

Preliminary Phytochemical Screening and Proximate Analyses of Leaf Extracts of *Newbouldia laevis* (Boundary tree)

*Ushie, O. A., Longbap, B. D., Ugwuja, D. I., Iyen, S. I., Azuaga, T.I., Uba, M.

¹Department of Chemical Science,
Federal University Wukari,
Nigeria

Email: ushie@fuwukari.edu.ng

Abstract

This work is designed to enrich the available scientific data on the phytochemistry and nutrient content of N. laevis leaves. The method of cold maceration was used in the extraction by serial exhaustive extraction method. The phytochemical screening of Newbouldia laevis was using through controlled experiment. Qualitative phytochemical screening and proximate analyses of Newbouldia laevis was studied using extracts of n-hexane, ethyl acetate, acetone and methanol which were obtained extract from powdered plant part. The extracts were subjected to qualitative phytochemical screening using standard procedure and the results shows that all the phytochemicals screened for were revealed in various leaf extracts. Alkaloids and flavonoids are present in all the extracts except ethyl acetate that did not show the presence of alkaloids. Only phlobatannins and tannins were absent in all the extract, steroid is present only in acetone. The proximate analysis revealed the nutritional composition of Newbouldia laevis to be 6.03% of moisture, 7.96% of ash, 9.81% of crude protein, 16.50% of fat, 33.40%. The diversity of phytochemical present suggested that N. laevis could serve as a source of drugs.

Keywords: *Newbouldia laevis*, phytochemistry, Nutrients,

INTRODUCTION

Natural products have traditionally played a pivotal role in drug discovery as medicinal plants are the most exclusive sources of life saving drugs for the majority of the world's population (Sagwan *et al*; 2010). Medicinal plants acts as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health (Ajayi and Ojelere 2013). Wagner *et al.*, 2009 pointed out that the universal role of plant in the treatment of diseases is exemplified by their employment in all the major branches of medicine irrespective of the underlying philosophical premises through history and continued to serve as basis for many pharmaceuticals today. The world health organization (WHO) has recognized the role of herbals or traditional medicine in primary health care, especially in developing countries and encouraged members of nations to develop national policies for proper identification, sustainable, exploitation, scientific development and appropriate utilization of herbal medicine appropriate for their view

*Author for Correspondence

situation (WHO *et al.*, 2005). *Newbouldia laevis* is native to tropical africa and grows from guinea savannahs to dense forests, on moist and well-drained soils. In nigeria, the bark is chewed and swallowed for stomach pains, diarrhea and toothache (Lewis and Manony *et al.*, 1977). The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile and as a vermifuge to round worms (Usman *et al.*, 2008). It has also been found useful for earaches, sore feet, chest pain, epilepsy and children's convulsion (Akunyili *et al.*, 2000). The leaf, stem and fruits have been used for febrifuge; wound dressing and stomach ache (Iwu *et al.*; 2000).

The challenges about the claims of the medicinal plants such as lack of scientific proof for authentication and most of the foreign drugs are expensive to purchase and many were not found within our locality. Phytochemical screenings are now seen as the first step toward the discovery of useful drugs that the nature has been identified as a potential source due to its diverse presence in plants (Ogbannia *et al.*, 2013). The plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered. *Newbouldia laevis* is one the plant which is used in traditional medicine for many years. To the best of our knowledge little or no work has been done on the plant *Newbouldia laevis* in Wukari Local Government of Taraba, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and nutrient content of *N. laevis* leaves. The aim of the study is to carry out analysis on the leaves of *Newbouldia laevis* (boundary tree) with a view of identifying the phytochemical and nutrient(s) content present.

MATERIALS AND METHODS

Sample Collection and Preparation

The leaves of *Newbouldia laevis* sample were collected from its natural habitat in the main town of Wukari Taraba state, Nigeria. The leaf samples were dried for two weeks at room temperature. The powdered plant sample part were store in an air tight labeled plastic container and were used for extraction purpose. The method of cold maceration was used in the extraction by serial exhaustive extraction method as described by (Pavia 1976). This involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compounds could be extracted. The leaf extract were prepared by soaking 100g of each of the sample in 250ml hexane for four days with frequent agitation until soluble matter was dissolved. The resulting mixture was filtered using filter paper and the filtrate was concentrated by evaporation using rotary evaporator. This was then kept in a vacuum oven overnight at room temperature to remove any residual solvent before the sample was weighed. The procedure was repeated on the residue using the following solvents; chloroform, ethyl acetate, acetone and ethanol sequentially in order of polarity. The extracts were kept in the refrigerator until required for testing.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Qualitative analysis of the crude extracts were carried out as described by, Tiwari, *et al.*, 2011, Ushie and Adamu, 2013, Kendeson *et al.*, 2019 and Ushie *et al.*, 2019, to identify the presence of the classes of Secondary Metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenes, steroids, phenol, etc).

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered and were subjected to Mayer's Test and Wagner's Test. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Glycosides

Extracts were subjected to test Modified Born Trager's Test for glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of Saponins

Extracts were dissolved individually in dilute Hydrochloric acid and filtered and were subjected to Froth Test and Foam Test. Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Flavonoids

Extracts were subjected to test Alkaline Reagent and Lead acetate Test Test for flavonoids. Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Anthraquinone

About 0.5g of the extract was boiled with 2ml HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH₃ Solution was added to the mixture and heated, formation of rose-pink colour indicated the presence of anthraquinone.

Detection of Terpenoids

The extract (0.2g) was mixed with 2ml chloroform, and 3ml concentrated H₂SO₄ was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids.

Detection of Phenol

To 1ml of leaf extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for Phlobatannins

A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins.

Test for Steroids

5 drops of concentrated H₂SO₄ was added to 1ml of each extract in a test tube. The solutions were observed for a red colouration indicated the presence of steroids in the extracts.

Proximate Analysis of *Newbouldia Laevis*

The air dried powdered leaves were taken for proximate analysis. The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of the samples were determined using standard methods of the Association of Official Analytical Chemists (AOAC, 2000). The analyses were done in triplicates. The proximate values were reported in percentage. Determination of moisture content was done by weighing the sample in crucible and drying in oven at 105 °C, until a constant weight was obtained, determination of ash content was done by ashing at 550 °C for about 3 hours. The kjeldah method was used to determine the protein content by multiplication of the nitrogen value with a conversion factor of 6.25. The crude fibre content of the samples was determined by digestion method and the crude fat was done by Soxhlet extraction method. Total Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition (100 %).

RESULTS AND DISCUSSIONS

Preliminary Result for Phytochemical Screening of *Newbouldia laevis*

The hexane, ethyl acetate, acetone and methanol extracts of the leaves of *N. laevis* were screened for the presence of some phytochemicals. The ethyl acetate, acetone, hexane, and methanol extracts of the *N. laevis* revealed the presence of such as alkaloid, anthraquinones, saponins, steroids, terpenes, flavonoid, tannins, phenol, glycosides, and phlobatannins. The results showed the absence of phlobatannins in all the extracts and steroids presence in acetone extract only but absence in all other extracts. These are presented in Table 1.

Table 1: Preliminary Result for Phytochemical Screening of *Newbouldia laevis*

Test sample	Reagents	HE	EAE	AE	ME
Alkaloid	Mayer's test	+	-	+	+
	Wagner's test	+	+	+	+
Flavonoids	Lead acetate + extract	-	+	+	+
	Alkaline NaOH + Extract	+	-	+	+
Steroid	Conc H ₂ SO ₄ + Extract	-	-	+	-
Phlobatannin	Extract + (2%) HCl in boiling water	-	-	-	-
Tannins	Extract +distilled +boiling water + FeCl ₃	-	-	-	-
Phenol	Extract + Distilled water + % FeCl ₃	+	+	+	+
Terpenoids	Extract + chloroform + conc H ₂ SO ₄	+	+	+	+
Saponnin	Froth	-	-	+	+
	Foam	-	-	+	+

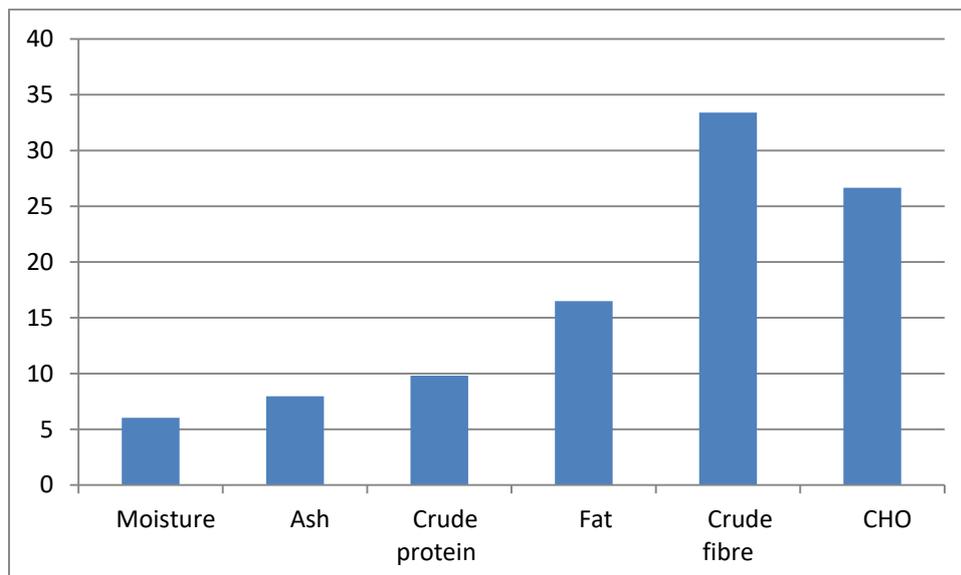
Key HE = Hexane,
EAE=Ethyl Acetate
AE =Acetone
ME = Methanol

Result for the proximate analysis of *Newbouldia laevis*

The proximate analysis of *N. laevis* leaves shows that extract was revealed to contain 6.03% of moisture content, 7.96% of ash, 9.81% of crude protein, 16.50% of fat, 33.04% of crude fibre and 26.66% of total carbohydrate. These are presented in Table 2.

Table 2 : Result for the proximate analysis of *Newbouldia laevis*

S/N	Element	Mean (%)
1	Moisture	6.03
2	Ash	7.96
3	Crude protein	9.81
4	Fat	16.50
5	Crude fibre	33.40
6	CHO	26.66



DISCUSSION

Phytochemicals are mostly secondary metabolites of plants that are often involved in plants protection against biotic or abiotic stresses. Secondary metabolites are used as especially chemical such as drugs, flavours, fragrances, insecticides, and dyes by human because of a great economic value. (Pagare *et al.*, 2015). Phytochemicals include flavonoid, tannins, saponins, alkaloid and glycosides and in this study it is revealed that *N. laevis* contains most of these constituents. They were obtained from the extract of *N. laevis* using ethyl acetate, hexane, acetone, and methanol in order of increasing polarity. The ethyl acetate, acetone, hexane, and methanol extracts of the *N. laevis* revealed the presence of such as alkaloid, anthraquinones, saponins, steroids, terpenes, flavonoid, tannins, phenol, glycosides, and phlobatannins. The results showed the absence of phlobatannins in all the extracts and steroids presence in acetone extract only but absence in all other extracts.

Saponins were detected in, acetone and methanol extracts. The presence of saponins in the leaves of *Newbouldia laevis* can be useful in treating inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Rita *et al.*, 2015). Also in nature, saponins appear to act as antibiotics that protect plants from microbes (Opara *et al.*, 2019). Terpenoids were detected in all the extracts. *Newbouldia laevis* can be used as antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Tawheed and Monika, 2014). Steroids were detected in only acetone extract. Steroids in plants have been shown to exhibits analgesic properties and responsible for central nervous system activities (Ahmed and Mohammad, 2014). Alkaloids are present in all the extracts except ethyl acetate extract. *N. laevis* can be as muscle relaxant property and

can be utilized for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive antifungal, antiinflammatory, antifibrogenic effect (Ghosal *et al.*, 1996).Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevitt *et al.*, 1996). The presence of alkaloids in the six medicinal plants makes them recommendable for patient as alkaloids possess a significant pharmacological property. The result revealed the presence of flavonoid in the extracts. Hence, *N. laevis* can be use to modifies the body's reaction to allergens, virus and caranogens. It has been reported to show anti-inflammatory, antifungi, antibacterial and antimicrobial activities based on the literature (Cushnie and Lamb 2005). Phenols are present in the extracts of *N. laevis*, thus can normally be involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as causative to plants colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet (Dai and and Mumper 2010). Also, phenolic compounds, can inhibit the absorption of amylase in the treatment of carbohydrate absorption, such as diabetes (Sales *et al.*, 2012)

The proximate analysis of *N. laevis* leaves shows that extract was revealed to contain 6.03% of moisture content, 7.96% of ash, 9.81% of crude protein, 16.50% of fat, 33.04% of crude fibre and 26.66% of total carbohydrate. Proximate analysis is conventionally used to assess the food value of feed substance (AOAC, 2000). The proximate analysis of *N. leaves* also showed it to contain protein, ash and moisture in reasonable as well as carbohydrate, fat and oil and fibre (Table 2). The leaves contain a significant amount of fibers which is necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al.*, 2000). Ash content determined is considerable high which suggest that the selected plant seeds could be good sources of mineral elements (Ajayi and Ojelre 2013). The ash content is a reflection of the amount of mineral elements present in the samples; therefore, the plants contained a good amount of minerals (Aborisade *et al.*, 2017). The leaves of *N. laevis* contain reasonable amount of carbohydrates are known to be important components in many foods, and the digestible carbohydrates are considered as an important source of energy. Carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the safeguarding and nourishment of life in plants and animals and also provide raw materials for many industries (Ebun-Oluwa and Alade 2007). The leave is a good source of carbohydrate when consumed because it meets the Recommended Dietary Allowance (RDA) values (F. N. D. (2002). The leaves of *N. laevis* also contain crude proteins as were revealed in the results. Jitendra *et al* 2013 pointed out that among nutrients, the human body requires proteins as the most important compounds because they aid in building cells and tissues and help in repairing the tissues in the body. A high protein diet is recommended for those thinking of building body or muscles. Many versatile plant proteins are used as medicinal agents as they are produced by using molecular tools of biotechnology (Jitendra *et al* 2013).

CONCLUSION

Plants have contributed immensely to the medical field and it has been the source of most drugs used for combating infections. The results obtained from the present study support the use of this plant parts in the traditional treatment. the presence of some phytochemical components which include;alkaloids, flavonoids, tannins, glycosides, steriods, phenols, terpenoids, and saponins. These phytochemicals contain medicinal properties such as anticancer, antitumor, anti-malarial, anti-diuretic, antipyretic. Also, this study has revealed

that the leaves of *Newbouldia laevis* contain nutritional components and vitamins which are essential to the body and can serve as food supplement.

RECOMMENDATION

Further research on antioxidant activities can be done on those samples, novel bioactive compounds can be derived from them after the identification, isolating the compounds and characterizing them using various spectroscopic techniques

REFERENCES

- Aborisade, A. B., Adetutu, A., Owoade, A. O. (2017) Phytochemical and Proximate Analysis of Some Medicinal Leaves. *Clinical Medicine Research*; 6(6): 209-214
- Ahmed O & Mohammad Ali., 2014. Qualitative and Quantitative Analysis of Phytochemicals of *Loranthus bengwensis* leaf. *Int. R. J. Pharm. Sci.*, 05(01): 1-3.
- Ajayi, I. A. and Ojelere, O. O. (2013) Phytochemical Screening, Proximate analysis and Anticancer Properties Molecules. 15(10): 7313-7352.
- Akunyili DN. 2nd NAAP Scientific conference (2000). Anticonvulsant Activity of the Ethanolic extract of *Newbouldia laevis*; pp. 155-158.
- AOAC 2000. Association of Official Analytical Chemists; Official method of analysis. 15th Edn, cathatica) seed. *Pakistan Journal of Nutrition*, Volume 6: 345-348.
- Cushnie T. P. and Lamb A. J. (2005). Antimicrobial activity of flavonoids: *International Journal of Antimicrobial Agents*. Volume 26:343-356.
- Dai, J. and Mumper, R. J. (2010) Plant Phenolics: Extraction, Analysis and Their Antioxidant Different Plants of North-Eastern Region of India. *J Biosci Bioeng*, 2:9-11
- Ebun-Oluwa and Alade A. S. (2007). Nutritional Potential of Berlandier Nettle spurge (*Jatropha evaluation of chemical component of leaves stalks and stems of sweet potatoes (Ipomoea batatas* poir). *Food Chem.*, 68: 359-367.
- F. N. D. (2002). Food and Nutrition Board, Institute of Medicines. National Academy of for the treatment of diarrhea in AIDS patients abstract. In program and Abstracts of the 36th Interscience conference on Antimicrobial Agents and Chemotherapy. *American Society for Microbiology*, Washington, D. C.
- Ghosal S., Krishna-Prasad B. N. and Lakshmi V. (1996). Antiamoebic activity of Piper longum fruits against *Entamoeba histolytica* in vivo. *Journal of Ethnopharmacology*. Volume 50:167-170.
- Ishida H, Suzuno H, Sugiyama N, Innami S, Todokoro T, Maekawa A (2000). Nutritional evaluation of chemical Component of leaves stalks and stems of sweet potatoes (*Ipomoea batatas* poir). *Food Chem.*, 68: 359-367
- Iwu (2001). Evaluation of the chemical composition of indigenous spices and flavoring agents. *Global J. Pure Applied Sci*. 7:455-459.
- Iwu MM. *Handbook of African Medicinal Plants*. London: CRC Press, Inc; 2000. p. 19.
- Jitendra Y. Nehete, Rajendra S. Bhambar, Minal R. Narkhede,¹ and Sonali R. Gawali (2013) Natural proteins: Sources, isolation, characterization and applications. *Pharmacogn Rev*. 7(14): 107-116.
- Kendeson A. C., Iloka S. G., Abdulkadir A. G., Ushie O. A, Abdu Z., Jibril S. & John S. T. (2019). Phytochemical Screening, Antimicrobial and Elemental Analyses of Crude Extracts from *Cocos nucifera* (Coconut) Shell. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, 5 (1b): 169 -175.
- Lewis WH, Manony PFE. *Medical Botany: Plants Affecting Man's Health*. New York, USA: John Wiley and Sons; 1977. p. 240.
- McDevitt J. T., Schneider D. M., Katiyar S. K. and Edlind F. S. (1996). Berberina: a candidate

- for the treatment of diarrhea in AIDS patients abstract. In program and Abstracts of the 36th Interscience conference on Antimicrobial Agents and Chemotherapy. *American Society for Microbiology*, Washington, D. C.
- Ogbonnia, S. O.; Mbaka, G. O.; Nwozor, A. M.; Igbokwe, H.N.; Usman, A.; Odusanya, P. A.; (2013). Evaluation of microbial purity and acute and sub-acute toxicities of a Nigerian commercial Polyherbal formulation used in the treatment of diabetes mellitus. *British Journal of Pharmaceutical Research*.3(4):948-962.
- Okwu, D.E. (2004) Phytochemical and Vitamin Content of Indigenous Species of South-Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment*, 6, 30-37.
- Opara I.J., Ushie O. A., Aondoyima.I and Onudibia M.E (2019). Phytochemical Screening, Proximate and Vitamin Composition of *Cucumis melo* Seeds (Sweet Melon). *International Journal of Research in Informative Science Application & Techniques (IJRISAT)*. Vol.No.3, Issue No.1 193122 – 193128
- Pagare, S., Bhatia, M., Tripathi, N., Pagare and Bansal, Y.K. (2015) Secondary Metabolites of Plants and their Role: Overview Current Trends in Biotechnology and Pharmacy. 9 (3) 293-304.
- Pavia, L.K. (1976). **A contemporary approach, Introduction to organic laboratory techniques**, N.B. saunders company Canada, 46, 50, 358, 599 - 605.
- Rita N., Baruah KK, Sarma S, Bhuyan R., Roy DC & Mithu D2015. Phytochemical Screening of Different Plants of North-Eastern Region of India. *J Biosci Bioeng*, 2:9-11
- Sagwan S, Rao DV and Sharma (2010). Phytochemical evaluation and quantification of primary metabolites of *Maytenus emarginata* (Willd.) Ding HOU. *J. Chem. Phar. Res.*, 2, 2010, 46-50
- Sales, P.M., Souza P.M., Simeoni L.A., Magalhães P.O., Silveira D (2012). α -Amylase Inhibitors A Review of Raw Material and Isolated Compounds from Plant Source. *J. Pharm. Pharm. Sci.* 15:141-183.
- Stray F 1998. *The natural guide to medicinal herbs and plants*. Tiger Books International, London, pp. 12-16
- Tawheed A & Monika T 2014. A comparative study on proximate composition, phytochemical screening, antioxidant and antimicrobial activities of *Linum usitatissimum* L. (flaxseeds). *Int. J. Curr. Microbiol. App. Sci.*, 3(4): 465-481.
- Usman, H. Yaro, A.H. and Garba, M.M.(2008). Analgesic and Anti-Inflammatory Screening of *Newbouldia laevis* Flower in Rodents. *Trends in Medical Research*, 3: 10-15.
- Ushie O A & Adamu. H.M (2010). Phytochemical Screening of *Borreria verticillata* Leaves.- *Journal of Agriculture, Biotechnology and Ecology* 3(1), 108-117
- Ushie, O. A., Abeng, F. E., Azuaga, T. I., Donatus, R. B., Ama, S. O. and Aikhoje, E. F. (2019). Phytochemical Screening and Bioactivity of Chloroform, Acetone and Ethyl Acetate, Extracts of *Haematostaphis Barteri*. *FUDMA Journal of Sciences (FJS)* 3(4), pp 138 -143
- World Health Organization (2005). *General guidelines for Methodologies on research and evaluation of traditional medicine*. World Health Organization. Pp.1378-1382.323