

PHYTOCHEMICAL SCREENING AND PROXIMATE ANALYSIS OF THE LEAF EXTRACTS OF *VITEX DONIANA*

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ABSTRACT

The method of cold maceration was used in the extraction by serial exhaustive extraction method which 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 compound could be extracted. The phytochemical screening of the crude extract of *Vitex doniana* showed that flavonoids, steroids, phlobatannins, alkaloids, saponins were found to be present. Alkaloids were detected in the methanol, n-Hexane, acetone and ethyl acetate extracts when Wagner's reagent was used. Flavonoids were detected in the methanol, hexane and acetone extracts when treated with NaOH and was present in all extracts with the exception of methanol extract when treated with lead acetate. Acetone extract indicated the presence of phlobatannins, but phlobatannins was absent in the hexane, ethyl acetate, and methanol extracts. Proximate analysis for the leaves of *Vitex doniana* revealed moisture content (7.04%), crude fibre (8.12%), crude protein (51.80%), ash (5.5%) fat (16.33%) and carbohydrate (11.21%) indicating high nutritional value.

INTRODUCTION

The use of plants for the treatment of diseases and maintenance of good health has been well researched. Kamba and Hassan (2010) reported that plants and plant-based products are the bases of many modern pharmaceuticals used today for the treatment of various ailments. Modern society is now embracing the use of plants and plant-based products to meet societal health needs due to the fact that indiscriminate use of commercial antibiotics commonly utilized in the treatment of infectious diseases has led to the development of multiple drug resistance with attendant adverse effect on the host (Gupta *et al.*, 2008). This emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical and veterinary applications

represents a serious public health concern (Keymanesh *et al.*, 2009). The resistance of bacteria and fungi to these drugs is becoming increasingly important (Lagnikaet *al.*, 2012). This resistance has led to the search for plants with antibacterial and antifungal activity in recent years. Other factors responsible for the use of plants in traditional as well as in modern medicine include safety, cost effectiveness (Koche *et al.*, 2011) and adulteration of synthetic drugs (Shariff, 2001). Such plants have been effectively used both in the treatment of infectious diseases to mitigated many of the side effects that are associated with synthetic antimicrobials (Perumalsamy and Ignacimuthu, 2000). The stem bark extract of *Vitex doniana* is used for the control of hypertension, treatment of stomach ache,

pains, disorders, indigestion and sterility (Ladeji and Okoye, 1996). It has also been used for the production of dyestuff for textile materials (Tadzabia, *et al.*, 2013 and Aiwonegbe *et al.*, 2017). The search for more nutrition sources among forest products has called for the proximate analysis of *Vitex doniana* (Adejumo *et al.*, 2013). Most foreign drugs are expensive to purchase and may not be easy to find in our locality. Phytochemical screenings are usually considered as the first step toward the discovery of useful drugs in which the nature has taken as a potential source to its divers in plants (Obgbannia *et al.*, 2013).

There are still over a thousand species of plants which contain substances of medicinal and nutritional value which are yet to be discovered. *Vitex doniana* is one of the plants which have been used in traditional medicine for decades of years. This study is designed to enrich the available scientific proof on the phytochemistry and proximate analysis of *V. doniana* leaves. This work was initiated to justify the claims of the traditional uses of the plants by performing a test of the active component through phytochemical screening and also to ascertain the nutritional value of *V. doniana* through proximate analysis. The results obtained from this research are useful because the extracts of *V. doniana* prove that indeed *Vitex doniana* has nutritional value and the use of this plant for medicinal purposes is safe. To the best of our knowledge little or no work has been done on the plant *Vitex doniana* in Taraba, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and nutrient content *Vitex doniana* leaves. Hence, this paper reports the phytochemistry and nutrient content *Vitex doniana* leaves on

some bacterial and fungal isolates. The aim of the study is to carry out preliminary phytochemical screening and proximate analysis on the leaves of *Vitex doniana* with a view of identifying the phytochemicals and nutrients content present.

MATERIALS AND METHOD

Sample Collection and Preparation

The leaves of *Vitex doniana* sample were collected from its natural habitat in the main town of Wukari Taraba state, Nigeria. The leaf sample was 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 extracts 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 to identify the presence of the classes of secondary metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenes, steroids, phenol, etc) as previously described (Tiwarriet *al.*, 2011; Ushie and Adamu, 2013; Kendesonet *al.*, 2019; Ushie *et al.*, 2019)

Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered and were subjected to Mayer's Test and Wagner's Test:

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

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.

Modified Born Trager's Test

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 ammoniacal 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**.

Froth 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.

Foam Test

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 Alkaline Reagent and Lead acetate for the detection of flavonoids

Alkaline Reagent Test

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.

Lead acetate Test

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 or the extract was mixed with distilled water and heated in 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. The 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 indicating the presence of steroids in the extracts.

Proximate Analysis of *Vitex doniana*

The powdered air dried leaves were taken for proximate analysis. The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of the samples were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). All determinations 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

Table 1: Results of Preliminary Phytochemicals Screening from leaves of *Vitex doniana*

Phytochemicals	Reagents	HE	EAE	AE	ME
Flavonoids	Alkaline Test	+	+	+	-
	Lead acetate test	+	-	+	+
Alkaloids	Mayer	+	-	+	+
	Wagner	+	+	+	+
Steroids	Extract+ H₂S₀₄ + Acetic acid	-	-	+	-
Tannins	Extract + H₂O + FeCl₃	+	-	-	+
Saponins	Froth test	-	-	+	+
	Foam test	+	-	+	+
Phlobatannins	Extract+ 2% HCl	-	-	+	-
Phenols	Extract+ H₂O+ FeCl₃	+	+	+	+

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

Table2 Proximate Percentage (%) Compositions of leaves of *Vitex doniana*

S/N	Element	Mean (%)
1	Moisture content	7.04
2	Crude protein	51.80
3	Crude fibre	8.12
4	Ash	5.50
5	Fat	16.33
6	Carbohydrate	11.21

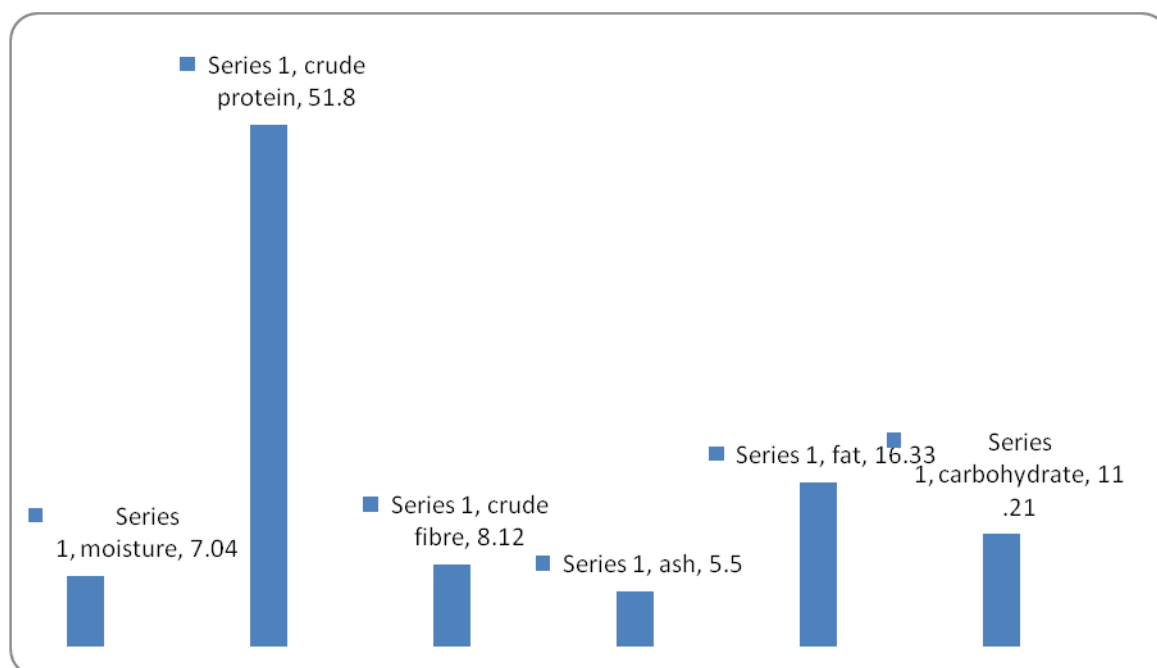


Figure 1: Histogram of Proximate % Composition of *Vitex doniana*

DISCUSSIONS

Preliminary Phytochemical Screening

The phytochemical screening of crude yields of *Vitex doniana* showed that flavonoids, steroids, phlobatannins, alkaloids, saponins were found to be present.

Tannins were found to be present in the methanol and hexane extracts. Hence, *Vitex doniana* can be a non-toxic and can they generate physiological responses in animals that consume them because of the presence of tannins (McDevitt *et al.*, 1996). The presence of tannin in the medicinal plant suggests that the *V. doniana* have muscle relaxant property and can be utilized for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Alkaloids were detected in the methanol, n-Hexane, acetone and ethyl acetate extracts. Alkaloids have been found to have microbiocidal effect and the major

anti-diarrheal effect is probably due to their effects on the small intestine and antihypertensive antifungal, antiinflammatory, antifibrogenic effect (Ghosal *et al.*, 1996). Alkaloids in plants are used in medicine as anaesthetic agents (Herourat *et al.*, 1998). Flavonoids were detected in the methanol, hexane and acetone extracts. Hence, *Vitex doniana* can be used to modify the body's reaction to allergens, virus and carcinogens. It has been reported to show anti-inflammatory, antifungal, antibacterial and antimicrobial activities based on the literature (Cushnie and Lamb, 2005).

Vitex doniana is important in pharmacy due to the presence of steroidal compounds which has a relationship with sex hormones (Okwu, 2001). Saponins were found to be present in the methanol extract and acetone extract. The presence of saponins in the seeds 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).

Phenols are present in the extracts of *Vitex doniana*, 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 Mumper, 2010). Also, phenolic compounds can inhibit the absorption of amylase in the treatment of carbohydrate absorption, such as diabetes (Sales *et al.*, 2012)

Proximate analysis is conventionally used to assess the food value of feed substance (AOAC, 2000). The proximate analysis of *Vitex doniana* 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 *Vitex doniana* contain reasonable amount of carbohydrates and are known to be important components in many foods. 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 leaf is a good source of carbohydrate when consumed because it meets the Recommended Dietary Allowance values (FND,2002). The leaves of *V. doniana* 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

From the results obtained from the analysis carried out on the leaves of this plant (*Vitex doniana*) we can conclusively say that this plant uncovers the possibility of it being a potent source of food nutrients and medicine. We can also conclude that since the leaves of *Vitex doniana* contains some significant phytochemicals such as flavonoids, phlobatannins, steroids, tannins, alkaloids, spaonins and phenols, it has some biochemical functions in the body and is an essential nutrient source. This plant is also a potent antibiotic and blood building agent as well as possesses medicinal value. Therefore, it could be

exploited for use in the formulation of cheap alternative antimicrobial drugs to cure or control human infectious diseases. This study has also showed that extracts of *Vitex doniana* contains significant levels of macro and micro nutrients i.e. carbohydrate, crude protein, crude fat, crude fibre, moisture, ash (minerals) therefore consumption of this plant is encouraged with exception of the aged due to the effect on the protein content in the liver of old people.

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
- Adejumo, O. E., Kolapo, A. L., Folain, A. O(2012). *Moringa oleifera* Lam. (Moringaceae) grown in Nigeria: *In vitro* antisickling activity on deoxygenated erythrocyte cells. *Journal of Pharmacy and Bioallied sciences*.4(2): 118-122
- Aiwonegbe, A. E., Iyasele, J.U. and Momodebe R. O. (2017). *Characterization of a natural dye produced from the alcoholic extract of African black plum (Vitex doniana) fruit pulp using wool fabric. Proceedings of the 16th Annual International Conference of Nigerian Material Congress, Material Science and Technology Society of Nigeria (MSN)*. pp105- 109.
- Ajayi, I. A. and Ojelere, O. O. (2013). *Phytochemical Screening, Proximate analysis and Anticancer Properties, Molecules*.15(10): 7313–7352.
- AOAC 2000. *Association of Official Analytical Chemists; Official method of analysis. 15th Edn, cathatica*) seed. *Pakistan Journal of Nutrition*, 6: 345-348.
- Cushnie T. P. and Lamb A. J. (2005). *Antimicrobial activity of flavonoids: International Journal of Antimicrobial Agents*. 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. Molecules* 15, 7313-7352
- 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 Laksmi V. (1996). *Antiamoebic activity of Piper longum fruits against Entamoeba histolytica in vivo. Journal of Ethnopharmacology*.50:167-170.
- Gupta, C., Amar, P., Ramesh, G., Uriya, C. and Kumari, A. 2008. *Antimicrobial activity of some herbal oils against common food-borne pathogens. African Journal of Microbiology Research*2, 258-261.

- Herourat D, Sangwin R.S, Finiaux M.A, Sangwan-Norrel B.S. (1988). Variations in the leaf alkaloid content of androgenic diploid plants of *Datura innoxia*, *Planta medica* *Journal of Medicinal Plants Research* .54:14-20.
- 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 Chemistry*, 68: 359-367
- Jitendra Y. Nehete, Rajendra S. Bhambar, Minal R. Narkhede, and Sonali R. Gawali (2013) *Natural proteins: Sources, isolation, characterization and applications. Pharmacognosy Reviews* 7(14): 107–116.
- Kamba, A.S. and Hassan, L.G. (2010). Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stem and root against some pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*(1), 57-64.
- Kenderson 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.
- Keymanesh, K., Hamedi. J., Moradi, S., Mohammadipanah, F. and Sardari, S (2009). *Antibacterial, antifungal and toxicity of rare Iranian plants. International Journal of Pharmaceutics*.5, 82-85.
- Koche, D. K., Bhadange, D. G. and Kamble, K. D. (2011). *Antimicrobial activity of three medicinal plants. Bioscience Discovery* 2(1), 69-71.
- Ladeji, O., Udo, F. V., Okoye, Z. S. C. 2004. *Activity of aqueous extract of the bark of Vitex doniana on some Uterine Muscle Response to Drugs. Phytotherapy Research* 19, 804 - 806.
- Lagnika, L., Amoussa, M., Adjovi, Y. and Sanni, A. (2012). *Antifungal, antibacterial and antioxidant properties of Adansonia digitata and Vitex doniana from Bénin pharmacopeia. J. Pharmacog. and Phytotherapy*.4(4), 44-52.
- 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 and Okwu, M. E. (2001). *Chemical composition of Spondia mombin plants. Journal of Sustainable*

- Agriculture and Environment* 6, 140-147.
- Opara I.J., Ushie O. A., Aondoyima.I and Onudibia M.E. (2019). *Phytochemical Screening, Proximate and Vitamin Composition of Cucumismelo Seeds(Sweet Melon).International Journal of Research in InformativeScience Application & Techniques (IJRISAT)*. 3(1) 193122 – 193128
- 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 K.K, Sarma S, Bhuyan R., Roy D.C and Mithu, D. (2015). *Phytochemical Screening of Different Plants of North-Eastern Region of India. Journal of Bioscience and Bioengineering* 2: 9-11
- Sales P.M., Souza P.M., Simeoni L.A., Magalhães P.O., Silveira D (2012). *α -Amylase Inhibitor: A review of Raw Material and Isolated Compounds from Plant Source. J. Pharm. Sci.* 15:141–183.
- Stray F (1998). *The natural guide to medicinal herbs and plants. Tiger Books International, London*, pp. 12-16
- Tadzabia, K., Maina, H.M., Maitera, O.N. and Osunlaja, A.A. (2013). *Elemental and physiochemical screening of Vitex doniana leaves and stem bark in Hong Local Govt. Area of Adamawa State, Nigeria.International Journal of Chemical Studies* (3), 150 -156.
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H.(2011). *Phytochemical Screening and Extraction: A Review. Internationale Pharmaceutica Scientia* 1(1):98-106.
- Ushie O A and 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