reason why *B. nivosa* is used as chewing stick and for the treatment of toothache and tooth infections [3](Onyegbule et al., 2014).These minerals and phytochemicals may have contributed to the therapeutic properties of *B. nivosa*.

Conclusion

The tremendous potentials in complementing the use of orthodox medicine with ethno-medicine will continue to be modified in line with modern trends in therapeutics. The wide distribution of herbal medicinal plants in the tropics especially Africa, needs to be explored and researched into, due to the huge economic and socio-cultural advantages they possess. The result of this study demonstrated the rich proximate, mineral and phytochemical contents of *B. nivosa*. It is important that pharmaceutical companies explore the rich potentials of this plant

Competing Interests

Authors have declared that no competing interests exist.

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