Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 9, Issue 2, 2021

Evaluation of the Nutritional Quality and Phytochemical Properties of Okra (*Abelmoschus esculentus*, *L.*) Seed Flour

[*1]A.J. Okanya, [2]S.S. Audu, [3]O.E. Igwe

[1,3]Department of Chemistry Education, Federal College of Education, Eha-Amufu, Enugu State, Nigeria

[2]Department of Chemistry, Nasarawa State University, Keffi, Nigeria

Corresponding Author: okanyaadaeze30@gmail.com.

ABSTRACT

In this research, Okra (*Abelmoschus esculentus,* L.) seed flour was analyzed for amino acids, and its phytochemical properties using standard analytical techniques for effective utilization of this crop in various food applications. Okra seed contained useful quantities of total essential amino acids (TEAA) of 47.32 % and Predicted-Protein Efficiency Ratio (P-PER) of 2.8. Okra seed also contained phytochemicals such as flavonoids (20.86 mg/100g), alkaloids (5.69 mg/100g), saponins (16.4 mg/100g), tannins (332.78 mg/100g), oxalate (0.43%) and phenolic compounds (27.05 mg/100g). These phytochemicals have health benefits and have been associated with lowering the incidences of diet related diseases. Phytochemicals have also been proven useful in attenuating infectious conditions. . The results showed that leucine was the most concentrated essential amino acid (7.87 mg/g). Generally, okra seed flour would be a good source of strengthening the immune system and also a source of essential amino acids in food formulation.

Keywords: *Abelmoschus esculentus*, Amino acids, Malnutrition, Phytochemicals

INTRODUCTION

Vegetable seeds are of great importance as food and also as commercial products. They are very good sources of most vitamins such as vitamin A, B, C and some organic acids like folic acids. Most minerals, such as iron, calcium and phosphorus are obtained from vegetable and fruit seeds [1]. Okra (*Abelmoschus esculentus*) is a herbaceous hairy annual plant of the mallow family *malvaceae* [2]. It is native to the tropics of the Eastern Hemisphere and is believed to have originated from Ethiopia. It is known by different names in different parts of the world. It is called *gumbo* in the United States of America, *lady's finger* in England, *bhindi* in India, *kenkase* in Ethiopia and *okro* in Nigeria [3].

Nutritionally, the richest part of okra plant is the dried seed as it is very rich in protein, oil and phytochemicals [4]. It is a very good source of dietary fibre, carbohydrate, magnesium,

manganese, potassium, vitamins, lipids and folate [5]. Its seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources [6]. The seeds also possess blood glucose normalization and lipid profiles lowering action in diabetic condition [7]. Consumption of vegetable and fruit seeds can help curtail heart diseases like stroke, high blood pressure and accumulation of cholesterol [8]. Phytochemicals are bioactive compounds found in vegetables, fruits, cereals, grains and plant-based beverages such as tea and wine [9]. Phytochemical consumption is associated with a decrease in risk of several types of chronic diseases due to their antioxidant and free radical scavenging effects [10].

The objectives of this research are to: (i) determine the amino acid content of okra seed flour. The essential amino acids determined include: leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, arginine, histidine, threonine. (ii) investigate the phytochemical properties of okra seed flour. The phytochemicals determined include: saponins, tannins, alkaloids, oxalates, flavonoids, phenolic compounds.

EXPERIMENTAL

Sample collection

Fresh okra pods were bought from a farmer in a village (*Angwan lambu*) in Keffi, Nasarawa State, Nigeria. The pods were identified at the Botanical Department of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, and were taken to Multi-Environmental Management Consultants Limited, Ikorodu, Lagos, where the analyses were conducted.

Sample preparation

The mature okra pods (fresh) were sliced and the seeds removed from the pods and oven dried at 50 ^{o}C for 72 hours. Then the stones and dead seeds were handpicked. The good seeds were milled into smooth powder using an electric grinder until it could pass through a 0.425 mm sieve mesh size. It was packed into airtight polyethylene plastic bags to reduce heat build-up and finally stored in the dessicator until needed for analysis [11].

Determination of amino acids

The sample (0.5 g) was weighed into a 250 cm^3 conical flask. The sample was defatted by extracting with 30 cm^3 of the petroleum spirit three times using a soxhlet extractor equipped with a thimble. It was then hydrolysed three times in order to achieve complete hydrolysis for the totality of amino acids recovery.

The pulverised and defatted sample was soaked with 30 cm^3 of 1 cm^3 potassium hydroxide solution and was placed in a tightly closed borosilicate glass container and put in the incubator for 48 hours at 110 ^oC. Alkaline hydrolysis was carried out, after which, the hydrolysate was neutralized to get pH in the range of 2.5- 5.0. The solution was purified by cation-exchange solid-phase extraction. This cation-exchange solid-phase extraction was achieved using a particulate sorbent (cellulose fibre) packed into columns of short length (called cartridges) containing porous, siloxane-bonded silica particles, sized 50 – 60 µm to allow sample processing by gentle suction. The cation exchanger (chlorine) was packed into the column through which the solution was made to flow. The column arrangement forced the ion- exchange reaction to go to completion in the desired manner. The solution flowing down the column continually met fresh exchanger, and the reaction went halfway in the first centimeter of the column, three- quarters completed in the second centimeter. In a short time, the exchangeable ions that entered the column were adsorbed.

Determination of Amino acid score

To determine the amino acid score (AAS), the essential amino acid composition (EAAC) was divided by the provisional amino acid (egg) scoring pattern (PAAESP) according to Belschant *et al.* [12].

Written as: $$AAS = \frac{EAAC}{PAAESP} \quad (1.1)$$

Predicted Protein Efficiency Ratio (P-PER)

The predicted protein efficiency ratio was calculated according to the equation developed by Alsmeyer *et al*. [13] as shown in equation (1.2):

$$P\text{-}PER = -0.468 + 0.454(Leu) - 0.105(Tyr) \quad (1.2)$$

Phytochemical Properties

Saponins Determination

The extraction was carried out by following the modified method of *Analytical Sciences* according to the method of Guo [14]. The sample was pulverised and the saponin was extracted three times with re-distilled methanol. The saponins were removed for 20 minutes with 20 cm^3 of the solvent with the help of the sonication. The combined extracts were concentrated to syrup under reduced pressure and then suspended in water. The suspension was extracted with chloroform, petroleum ether and 1-butanol saturated with water, successively to give the respective extract after removal of the solvent. The combined extract was filtered and concentrated to 1 cm^3 in the vial for gas chromatography analysis, 1 µL of the extract was injected into the injection port of GC, and the reading for the saponin content was recorded.

Determination of Tannins

The extraction was carried out by following the modified method of Swain [15]. The pulverised sample (0.2 g) was measured into the 50 cm^3 borosilicate beaker. The 50 % methanol was added (20 cm^3) and covered with paraffin and put in a water bath at 80 ^oC for 1 hour. The content was stirred with a glass rod to avoid lumping. The extract was quantitatively filtered using a double-layer Whatmann No. 1 filter paper into a 100 cm^3 volumetric flask using 50 % methanol to rinse. This was concentrated to 2 cm^3 in the borosilicate vial for the gas chromatography analysis. 1.0 micro litre was injected into the injection port of the gas chromatography. The reading obtained was recorded for the tannin content.

Determination of Alkaloids

The alkaloid extraction was carried out according to the modified method of Ngounou *et al.* [16]. The pulverised sample was weighed and kept for analysis. The pulverised sample (5 g) was macerated in hexane of 25 cm^3 for about 72 hours. The extract was filtered and the residue air-dried. It was later treated with 10 % NH_3 and macerated in $CHCl_3$ for 24 hours. After the filtration and evaporation at reduced pressure, the crude extract which resulted was treated with 5 % aqueous HCl of about 7.5 mL. The aqueous phase was made alkaline with aqueous NH_3 and extracted three times with $CHCl_3$, the $CHCl_3$ fraction was washed with water.

The extract was poured into the round bottom flask of the rotary evaporator arrangement. It was separated by driving the solvent off the extract. Then the concentrated extract was dried of

water by using the anhydrous sodium sulphate before gas chromatography analysis. The concentration of alkanoids was obtained from the readings gotten from the GC.

Determination of Flavonoids

The flavonoids extraction was carried out according to the modified method of Millogo Kone *et al* [17]. The powdered sample (50.00 g) was weighed and transferred to a stopped flask and treated with ethanol until the powder was fully soaked. The flask was shaken every hour for the first six hours and then it was put aside and then shaken after 24 hours. This process was repeated for three days after which the extract was filtered. The extract was taken and evaporated to dryness using nitrogen steam. The concentrate (0.5 g) was weighed into a 250 cm^3 conical flask capacity with the addition of 100 cm^3 of de-ionised water and boiled for 10 min. The flavonoids extract was gotten by pouring 100 cm^3 of the boiling methanol: water (70: 300 v/v) onto the materials. The homogenate was allowed to stand for about four hours and then filtered through a filter paper (Whatmann No.1). The filtrate was derivatised for volatility in gas chromatography analysis. The mixture was concentrated to 2 cm^3 in Agilent vial for gas chromatography analysis. The concentration of flavonoids was obtained from the readings gotten from the GC.

Determination of Oxalate

The modified method of Day and Underwood [18] was employed for the analysis of oxalate. The sample (1.0 g) was weighed into a 100 cm^3 borosilicate glass flask, 75 $cm^3$of 3 mol/dm^3 H_2SO_4 was added and the solution was stirred carefully at intervals with a magnetic stirrer for about 1 hour and later filtered with Whatmann No 1 filter paper. The sample filtrate (25 cm^3) was collected and then titrated at a hot condition of 80 ^oC – 90 ^oC against 0.1mol/dm^3 $KMnO_4$ solution to the end point. A colour change was observed which showed the end point and the reading of the burette was taken when the red colour remained steady for some seconds. The concentration of oxalate (mg/g) in each of the sample was gotten by multiplying the burette reading by 11.5 (11.5 = dilution factor. The permanganate was diluted by 11.5).

Determination of Phenolic Acid

Two stage extraction procedures were employed for the effective removal of the polyphenols/ phenolic compounds:

Stage 1:

The sample (50.0 mg) was extracted using 5 $cm^3$of 1 mol/dm^3 NaOH for 16 hours on a shaker at room temperatures [19, 20]. After extraction, the sample was centrifuged (5000 X g), rinsed with water, centrifuged again and the supernatants were combined and placed in a disposable glass test tube and heated at 90 ^oC for 2 hours to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4 mol/dm^3 HCl to pH < 2.0, diluted to 10 cm^3 with deionised water and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted further in stage 2.

Stage 2:

The residue from stage 1 (above) was further extracted with 5 cm^3 of 4 mol/dm^3NaOH, and heated to 160 ^oC in Teflon. After cooling, the mixture was filtered and the supernatant was collected. The residue was then washed with deionised water. The supernatants were combined and adjusted to pH <2.0 with 4mol/dm^3 HCl. The filtrates were combined for further purification.

Purification of Extracted Phenolic Acids

An aliquot (10 cm^3) of the different supernatants were passed through a conditioned Varian (Varian Association Harber City, CA). Bond Elut PPL (3cm^3 size with 200 mg packing) solid-phase extraction tube at ~5 cm^3/min attached to a visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (0.60 KPa) until the resin was completely dried after which the phenolic acids (Pas) were eluted with 1 cm^3 of ethyl acetate into gas chromatography auto sampler vials. The Premium Power Led tubes (PPL tubes) were conditioned by first passing 2 cm^3 of ethyl acetate and then 2 cm^3 water (pH of 2.0). The phenolic acid standards used were purchased from Aldrich (Aldrich Chemical Co, Milwaukee, WI). The result for the phenolic acid was obtained from the readings gotten from the GC.

The GC conditions for the phytochemical analyses are given thus: The gas chromatography model HP 6890 powered with ChemStation Rev. A09.01 (1206) software was used. Split injection and a split ratio of 20:1 with Nitrogen as the carrier gas was applied.

These analyses were carried out at MultiEnvironmental Management Consultants Limited, Ikorodu, Lagos.

RESULTS AND DISCUSSION

Table 1: Amino acid composition (g/100g protein) of okra seed flour

Amino acid	Concentration (g/100g protein)
Leucine[e]	7.87
Lysine[e]	6.54
Isoleucine[e]	4.40
Phenylalanine[e]	3.99
Tryptophan[e]	1.14
Valine[e]	4.35
Methionine[e]	1.35
Proline	4.28
Arginine[e]	5.45
Tyrosine	2.98
Histidine[e]	2.71
Cystine	1.51
Alanine	3.67
Glutamate	15.52
Glycine	3.58
Threonine[e]	4.28
Serine	3.90
Aspartate	12.40
P-PER	2.8
Leu/ Ile	1.79

P-PER = Calculated predicted protein efficiency ratio; Leu/ Ile = Leucine to isoleucine ratio

[e] Essential amino acids

Table 2: Concentrations of total amino acids (g/100g crude protein) of okra seed flour

Total Amino Acid type	Concentration
Total Amino Acid (TAA)	88.90
Total Non-Essential Amino Acid (TNEAA)	47.84
Total Non-Essential Amino Acid (% TNEAA)	53.81
Total Essential Amino Acid (TEAA)	
(With Histidine)	42.07
(% TEAA) with Histidine	47.32
Total Essential Amino Acid (TEAA)	
(Without Histidine)	39.36
(% TEAA) without Histidine	44.27
Essential Aliphatic Amino Acid (EAAA)	80.8
Essential Aromatic Amino Acid (EArAA)	8.10
Total Neutral Amino Acid (TNAA)	62.81
Total Neutral Amino Acid (% TNAA)	70.65
Total Acidic Amino Acid (TAAA)	27.92
Total Acidic Amino Acid (% TAAA)	31.40
Total Basic Amino Acid (TBAA)	14.70
Total Basic Amino Acid (% TBAA)	16.54
Total Sulphur Amino Acid (TSAA)	2.86
% Cysteine in TSAA	52.80

Table 3: Amino acid scores of okra seed flour

EAA	[a]PAAESP (g/100g protein)	EAAC	AAS
Isoleucine	4.0	4.4	1.1
Leucine	7.0	7.9	1.1
Lysine	5.5	6.5	1.2
Met + Cys (TSAA)	3.5	2.9	0.8
Phe + Tyr	6.0	7.0	1.2
Threonine	4.0	4.3	1.1
Tryptophan	1.0	1.1	1.1
Valine	5.0	4.4	0.9
Total	36.0	38.5	8.5

EAA = Essential amino acid; PAAESP = Provisional amino acid (egg) scoring pattern

[a]Source = Belschant *et al.* `[12]; EAAC = Essential amino acid composition (see Table 4)

AAS = Amino acid score

Table 4: Phytochemical properties of okra seed flour

Parameter	Value
Saponins (mg/100g)	16.4
Tannins (mg/ 100g)	332.78
Alkaloids (mg/ 100g)	5.69
Oxalates (%)	0.43
Flavonoids (mg /100g)	20.86
Phenolic compounds (mg / 100g)	27.05

Amino acid composition of okra seed flour

The amino acid profile of okra seed is shown in Table 1, listing the concentrations of 18 amino acids. Among these amino acids, 10 essential amino acids were found. Leucine constituted the highest essential amino acid (EAA) in okra seed flour, while glutamic acid was the most concentrated amino acid as expected in vegetables [21, 22]. The least concentrated amino acid

was tryptophan (1.1). The predicted protein efficiency ratio (P–PER) is one of the quality parameters useful for protein evaluation. The P–PER value (2.8) in this report compared with values reported by Aremu *et al.* [23] for *Kerstingiella geocarpa* and *Phaseolus coccineus* protein concentrates (2.24 – 2.36). This satisfied FAO requirement [24]. The Leu/ Ile ratio in this report was 1.79. This low ratio is good because leucine /isoleucine balance is very important, rather than a dietary excess of leucine alone.

Evaluation report based on concentrations of total amino acids of okra seed flour is shown in Table 2. This is important because the nutritive value of a protein depends primarily on its capacity to satisfy the needs for nitrogen and essential amino acids [25]. Total Amino Acids (TAA), and Total Essential Amino Acids (TEAA) were 88.90 and 42.07 respectively. The value of TEAA (42.07) reported in this study agrees closely with that reported by Oshodi *et al.* [25] for TEAA of *A*. e*sculentus* (40.6). It is also similar to that of soyabean (46.5 %) [26], suggesting that *A. esculentus* contains a significant quantity of essential amino acids and is therefore a good food supplement. The Essential Aromatic Amino Acid (EArAA) was 8.1. This value falls within the ideal range (6.8 – 11.8 g/100g) suggested for infant protein by Oshodi *et al*. [26].

Table 2 also showed that the TAAA was 27.92. This was found to be higher than the TBAA (14.7), suggesting that the protein is probably acidic in nature [23]. Result of the EAA scores of okra seed flour based on the provisional amino acid scoring pattern [27] is displayed in Table 3. The essential amino acid scores greater than 1.0 were Ile, leu, lys, phe + tyr, thr and try. The limiting AAs for the sample were met + cys (TSAA) and valine. This agrees with the report of FAO/WHO [27] that the EAA most often acting in a limiting capacity are lys, met and cys.

Phytochemical Properties of Okra Seed Flour

The concentrations of the phytochemical properties of okra seed are presented in Table 4. The phytochemical analysis showed that okra seed sample contained phenolic compounds, flavonoids, alkaloids, tannins, saponins and oxalates.

The value of phenolic compounds was 27.05 mg/100g. This was higher than that of bambara groundnut seed flour (5.32 mg/g) reported by Mohammed *et al*. [28]. One of the major polyphenol characteristics is its radical-scavenging capacity, antioxidant properties, and the ability to interact with proteins [9]. The high antioxidant capacity makes polyphenols an important key factor which is involved in the chemical defence of plants against pathogens and

predators and in plant-plant interferences [29]. Arapitsas [30] reported that okra seeds are rich in phenolic compounds with important biological properties like catechin oligomers, flavonol derivatives, and hydroxycinnamic derivatives. These properties, along with the high content of proteins, carbohydrates, glycol-protein, and other dietary elements enhance the importance of this foodstuff in the human diet.

The flavonoids value was 20.86 mg/100g. This value was very much higher than that of bambara groundnut seed flour (0.67 mg/g) reported by Mohammed *et al*. [28]. Flavonoids can play an important role in decreasing disease risk through various physiological mechanisms. Some of these include antiviral, anti-inflammatory, antimicrobial, and antioxidant effects [31]. Mechanisms responsible for improvements in heart disease risk include decreased blood pressure, and improvements in lipid and insulin resistance [32]. Certain clinical studies have documented relationships between flavonoid consumption and decreased cancer risk. For example, research has shown a relationship between the reduction of colorectal cancer risk, which is the third most common type of cancer in the world, and the consumption of dietary flavonoids [33]. Additionally, the Flaviola Health Study reported that cocoa flavanol intake can improve endothelial function in those with cardiovascular risk factors and disease [9].

The level of tannins was 332.78 mg/100g. This value is much higher than the value reported for *sesbania* seed (2.25 %) [34]. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation [35]. Tannins have also been reported to exert other physiological effects, such as to reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses [36].

The saponins content was 16.4 mg/100g. This value is higher than values reported by Hossain & Becker [34] for *sesbania* seeds (1.46 %). Saponins offer tremendous health benefits. Studies have shown that they strengthen the immune system, promote normal cholesterol levels, and support overall wellness [37]. Preliminary research from a 2010 study concluded that saponins may offer a therapeutic benefit for kidney or urinary stones [38]. Saponins have also been found to promote balanced blood sugar and support normal bone density [39; 40].

The alkaloids content was 5.69 mg/100g. This value was slightly lower than that of *Lablab purpureus* seed (6.2 %) reported by Shaahu *et al*. [41]. Many of the alkaloids are useful as drugs. The drugs may be either purified natural alkaloids or chemically modified alkaloids [42].

Plants use alkaloids as defence chemical agents against herbivores and pathogens [42]. Newly discovered alkaloids such as berberine and galanthamine, used as antidiabetics and antioxidants are becoming better known [43]. Berberine regulates glucose and lipid metabolism via multiple pathways to restore insulin sensitivity [44]. Alkaloids belonging to beta-carboline group possess antimicrobial, anti-HIV and antiparasitic activities [45].

The oxalate value was 0.43 %. This value was lower than that of *soybean* (2.54 mg/g) and *pigeon pea* (2.86 mg/g) reported by Aletor and Omodara [46]. Oxalate is required in human metabolism for the formation of uracil and orotic acid. Uracil is a component of RNA which is common to all cells in the human metabolism.

CONCLUSIONS

In this study, dried okra seed flour was analysed for its nutritional composition as a healthy source of food. Its phytochemical properties were also analysed. The parameters assayed include amino acids and phytochemical composition of the seed flour. The amino acid profile of *Abelmoschus esculentus* seeds indicates that the seed flour evaluated contains all the essential amino acids necessary for human or animal nutrition. In this study, leucine constituted the highest essential amino acid, while glutamic acid was the most concentrated amino acid. The predicted protein efficiency ratio which is one of the quality parameters used for protein evaluation, is within the FAO/WHO/UNU standard. The value of TEAA (42.07) reported in this study suggests that *A. esculentus* seeds contain a significant quantity of essential amino acids and is therefore a good food supplement. The phytochemical analysis showed that okra seed sample contained phenolic compounds, flavonoids, alkaloids, oxalate, tannins and saponins. These phytochemicals possess antioxidant, anti-inflammatory, antimicrobial and antiviral properties. They provide desirable health benefits beyond basic nutrition, they play a crucial role in curtailing some chronic diseases.

REFERENCES

[1] Aremu, M.O., Opaluwa, D. O., Bamidele, T. O., Nweze, C.C., Ohale, I. M., & Ochege, M. O. (2014). Comparative evaluation of nutritive value of okra (*Abelmoschus esculentus*) and Bush mango (*Irvingia gabonensis*) fruits grown in Nasarawa State, Nigeria. *J. Food Sci. and Quality Manag.* 27, 3-8.

[2] Naveed, A., Khan, A.A., & Khan, I.A. (2009). Generation mean analysis of water stress tolerance in okra (*Abelmoschus esculentus* L.). *Pak. J. Bot*., 41, 195-205.

[3] Nzikou, J., Mvoula, T. M., & Matouba, E. (2006). A study on gumbo seed grown in Congo Brazzaville for its food and industrial applications. *Afr.J. of Biotech.,*5 (24), 2469-2475.

[4] Adelakun, O. E., Oyelade, O. J., Ade-Omowaye, B.I.O., & Adeyemi, H. (2009). Chemical composition and the antioxidative properties of Nigerian okra seed (*Abelmoschus esculentus* Moench) flour. *J. Food Chem. Toxicol*. 47(6), 1123-1126.

[5] Habtamu, F., Ratta, N., Haki, G. D., Woldegiorgis, A. Z., & Beyene, F. (2014). Nutritional quality and health benefits of okra (*Abelmoschus esculentus*).A review. *J. Food. Sci. Qual. Manag*., 33, 87–96.

[6] Oyelade, O. J., Ade-Omowaye, B. I. O., & Adeomi, V. F. (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. *J. Food Eng*., 57, 111-114.

[7] Sabitha, V., Ramachandran, S., Naveen, K. R., & Panneerselvam, K. (2011). Antidiabetic and antihyperlipidemic potential of *Abelmoschus esculentus* (L.) Moench.in streptozotocin-induced diabetic rats. *J Pharm Bioallied Sci.,* 3(3), 397 - 402.

[8] Etudeko, I. (2003). Conventional and traditional use of plants. Ethnobotany. The Verdict Press, p. 55.

[9] Ashley, A. T. (2016). Nutrition. http://nutrition.ucdavis.edu/centres-programs/rhnc. Accessed August 22, 2021.

[10] Zhang, Y. J. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *J. Molecules* 20(12), 21138-21156.

[11] Aremu, M.O., Opaluwa, D. O., Bamidele, T. O., Nweze, C.C., Ohale, I. M., & Ochege, M. O. (2014). Comparative evaluation of nutritive value of okra (*Abelmoschus esculentus*) and Bush mango (*Irvingia gabonensis*) fruits grown in Nasarawa State, Nigeria. *J. Food Sci. and Quality Manag.,* 27, 3-8.

[12] Belschant, A.A., Lyon, C. K., & Kohler, G. O. (1975). Sunflower, safflower, sesame and castor proteins in food protein sources. Cambridge University Press, Cambridge, UK. Pp., 79-104.

[13] Alsmeyer, R. H., Cunigham, A. E., & Hapich, M. I. (1974). Equations predicted (PER) from amino acid analysis. *J.Food Tech*., 28, 34-38.

[14] Guo M., Zhang L., & Liu Z. (2009). Analysis of saponins from leaves of *Aralia elata* by liquid chromatography and multi-stage tandem mass spectrometry. Analytical Science 25(6): 753-758.

[15] Swain, T. (1979). Tannins and lignins. In: G.A. Rosenthal and D.H. Janzen, eds. Herbivores and their interaction with secondary plant metabolites. Academic Press, New York. Pp. 657- 682.

[16] Ngounou, F. N., Maifouo, R. N., Tapondjou, L. A., Lontsi, V., Kuete, V.,Penlap, V., Etoa, F. X., Duboisaid, M. A., & Sondengam, B. L. (2005). Antimicrobial diterpenoid alkaloids from *Erythrophleum suaveolens*. *Chem. Soc. Ethiopia,* 19, 221-226.

[17] Millogo-Kone, H., Lompo, M., Kini, F., Asimi, S., Guissou, I. F., & Nacoulma, O. (2009). Evaluation of flavonoids and total phenolic contents of stem bark and leaves of *Parkia biglobosa* (Jacq.), free radical scavenging and antimicrobial activities. *Res. J. of Med. Sci.*, 3(2), 70–74.

[18] Day, R. A., & Underwood, A. L. (1986). Quantitative Analysis. 5th Edition, Prentice Hall Publication, Upper Saddle River, 701.

[19] Kelley, T. R., Ma, Z., & Xu, W. (1994). Revision of the structure of alatinone to emodin. *Phytochemistry.* 36: 253-254.

[20] Provan, G.J., Scobbie, L., & Chesson, A. (1994). Determination of phenolic acids in plant cell walls by microwave digestion. *J. Sci. Food Agri*., 9, 63-65.

[21] Aremu, M.O., Olonisakin, O., Bako, D.A., & Madu, P.C. (2006b). Compositional studies and physicochemical characteristics of cashew nut (*Anarcadium occidentale*) flour. *Pak. J. Nutr*., 5(4), 328–333.

[22] Aremu, M.O., Olaofe, O., Basu, S.K., Abdulazeez, G., & Acharya, S.N. (2010). Processed cranberry bean (*Phaseolus coccineus*L.) seed flour for the African diet. *Can. J. Plant Sci*., 90, 719-728.

[23] Aremu, M.O., Olaofe, O., & Orjioke, C. A. (2008). Chemical composition of bambara groundnut (*Vigna subterranean*) kersting groundnut (*Kerstingiella geocarpa*) and scarlet runner bean (*Phaseolus coccineus*) protein concentrates. *La Rivista Italiana Delle Sostanze Grasse*, 85, 56-62.

[24] FAO/WHO/UNU (1985). Energy and protein requirements. Expert report of a joint FAO/WHO/UNU ConsultationWorld health organization technical report series, No. 724, WHO, Geneva, Switzerland.

[25] Oshodi, A.A., Olaofe, O., & Hall, G. M. (1993). Amino acid, fatty acids and mineral composition of pigeon pea. *Int. J. Food Sci. and Nutr*., 43,187-191.

[26] Oshodi, A. A., Esuoso, K. O., & Akintayo, E. T. (1998). Proximate and amino acid composition of some underutilized Nigerian legume flour and protein concentrates. La Rivista Italiana Delle Sostanze Grasse, 75, 409 -412.

[27] FAO/WHO (1991). Protein quality evaluation report of joint FAO/WHO expert consultation FAO.Series 689, FAO, Rome, Italy.

[28] Mohammed, A., Dikko, A. U., Audu, M., Mohammed, B. S., & Adeboye, M. K. A. (2016). Evaluation of some nutrients in cowpea vine and litter for soil fertility improvement in Sudan Savanna. *Nigerian J. of Agriculture, Food and Environment.* 12(4), 91-97.

[29] Dixon, R. A., & Paiva N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*. 7, 1085-1097.

[30] Arapitsas, P. (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. *J. Food Chem*., 110, 1041-1045.

[31] Somerville, V. S. (2016). Effect of flavonoids on upper respiratory tract infections and immune function: a systematic review and meta-analysis. *Adv. Nutr*. 7(3), 488-497.

[32] Sansone, R. (2015). Cocoa flavanol intake improves endothelial function and Framingham risk score in healthy men and women: a randomized, controlled, double-masked trial: the flaviola health study. *Br. J. Nutr*. 114(8), 1246-1255.

[33] Koosha, S. (2016). An association map on the effect of flavonoids on the signaling pathways in colorectal cancer. *Int. J. Med. Sci.,* 13(5), 374-385.

[34] Hossain, M.A., & Becker, K. (2001). Nutritive value and antinutritional factors in different varieties of Sesbania and their morphological fractions. *J. Food Chem.,* 73, 421-431.

[35] Chung, K. T., Lu, Z., & Chou, M. W. (1998). Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *J. Food Chem. Toxicol*., 36, 1053-1060.

[36] Okuda, T., Yoshida, & Hatano, T. (1995). Hydrolyzable tannins and related polyphenols. *Progress in the chemistry of organic natural products*. 66, 1-117.

[37] Shi, J., Arunasala, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponin from edible legumes: Chemistry, processing and health benefits. *J. of Medicinal Food* 7(1), 67-78.

[38] Chaudhary, S., Khosa, R. L., Jha, K. K., & Verma, N. (2010). Evaluation of antidiabetic activity of cressa cretica linn in alloxan induced diabetes in rats. *Pharmacologyonline*. 3: 181-188.

[39] Nawel, M., Mohamed, E. A. D., Hocine, A., & Boufeldja, T. (2011). Hypoglycaemic effect of *Berberis vulgaris* L. in normal and streptozotocin-induced diabetic rats. *Asian Pac. J. Trop. Biomed.* 1(6), 468-471.

[40] Ojewole, J., & Adewole, S. (2009). Protective effects of *Annona muricata* linn (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. *Afr. J. Trad. CAM* 6(1), 30-41.

[41] Shaahu, D. T., Kaankuka, F. G., & Okpanachi. (2015). Proximate, amino acid, anti-nutritional factor and mineral composition of different varieties of raw lablab purpureus seeds. *Intl. J. Sci. Technol. Res.* 4(4), 157-161.

[42] Raffauf, R. F. (1996). Plant Alkaloids. A guide to their discovery and distribution. Brittonia 50, 55. Hawkworth Press, Inc., New York.

[43] Cushnie, T. T., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int. J. Antimicrob. Agents,* 44, 377-386.

[44] Yang, X., Yuan, L., & Xiong, C. (2014). Abacopteris penangiana exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative. *J. Ethnopharmacol* 157: 105-113.

[45] Bouayad, N., Rharrabe, K., Lamhamdi, M., Nourouti, N. G., & Sayah, F. (2011). Dietary effects of harmine, a β-carboline alkaloid on development, energy reserves and amylase activity of plodia interpunctella hubner [Lepidoptera: pyralidae]. *Saudi J. Bio. Sci.,* 19(1), 73-80.

[46] Aletor, V.A. & Omodara, O. A. (1994). Studies on some leguminous plants, with particular reference to their proximate, mineral and anti-nutritional constituents. *Anim. Feed Sci. Tech*., 46, 343-346