

Original Research Article

Nutritional and Phytochemical content of *Cissus populnea* (Okoho) Stem Bark

Abstract

In this study, the nutritional and chemical contents of the stem bark of *Cissus populnea* were determined. The analyses were carried out using standard methods. The proximate analyses showed that the sample contains crude protein ($1.49 \pm 0.02\%$), crude fibre ($22.13 \pm 0.62\%$), crude fat ($13.07 \pm 0.26\%$), carbohydrate ($56.04 \pm 0.78\%$), ash ($4.605 \pm 0.23\%$) and moisture ($2.665 \pm 0.33\%$). The vitamin content comprise of vitamin A (2.805 ± 0.03 mg/g), β -Carotene (1.795 ± 0.03 mg/g), vitamin B1 (0.93 ± 0.02 mg/g), vitamin B2 (1.755 ± 0.15 mg/g), vitamin B9 (1.67 ± 0.10 mg/g), vitamin C (2.235 ± 0.17 mg/g), vitamin D (2.02 ± 0.1 mg/g), vitamin E (1.41 ± 0.08 mg/g) and vitamin K (2.425 ± 0.21 mg/g). The phytochemical screening revealed the presence of alkaloids (1.551 ± 0.03 mg/g), flavonoids (0.761 ± 0.10 mg/g), saponins (2.208 ± 0.07 mg/g), steroids (1.452 ± 0.04 mg/g), tannins (1.987 ± 0.01 mg/g), terpenoids (1.530 ± 0.09 mg/g) and phenols (0.447 ± 0.06 mg/g). Glycoside was not detected. The results indicate that the stem bark of *Cissus populnea* is a good source of food nutrients and phytochemicals. These phytochemicals may be responsible for the medicinal properties of the plants.

Keywords: *Cissus populnea*, phytochemical, proximate, vitamin, stem, bark

INTRODUCTION

The use of natural products from indigenous plants as therapeutics in ethno-medicine and for nutritional purposes has led to great increase in interest among scientists to search for bioactive components [1, 2] that are beneficial to man. Recently, the interest in natural products from plants and their use has increased tremendously even in areas where conventional medicines are very much available. Medicinal plants are sources of raw materials for pharmaceutical drug formulation [3]. A significant percentage of medicinal plants used by the rural populace in Africa are affordable when compared to the high cost of conventional drugs.

Presently in Nigeria, natural products such as different parts of the plants are the most readily available sources of nutrients [4] and also serve as the most affordable source of medicine in terms of cost. Nigeria is enriched with different types of useful plants whose fruits, seeds, stems, roots and leaves serve various important roles in medicine and nutrition [5]. Among these is *Cissus populnea* [6], which belongs to the family of *Vitaceae*; a woody climber [7]. The genus *Cissus* comprises of about 350 species and it is native to west tropical Africa. In Nigeria, it is commonly found in the Northern and Southern parts. The common/local names include 'Okoho' (Idoma and Igala), 'Orogboloajara' (Yoruba) and 'Dafaaraa' (Hausa) [8]. The plant is 2 to 3m high semi-climber which grows in the savannah and is widely distributed in Senegal, Sudan, Uganda, Abyssinia and Nigeria [9]. *Cissus populnea* is a tropical medicinal plant used to correct male infertility factor in South-western part of Nigeria. The plant is also called food gum [10]. The gum is used for soup and as soup thickener. It is also widely used as medicine for the treatment of venereal diseases and indigestion and as drug binder [11]. From reports, it has antimicrobial activities which may cure many sexually transmitted infections that could be responsible for male infertility [12]. It is used as diuretic in Benin Republic [13]. Extracts from the root of the plant have been used for the treatment of skin disease, boils, infected wounds and for treating urinary tract infections, thus suggesting anti bacteria activity [14]. *Cissus populnea* are mucilaginous yielding a visci sap from a freshly cut stems. The root is used in part of Nigeria as an arrow-poison antidote [15]. It is usually powdered and added to "daddawar" batso (Hausa) [5]. In Niger, Kogi, Plateau, Kwara and Benue states of Nigeria, the plant is used for making vegetable soup for post natal stoppage of blood flow [16]. Reports from studies of herbaria collections shows that the plant is confined to the savannah zone of the country and thus is more abundant in the northern region where it is used by the Fulani to feed cattle, ostensibly to increase milk production [17]. The fibre is also used for binding material and for making papers and baskets. Although reportedly, *Cissus populnea* has been used in ethno-medicine for treatment of various diseases especially infertility, there is need to evaluate it for its nutritional and chemical compositions. This study is therefore aimed to determine the nutritional and phytochemical constituents of the stem bark of *Cissus populnea* in order to validate its nutritional and medicinal use.

MATERIALS AND METHOD

Sample Collection and Identification

Stem barks of *Cissus populnea* were collected from farmland in Gboko, Benue State, Nigeria. They were authenticated at Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Nigeria.

Sample Preparation

The stem barks were dried at room temperature to a constant weight. The dried barks were then pulverized to powder using an electric grinding machine. The powdered material was stored in air-tight containers. Five hundred grams (500g) of the powdered sample was extracted using 1500ml of distilled water for 48hrs with continuous stirring.

Proximate Analysis

Ash, crude fibre and moisture were determined according to the methods of AOAC [18]. Crude protein was determined using micro-Kjeldahl method. Carbohydrate was determined using difference method as reported by Onyeike et al [19].

Vitamin Analysis

The vitamin contents were determined using the method of AOAC [20].

Phytochemical Analysis

The qualitative and quantitative phytochemical compositions were determined.

Qualitative phytochemical analysis

The qualitative phytochemical analysis was carried out in order to ascertain the presence of plant secondary metabolites. Tannins, alkaloids, flavonoids and phenols were determined using the method of Trease and Evans [21]. Saponins, glycosides, terpenoids and steroids were determined using the method of Sofowara[22].

Quantitative phytochemical analysis

Alkaloids, terpenoids and glycosides were determined according to the method of Harbone[23]. Flavonoids and steroids were determined using the method of Bohn and Kocipal-Abyassa[24]. Tannins were determined using the method of Van-burden and Robison [25]. Saponins were determined using the method of Obdoni and Ochuko[26].

Statistical Analysis

Data was analyzed using analysis of variance (ANOVA). Results were expressed as mean \pm standard deviation (SD) of duplicate determinations.

RESULTS

Proximate Composition

The result of the proximate composition is presented in Table 1. From the result, carbohydrates were the predominant content while crude protein was the lowest.

Table 1: Proximate composition of the stem bark of *Cissus populnea*.

PARAMETERS	COMPOSITION (%)
Moisture Content	2.665±0.33
Ash content	4.605±0.23
Crude Fat	13.07±0.26
Crude Fibre	22.13±0.6
Crude Protein	1.49±0.02
Carbohydrate	56.04±0.78
Total energy (Kcal/100 g)	347.75

Values are presented as means ± SD of duplicate analysis.

Vitamin Content

The result of the vitamin analysis of *Cissus populnea* stem bark is presented in Table 2. Vitamin A was the most predominant vitamin with 16.456 % of all the vitamin content of the stem bark. Vitamin B1 was the least with only 5.456 % concentration.

Table 2: The vitamin content of *Cissus populnea* stem bark

Vitamins	Concentration	
	Mg/g	%
Vitamin A	2.805±0.03	16.456
B-Carotene	1.795±0.03	10.531
Vitamin B1	0.930±0.02	5.456
Vitamin B2	1.755±0.15	10.296
Vitamin B9	1.670±0.10	9.797
Vitamin C	2.235 ±0.17	13.113
Vitamin D	2.020±0.10	11.851

Vitamin E	1.410±0.08	8.272
Vitamin K	2.425±0.21	14.228

Values are presented as means ± SD of duplicate analysis.

Phytochemical Content

Table 3 shows the result of the qualitative and quantitative phytochemical analysis of the stem bark of *Cissus populnea*. The stem bark contained high concentrations of saponin, tannins, terpenoids, alkaloids and steroids which contributed more than 10% of the phytochemicals present. Phenols and flavonoids were the lowest with less than 10% concentrations. Glycosides were not detected.

Table 3: Qualitative and quantitative phytochemical composition of the stem bark of *Cissus populnea*.

Phytochemicals	Concentration		
	Qualitative	Quantitative (mg/g)	%
Alkaloids	++	1.551±0.03	15.579
Flavonoids	+	0.761±0.10	7.644
Saponin	+++	2.208±0.07	22.178
Steroids	++	1.473 ±0.04	14.790
Tannins	+++	1.987±0.01	19.958
Glycosides	ND	ND	ND
Terpenoids	++	1.530±0.09	15.368
Phenols	+	0.4465±0.06	4.485

Values are presented as means ± SD of duplicate analysis.

DISCUSSION

The result of the proximate analysis shown in Table 1 revealed the compositions of the carbohydrates, crude protein, crude fat, crude fibre, ash and moisture. The compositions of the constituents are in the following order: Carbohydrates (56.04%) > Crude fibre (22.13%) > Crude fat (13.07%) > Ash (4.605%) > Moisture (2.665%) > Crude protein (1.49%). These concentrations show that the stem bark of *Cissus populnea* is nutritionally rich especially in carbohydrates and crude fibre. The carbohydrate content is higher than that of the stem bark of *Maerua angolensis* (6.60%) as reported by Williams et al [27] but lower than the Recommended Dietary Allowance (RDA) of 130 g [28]. Nevertheless, the carbohydrate content of stem bark of *Cissus populnea* is moderate and it is a good source of energy as carbohydrates are the primary sources of energy that the body needs to carry out biological functions. Carbohydrates generate and supply energy to various cells in the body such as brain, muscle and blood as they are essential nutrient required for adequate diet [29, 30]. The

calorific value of *Cissus populnea* was 347.75 Kcal/100 g. This value is higher than the reported value of 169.81 Kcal/100 g for stem bark of *Maerua angolensis* and 243.26 Kcal/100 g of *Eucalyptus tereticornis* but lower when compared to 420.20 kcal/100 g and 440.70 kcal/100 g of *Ricinus communis* and *Tribulus Terrestris* L respectively [27, 31]. An average person requires 2000-3000 kcal per day [32]. Although the energy value is less than the required value but the plant can contribute greatly to the energy requirement of the body. Plants with a high calorific value can be considered as a good diet and indicates that they can be used as food or may be included as a part of dietary supplements [33]. Crude fibre was the second highest in composition. The crude fibre content of 22.13% is lower than that of stem bark of *Maerua angolensis* (48.51%) but comparable with that of *Peganum harmala* L. (21.10%) [27]. Crude fibre aids digestion and absorption of glucose and fat but its presence in high level can cause gastro-intestinal disturbances and decreased nutrient usage [34] because it is largely made up of high content of cellulose and a little lignin which is indigestible in human [35]. Increase in fiber consumption has been attributed to a decrease in the occurrence of digestive disorders and cardiovascular diseases as well as colon cancer, diabetes, hypertension and obesity [36, 37]. The crude fat content was 13.07% and relatively high. This is higher than 6.25% of *Maerua angolensis* stem bark crude fat content [27]. The high crude fat content of *Cissus populnea* is not desirable as a diet that provides 1–2 % of its calorific energy as lipid is said to be sufficient to human beings in that excess lipid consumption results in cardiovascular disorders [38] and obesity. However, lipid is needed in diet because it serves as a good source of energy, aids in transport of fat-soluble vitamins, contributes to important cell processes, insulates and protects internal tissues [39, 40]. Crude fats are the principal sources of energy. One gram provides 9.0 kcal of energy and so, 100 g of the stem bark crude fat of *Cissus populnea* should provide about 117.63 kcal of energy. Ash content is an indication of the mineral content of food [41]. From this study the ash content in *Cissus populnea* was low (4.605%). This value is lower than the reported values of 13.67% for the stem of *Ocimum gratissimum* [42] and 13.38% for the stem bark of *Maerua angolensis* [27]. The moisture content obtained was 2.665%. Moisture content is a measure of the food's water activity [43, 44]. It is an indication of stability and susceptibility of a food material microbial contamination [45]. The moisture content of the stem bark of *Cissus populnea* is lower than that of the stem bark of *Maerua angolensis* (3.58%) and within the range of values taken as safe limit for storage of plant food materials [46]. This low content indicates that it has a long shelf life and will not be prone to microbial growth. The crude protein content obtained for *Cissus populnea* was 1.49%. This is low and lower than that of the stem bark of *Maerua angolensis* (21.79%) as reported by Williams et al [27]. It is also less than the recommended dietary allowance (RDA) for protein (56 g) for individual weighing 70 kg and 46 g for adult weighing 50 kg, children may consume 18 – 20 grams/day [47]. This low protein content indicates that the stem bark of *Cissus populnea* is a poor source of protein and implies that its potential use for food and formation of animal feed is limited.

The stem bark of *Cissus populnea* contained varied concentrations of vitamins as shown in Table 2. Vitamins are micronutrients which the body needs for essential bodily functions such as enzyme reactions and in metabolic processes. In this study, vitamin A was obtained in highest concentration of 2.805 mg/g which is 16% of the total vitamin content of the stem bark of *Cissus populnea*. Vitamin A is needed for good vision, cell growth, healthy immune system and possess anti-cancer property [48]. Also, β -carotene; a precursor of vitamin A was also found in the stem bark of *Cissus populnea*. β -carotene is very important in strengthening of the immune system [49]. The B-vitamins act as co-enzymes and are involved in macronutrient metabolism. Vitamins C and E are antioxidants which protect the body cells

Formatted: Font (Default) Times New Roman, 12 pt, Italic, Font color: Black

from deleterious effects of free radicals and oxidative damage [50]. Vitamin D aids in many biological effects such as increased uptake of calcium, magnesium and phosphate [51].

Table 3 shows qualitative and quantitative phytochemical composition of the stem bark of *Cissus populnea*. The study revealed the presence of alkaloids, flavonoids, saponin, steroids, tannins, terpenoids and phenols. The presence of these phytochemicals is an indication that the stem bark of *Cissus populnea* could be a good source of bioactive compounds useful for medicinal purposes. Reports from various studies show that alkaloids possess antimalarial, anticancer, antiasthma [52], antiarrhythmic, vasodilatory [53], analgesic, hypoglycemic and antibacterial activities [54]. Flavonoids are antioxidants and possess anti-inflammatory, anti-allergic [55], anti-microbial, anti-diarrheal and anti-cancer properties. Saponins possess anti-cancer property [56], form complexes with dietary cholesterol in the intestinal walls, preventing their uptake, and hence lowering the amount of circulating cholesterol [57]. Steroids are important in pharmacology due to their relationship with sex hormones [58]. Tannins aid in speeding up blood clotting processes, reduction of blood pressure, modulation of immune-response and in reduction of plasma lipid [59]. Terpenoids are useful in the management and treatment of malaria, ulcer and cancer; also possess antimicrobial and diuretic activity [60]. Volatile terpenes are produced by plant extracts either to attract specific insects for pollination or repel certain preys which eat these plants [55]. Phenols also possess antioxidant activities and anti-carcinogenic properties [60].

CONCLUSION

The results from this study showed that the stem bark of *Cissus populnea* contained nutritive and medicinal compounds. The nutritional properties could be utilized in food supplementation for humans and animals. From the energy value and high content of carbohydrates, the plant can contribute in meeting daily energy needs. The plant also contained appreciable concentrations of vitamins which are important in metabolic processes. The antioxidant properties of the vitamins could be utilized in protecting the body tissues from deleterious effects of free radicals and the associated degenerative diseases. The presence of the phytochemicals is an evidence that the plant possess medicinal properties and this study serves as a scientific proof and validation for the numerous usage in traditional medicine.

REFERENCE

1. Oktay M, Gülçin I, Küfrevioğlu OI. Determination of invitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Journal of Advanced Scientific Research*, 2003; 36: 263 - 271.
2. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. *Food Chemistry*, 2004; 88: 293-297.
3. World Health Organisation (WHO): *Traditional Medicine Strategy*. Geneva; 2014; 10(6): 15 – 20.
4. Thompson HC, Kelly WC. *Vegetable Crops*. 5th Ed. New Delhi: Mac Graw Hill Publishing Company Ltd, 1990; 120-125.
5. Onojah, PK, Salawu, O.W, Umar .S. Proximate and PhytoChemical Screening of *Cissus populnea*. *Research Journal of Science and IT Management*. 2013; 02(3):1-5.
6. Inovova, D; D, Gerova; T, Chervenkor and T. Tankova. 2005. *J Ethnopharmacol*, pp. 154-150.

7. Li, K. (1998). Vitaceae: in lichenan (ed). *Fl. Republic popularis Sin.* 48(2): u-vu, 1-3, 12-208.
8. Burkill, H.M. . 2000. The useful plant of West tropical Africa, Vol.5 Royal Botanic Garden Ken, 296-297.
9. Hutchinson J, Dalziel J M. Flora of West tropical Africa. Second edition. Ii part 2. Millbert. London: Crown Agents for Oversea Government and Administration; 1958. pp. 672–683.
10. Chukwu, A.C.O. (1989). Primary evaluation of *Cissuspopulnea* gum as binder in sodium sachy late tablet formulation. *Drug development industry pharm.* 15(2): 325—330.
11. Iwe, M.O. and C. Atta. 1993. Functional properties of active ingredient of *Cissuspopulnea*. Guill&Perr. pp. 29-53.
12. Ojekale, A.B., Lawal, O.A., Lasisi, A.K. and Adeleke, T.S. (2004) phytochemistry and spermatogenic potential of aqueous extract of *Cissus Populnea*. Guill and perstem bark. *Sd World J.* 2006, 6: 2140 - 2146.
13. Belmain, SR, Golo, P, Andan, HF, Atarigiya, H, Chare, FA, Carr, P. “Toxicity and repellency of ethnobotanicals used in Ghana as post -harvest protectants,” *Phytoparasitic.* 2000; 28(1): 87–90.
14. Koné WM, Kamanzi Atindehou K, Terreaux C, Hostettmann K, Traoré D, Dosso M, “Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity,” *Journal of Ethnopharmacology.* 2004; 93(1): 43–49.
15. Gill LS. Ethno medicinal uses of plant in Nigeria. Uniben Press Nigeria. 1992; pp. 212.
16. Soladoye, M. and E. Chukwuma, Quantitative phytochemical profile of the leaves of *Cissuspopulnea* Guill. & Perr. (Vitaceae)-an important medicinal plant in central Nigeria. *Archives of Applied Science Research,* 2012. 4(1).
17. Brotherton JGH. The nomadic Fulani-Nigeria field. 1969, pp. 126-136.
18. AOAC. Official method of Analysis 16th Edition, Association of official Analytical Chemists, Washington D.C US. 2000;200-210.
19. Onyeike EN, Ehirim FC. Chemical and sensory evaluation of melon fungus (*Pleurotus tuber regium*) and melon funguscake. *Journal of Biochemistry and Molecular Biology.* 2001;16(1):77-81.
20. AOAC, Official methods of analysis 18th ed, Association of Official Analytical Chemists, Washington, DC, USA; 2005.
21. Trease GE, Evans MC. Textbook on pharmacognosy (13th Ed). Bailliere Tandal and Causel, London. 1989;144-148.
22. Sofowora A. Screening Plants for Bioactive Agents. In: Medicinal plants and traditional medicines in Africa. 2nd ed. Spectrum Books Ltd., Sunshine House, Ibadan. 1993;81-93:134-156.
23. Harborne JB. Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. Chapman and Hall Ltd, London. 1973;49-188.
24. Bohn BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycynium*. *Pacific Science.* 1994;48:458-463.

25. Van-Burden TP, Robinson WC. Formation of complexes between protein and tannic acid. *Journal of Agricultural Food Chemistry*. 1981;1:77.
26. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*. 2001;8b:203-208.
27. Williams ET, Nachana'a T, Attama C. Phytochemical Screening, Elemental and Proximate Analysis of *Maerua angolensis* (Capparaceae) Stem Bark. *International Journal of Biochemistry Research & Review*. 2019; 27(4): 1-10.
28. Pearson D. *Chemical analysis of foods*. (7th ed) Churchill, Livingstone, London. 1976;218-336.
29. Emebu PK, Anyika JU. Proximate and Mineral Composition of Kale (*Brassica oleracea*) Grown in Delta State, Nigeria. *Pakistan Journal of Nutrition*, 2011; 10(2): 190-194.
30. Ejelonu BC, Lasisi AA, Olaremu AG, Ejelonu OC. The chemical constituents of calabash (*Crescentia cujete*). *African Journal of Biotechnology*, 2011; 10(84): 19631-19636.
31. Ani ON, Asogwa KK, Onyishi CK, Ujah II and Ebulue MM. Nutritional profile, bioactive compound content and antioxidant activity of ethanol leaf extract of *Eucalyptus tereticornis*. *European Journal of Biomedical and Pharmaceutical sciences*. 2020; 7(5): 61-73.
32. Meda NT. Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *maerua angolensis* of Burkina Faso. *Journal of Applied Pharmaceutical Science*. 2013;3(02):036-042.
33. Datta S, Sinha BK, Bhattacharjee S, Seal T. Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. *Heliyon*, 2019; 5(3): e01431.
34. Oladiji AT, Mih FO. Proximate composition mineral and phytochemical constituents of *Eleusine coracana* (finger millet). *African Journal of Biotechnology*, 2005; 4(12): 1440-1441.
35. Mann A, Otori AA. Determination of chemical composition, minerals and antinutritional factors of two wild seeds from nupeland, north central Nigeria. *American Journal of Chemistry and Application*. 2014;1(1):20-26.
36. Food and Agriculture Organization. *Roots, Tubers, Plantains and Bananas in Human Nutrition*. FAO Corporate Document Repository, Rome, 1990. <https://www.fao.org/docrep/t0207e/T0207Eo8.htm>.
37. Scientific Advisory Committee on Nutrition. Draft SCAN position statement on dietary fiber and health and the dietary fiber definition. SACN/08/20. https://www.sacn.gov.uk/pdfs/final_draft_sacn_statement_on_dietary_fibre_2008.
38. Kris-Etherton PM., Hecker KD., Bonanome A, Coval SM., Binkoski AE., Hilpert KF., Griel AE., Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *PubMed*. 2002; 9: 71-88.
39. Pamela CC, Richard AH, Denise RF. *Lippincott's Illustrated Reviews Biochemistry* 3rd Ed, Lippincott Williams and Wilkins, Philadelphia, 2005; 335-388.

Formatted: Spanish (Mexico)

Formatted: Spanish (Mexico)

40. Jones MM, Johnson DO, Netterville JT, Wood JI, Joesten M. Chemistry and Society 5th ed. Sanders college publishers U.S.A., 1985; 521-577.
41. Olusanya, J.O, Essentials of food and nutrition. 1st edition, Apex Books limited, Lagos, 2008, pp. 36-77.
42. Idris S, Iyaka YA, Ndamitso MM, Paiko YB. Nutritional Composition of the leaves and stems of *Ocimum gratissimum*. Journal of Emerging Trends in Engineering and Applied Sciences. 2011; 2(5):801-805.
43. Olutiola PO, Famurewa O, Sonntag HG. An Introduction to General Microbiology, a Practical Approach. Germany: Heidelberg Verlaganstalt and Druckerei GmbH Heidelberg, 1999.
44. Pearson A. Vitamins in fruits. The Biochemistry of fruit and other products. Academic press; New York, 1994; 369-384.
45. Uraih, N and Izuagbe, Y. Public Health Food and Industrial Microbiology Nigeria: Uniben Press. URL:<http://www.fao.org/docrep/005/ac45/eob.htm#fn/11>. URL:<https://www.spectracell.com/media/patient-brochureheart-disease.pdf>. 1990.
46. Umar KS, Hassan LG, Ado Y. Mineral composition of *Detarium microcarpum* Grown in Kwatarkwashi, Zamfara State, Nigeria, Inter, J. Pure Appl. Sci., 2007; 1(2): 43-48.
47. Mann A, Otori AA. Determination of chemical composition, minerals and antinutritional factors of two wild seeds from nupeland, north central Nigeria. American Journal of Chemistry and Application. 2014;1(1):20-26.
48. Brett, J. Health vitamins and supplements facts, 2013. www.howstuffworks.com.
49. Achikanu CE, Ani ON, Akpata EI. Proximate, vitamin and phytochemical composition of *Cucumis metuliferus* seed. International Journal of Food Science and Nutrition. 2020; 5(3): 20-24.
50. Guyton C, Hall JE. Textbook of medical physiology. Elsevier publisher, Philadelphia, India. 2006; 11:113-115.
51. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American Journal of Clinical Nutrition. 2004; 80(6):1678S-88S.
52. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. Current Topics in Medicinal Chemistry. 2014; 14(2):239-252.
53. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A. Multitarget drugs of plants origin acting on Alzheimer's disease. Current Medicinal Chemistry. 2013; 20(13):1686-93.
54. Qiu S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y et al. Natural alkaloids: basic aspects, biological roles, and future perspectives. Chinese Journal of Natural Medicine. 2014; 12(6):401-406.
55. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. Journal of Clinical Investigation. 2001; 107(2):135-42.
56. Sun H, Xie Yong, Ye Y. Advances in saponin-based adjuvants. Vaccine. 2009; 27(12):1787-1796.
57. Onyegeme-Okerenta BM, Nwosu T, Wegwu MO. Proximate and phytochemical composition of leaf extract of *Senna alata* (L) Roxb. Journal of Pharmacognosy and Phytochemistry. 2017; 6(2):320-326.

Formatted: Italian (Italy)

Formatted: Font: (Default) Times New Roman, 12 pt, Font color: Black, Spanish (Mexico)

58. Okwu, DE. Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure Applied Science*. 2001; 7(3):455-459.
59. Chung KT, Wong TY, Wei C, Huang Y, Lin Y. Tannins and Human Health: A Review. *Critical Reviews in Food Science and Nutrition*. 1998; 38(6):421-64.
60. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and humans. *Journal of Medicinal Plants Research*. 2011; 5(31):6697-6703.

UNDER PEER REVIEW