International Journal of Biochemistry Research & Review

20(2): 1-8, 2017; Article no.IJBCRR.37965
ISSN: 2231-086X, NLM ID: 101654445

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

O. R. Ezeigbo[1*], I. Nwachukwu[1], C. A. Ike-Amadi[2] and J. B. Suleiman[3]

[1]Department of Biology/Microbiology, Abia State Polytechnic, Aba, Nigeria.
[2]Department of Chemistry/Biochemistry, Abia State Polytechnic, Aba, Nigeria.
[3]Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ORE designed the study and managed the analyses of the study. Authors IN and CAIA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JBS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/37965
Editor(s):
(1) Halit Demir, Professor, Department of Chemistry, Faculty of Art and Science Yuzuncu, Yil University, Turkey.
Reviewers:
(1) Divya S. Rajan, Christian College, India.
(2) Nandutu Agnes Masawi, Makerere University, Uganda.
(3) Zeliha Selamoglu, Nigde Ömer Halisdemir University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/22380

Original Research Article

Received 4th November 2017
Accepted 11th December 2017
Published 20th December 2017

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 chemicals 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. The extracts are natural analgesic, anti-inflammatory and antimicrobial agents and could have therapeutic potentials in management of various chronic 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 methods. 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

**Corresponding author: E-mail: obyezeigbotxt1@yahoo.com;*

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.

Keywords: Phytochemical; mineral; proximate composition; Breynia nivosa; leaf.

1. INTRODUCTION

The practice of complementary and alternative medicine is now on the increase in developing countries. This is in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine [1,2]. For many years, people depended exclusively on leaves, flowers and barks of plants for medicine. The increasing discovery of more medicinal plants has demanded for increased scientific scrutiny of their bioactivity so as to provide data that will help physicians and patients make wise decision before using them. There is increased evidence to proof that medicinal plants may represent an alternative and reliable treatment for infectious diseases. Recently, studies are conducted to demonstrate the importance of natural antioxidants in human health. The purpose of these studies is (a) to identify the important plants and extract from them and (b) to demonstrate the effect of these extracts on human and environmental health [3]. Although synthetic drugs have come into use, in many instances they are replicas or modifications of chemicals identified in plants.

Breynia nivosa is a tropical shrub, about 2 m high and primarily used for its attractive foliage. It is found in gardens and public places. It has small mottled multi-coloured variegated leaves with white, green and red colouration [4]. Ethnomedically, *Breynia nivosa* is used in folklore for the treatment of headache, toothache and tooth infections [5] 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 [6], while the leaves are use as antimicrobials [6,7], analgesic, anti-inflammatory and antioxidants activities [5]. Amadi et al. [8] 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* can be used for cleansing of oral cavity, maintenance of oral hygiene and prevention of dental caries. The active ingredients are the secondary metabolites (phytochemicals) synthesized in plants' biochemical pathways that are not necessary for plant growth but have biological and pharmacological importance [8]. These secondary metabolites, ubiquitous in plants, are considered as a main part of the human diet as well [9]. Most of these compounds present in natural foods may reduce the risk of important health defects because of their antioxidant properties [8]. This study aimed at evaluating the phytochemical, proximate, mineral properties of *B. nivosa* leaf.

Fig. 1. *Breynia nivosa* leaves
Source: www.missouribotanicalgarden.org

2. MATERIALS AND METHODS

2.1 Collection of Samples and Analysis

Fresh leaves of *B. 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. [10], the plant

materials were air-dried in the laboratory for two days and then grounded into powder using a food blender. 50 g 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 40℃ using rotary evaporator.

2.2 Phytochemical Screening of the Leaves of *B. novisa* [11-13]

2.2.1 Test for alkaloid

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

2.2.2 Test for tannins

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

2.2.3 Test for steroids

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

2.2.4 Test for phenols

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

2.2.5 Test for flavonoids

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

2.2.6 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.

2.2.7 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.

2.2.8 Test for oxalate

A part of the sample was heated with 2 mL 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.

2.3 Quantitative Determination of the Phytochemicals

2.3.1 Determination of alkaloids

The gravimetric method of Harborne [14] 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 NH_4OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off and washed with 1% NH_4OH solution and weighed. The precipitate was dried at 60℃ for 30 minutes and reweighed. By weight difference, the weight of alkaloid was determined.

2.3.2 Determination of tannins

The methods of Van-Burden and Robinson [15] 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 filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.IN HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.3.3 Determination of steroids

The steroid content of *B. nivosa* leaves was determined using the method described by Harborne [14]. Five grams (5.0 g) of the powdered leaf sample was hydrolyzed by boiling in 50 mL hydrochloric acid 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℃ 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.

2.3.4 Determination of phenols

This was determined using the method described by Oberlease [16]. The sample was first extracted with 0.2 mL of NH_4Cl. 0.5 mL of the extract solution was pipette into a test tube fitted with a ground glass stopper. 1 mL 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 for 15 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 1.5 mL of 2, 2, bipyrindine solution was added and the absorbance measured at 519 nm against distilled water.

2.3.5 Determination of flavonoids

Flavonoid in the sample was determined by the acid hydrolysis gravimetric method of Harborne [14]. 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.

2.3.6 Determination of saponin

The method used was described by AOAC [17]. About 5 g 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.

2.3.7 Determination of oxalate

Oxalate is determined by the method of Munro and Basir [18]. The oxalate was extracted with dilute HCl at 50℃ 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 2.2 mg of oxalate.

2.3.8 Determination of hydrogen cyanide

This was carried out according to AOAC [19]. 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 1 mL of 0.02 $AgNO_3$ as equivalent to 1.08 mg HCN.

2.4 Determination of Proximate Composition

2.4.1 Determination of moisture content

Moisture content was determined by the gravimetric method described by James [20]. 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℃ for 3 hours. It was cooled in 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
W_1= weight of empty moisture can.
W_2 = weight of can and sample before drying.
W_3 = weight of can and sample after drying to constant weight.
% dry mass= 100- % moisture

2.4.2 Determination of crude fibre

This was determined by the Wende method [20]. Five grams of the sample was defatted and boiled in 200 mL of 1.25% H_2SO_4 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℃ for 3 hours. After cooling in desiccators, the sample was reweighed (W_2) and then put in a muffle furnace and burned at 550℃ 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 W_1 – weight of sample
W_2 = weight of crucible + sample after washing and drying in oven
W_3 = weight of crucible + sample as ash

2.4.3 Determination of crude protein

This was determined by kjeldahl digestion method [20]. About 0.5 g 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 100mL flask and made up to a mark in the flask with distilled water. A 100 mL 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 10 mL of 4% boric acid solution containing 3 drops of mixed indicator, bromo cresol green and methyl red. A total of 50 mL 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:

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

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- blank
% crude protein = %N X 6.25

2.4.4 Determination of fat content

The method used was described by Pearson [21]. 2 g of sample was wrapped in Whatman No 1 filter paper and put in a soxhlex reflux flask containing 200 mL 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 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℃ for 3 minutes 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

2.4.5 Determination of carbohydrates

The carbohydrate content was calculated by difference as the nitrogen free extraction (NFE), a method described by James [20]. 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
CP= crude protein
Ash= % ash

2.4.6 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 [20].

2.5 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.

3. RESULTS

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

Table 1. The Phytochemical composition of *Breynia nivosa*

Phytochemical composition	Quantity (mg/100 g)
Alkaloids	3.29± 0.01
Flavonoids	1.51± 0.00
Oxalates	0.24± 0.02
Saponins	0.79± 0.00
Phenols	0.24± 0.01
Sterols	0.16± 0.10
Cyanogenic glycosides	5.34± 0.02
Tannins	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%).

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

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. Mineral content of *B nivosa* leaf extract

Mineral composition	Quantitative composition (mg/100 g)
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

4. 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 [22]; Onyebule et al. [5] and Ezemokwe et al. [23]. These phytochemical compounds have been implicated in antimicrobial activity and the treatment of other human diseases [5,24,25]. The secondary metabolites (phytochemicals) obtained from this study were in quantities that are not necessarily toxic [18]. According to Sodipo et al. [26] 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.

The quantitative proximate composition also showed the leaf of *B. nivosa* rich in carbohydrate (47.79± 0.00 mg/100 g), crude protein (16.84± 0.01 mg/100 g), 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 [6]. These minerals and phytochemicals may have contributed to the therapeutic properties of *B. nivosa*.

5. 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*. Previous studies have confirmed *B. nivosa* to possess anti-inflammatory, analgesic, and antimicrobial properties and this provides the scientific basis for the ethnomedicinal utilization of this plant. It is therefore important that pharmaceutical companies explore the rich potentials of this plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vijaya K, Ananthan S. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. J. Altern. Complement Med. 1997;3:13-20.
2. Dilhuydy JM. Patient's attraction to complementary and alternative medicine (CAM): A reality which physicians can neither ignore nor deny. Bull. Cancer. 2003;90:623-628.
3. MiKo K, JaeSung P, SangBin. Antioxidant activity and cell toxicity of pressurized liquid extracts from 20 selected plant species in Jeju, Korea. Food Chemistry. 2010;122:546-552.
4. Smith B. Flora vitiensis nova: A new flora of Fiji. National Tropical and Botanical Garden Garde, Lanai, Kania, Hawaii. 2002; 2:466-46.
5. Onyegbule FA, Ilouno IO, Eze PM, Abba CC, Chigozie VU. Evaluation of the analgesic, anti-inflammatory and antimicrobial activities of leaf extracts of *Breynia nivosa*. Chem Sci Rev Lett. 2014; 3(12):1126-1134.
6. NNMDA. Medicinal Plants of Nigeria: South-East Nigeria. Nigeria Natural Medicine Development Agency, Federal Ministry of Science and Technology. 2008;1:90.
7. Zeliha S. Polyphenolic compounds in human health with pharmacological properties. Journal of Traditional Medicine and Clinical Naturopathy. 2017;6:4.
8. Amadi ES, Oveka CA, Onveagba RA, Ugbogu OC, Okoli I. Antimicrobial screening of *Breynia nivosus* and *Ageratum conzynoides* against dental caries organisms. J Bio Sci. 2007;7(2): 354-358.
9. Gazor R, Asgari M, Pasdaran A, Mohammadghasemi F, Nasiri E, Roushan ZA. Evaluation of hepatoprotective effect of *Acantholimon gilliati* aerial partmethanolic extract. Iran J. Pharm. Res. 2015;16:135-141.
10. Oyagade JO, Awotoye JT, Adewunmi A, Thorpe HT. Antimicrobial activity of some Nigerian medicinal plants: Screening for antibacterial activity. Biological Recourses Community. 1999;11(3):193-197.
11. Harborne JB. Phytochemical methods. Chapman and Hall, Ltd., London. 1973; 49-188.
12. Trease GE, Evans WC. Pharmacognosy, Williams Charles Evans, 13th Edition. Balliere Tindall, London. 1989;176-180.
13. Sofowora A. Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. 1993;134-156.
14. Harborne JB. Methods of extraction and isolation: Phytochemical methods (3rd edition). Chapman and Hall, London. 2000; 60-66.
15. Van-Burden T, Robinson W. Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 1981;1:77.

16. Oberlease O. Phytates in: toxicant occurring naturally in food. Natural Academy of Science, Washington DC. 2003;363-371.
17. AOAC Association of Official Analytical Chemists. Method of Analysis, 17th Edition. Washington D. C. 2000;12-20.
18. Munro A, Bassir O. Oxalate in Nigerian vegetables. W. A. Journal of Biological and Applied Chemistry. 1969; 12(1):4–8.
19. AOAC Association of Official Analytical Chemists. Method of Analysis, 12th Edition. Washington D. C.; 1975.
20. James CS. Analytical chemistry of foods, 5th edition. Blackie Academic and Professional, Chapman and Hall, Western Cleddens Road Bishop Brings, Glassgow. 2000;140-144.
21. Pearson NR. Chemical analysis of foods, 7th edition. Edinburgh Churchil Livingstone. 2006;70-75.
22. Ezekwesili CN, Ogbunugafor HA. Blood glucose lowering activity of five Nigerian medicinal plants in alloxan-induced wistar albino rats. Animal Research International, 2015;12(2):2150-2158.
23. Ezemokwe IC, Onyegbule FA, Anwachaepe AU. Anti-inflammatory antimicrobial and stability studies of poly-herbal mouthwashes against Streptococcus mutans. Journal of Pharmacognosy and Phytochemistry, 2016;5(5):354-361.
24. Prasad RN, Viswanathan J, Devi R, Nayak V, Swetha VC, Archana BR, Parathasarathy N, Rajkumar J. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman.* Medicinal Plants Research. 2008;2(10):268-270.
25. Kokon JE, Koofreh D, Azare BA. Antimalarial activities of *Breynia nivosa*. Journal of Herbal Drugs. 2015;5(4):b168-172.
26. Sodipo OA, Akiniyi JA, Ogunbano US. Studies on certain characteristics of extracts of bark of *Pausinystalia johimbe* and *Pausinystalia macroceras* (K. Schum.) Pierre ex Beille. Global Journal of Pure and Applied Sciences. 2000;6(1):83–87.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22380