Asian Journal of Agriculture and Food Sciences (ISSN: 2321 – 1571)
Volume 06 – Issue 03, June 2018

Effects of Methanol Extraction on Some Nutritional and Antinutrient Contents of *Xylopia aethiopica* Fruits from Enugu State, Nigeria

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ABSTRACT--- ***Mineral contents and amino acid and fatty acid profiles of Xylopia aethiopica fruits from Enugu State, Nigeria were evaluated in this study. The amino acid and fatty acid profiles, phytochemical, proximate, mineral, anti-nutrient and vitamin contents of X. aethiopica fruits were determined using standard methods. The proximate, vitamins, phytochemical, anti-nutrient of both the crude and methanol extract were determined while the mineral and amino acid profile were determined in the crude fruit powder while fatty acid profile was determined in the oil extract of the fruits. Results show that the predominant amino acids present include proline, alanine, norleucine, isoleucine, glycine, threonine, cysteine, methionine, and tryptophan. Also, the most abundant fatty acids include arachidonic acid, dihomoϒ-linolenic acid, palmitic acid, caproic acid, linoleic acid, palmitoleic acid, stearic acid and myristic acid. The mineral contents of X. aethiopica fruits detected in high amount were calcium, iron, potassium and magnesium. Sodium, zinc and manganese were detected in lower amounts. However, lead, silicon, nickel and chromium were not detected. The result of the proximate analysis shows moisture, total protein, crude ash, total fat, crude fibre, and carbohydrate contents. Anthraquinone was not detected in both crude and methanol extract of the fruit. The concentration of flavonoids, steroids, anthocyanin and terpenoids were significantly ($p < 0.05$) higher in crude sample when compared with methanol extract. The levels of carotenoid and glycosides were significantly ($p < 0.05$) higher in the methanol extract when compared with crude sample. Vitamins B1, B3, B6, B9, B12, C and E levels were significantly ($p < 0.05$) higher in crude extract when compared with methanol extract. However, vitamin A content was significantly ($p < 0.05$) higher in methanol extract when compared with crude extract. Results obtained showed that X. aethiopica fruits are rich in nutrients and that methanol extraction decreased the anti-nutrient contents of the fruits thereby increasing the bioavailability of the nutritive factors. However, the quantities of some of the nutritive factors were reduced after extraction.***

Keywords-- *Xylopia aethiopica*, vitamins, amino acids, fatty acids, proximate, mineral, phytochemical, solvent extraction

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1. INTRODUCTION

The use of medicinal plants is as old as man. The action and efficacy of these plants have been attributed to the presence of some secondary plant metabolites [1]. One of the most used medicinal plants in Africa and other parts of the world is *Xylopia aethiopica* (Annonaceae). It is an evergreen, aromatic tree that grows up to 15-30m high and is commonly known as "spice tree," "Africa pepper," "Ethiopian pepper" or "Guinea pepper". Its fruit is a black berry containing 4-9 peppery seeds; both the seeds and are used as mixed spice with great nutritional and medicinal values [2]. This specie is found in countries like Angola, Ghana, Senegal, Benin, Burkina-Faso, Guinea, Sierra-Leone, Cameroon, South Sudan, The Central Africa Republic, Ivory Coast, Tanzania, Kenya, Togo, The Democratic Republic of Congo, Mozambique, Liberia, Zambia, Nigeria, Ethiopia, Gabon, The Gambia, Uganda, Sao Tome and Principe [3]. The local names of *X. aethiopica* and the languages include: Akan-Asante of Ghana (hwenteaa), Arabic (Fulful as-Sudan, Hab al-Zelim), Dutch(Granen Van Selim) Edo (Unien), English (Grains of Selim, Ethiopian pepper, Moor pepper, Kani pepper, Senegal pepper, Negro Pepper, African pepper, Guinea pepper), Efik/Ibibio (Atta), Estonian (Etioopia Ksüloopia), Ewe of Ghana (Etso), French (Piment noir de Guinée, Kili, Graines de Selim, Poivre de Sénégal), German (Selimskörner, Senegalpfeffer, Mohrenpfeffer, Kanipfeffer, Negerpfeffer), Greek (Αφρικάνικο (Afrikaniko Piperi)), Hausa (Kimba, Chimba), Hungarian (Arabbors, Borsfa), Igbo (Uda, Oda), Korean (Kusillipia), Lithuanian (Juodieji pipirai), Nupe (Tsunfyányá), Polish (Pieprz murzyński), Portuguese (Pimenta-da-áfrica, Pimenta-do-congo), Russian (Кмба перец

(Kumba Perets), Мавританский перец (Mavritanskij Perets)), Swahili (Mchofu), Wolof (Ndiar) and Yoruba (Eeru, Erunje) [4].

Xylopia aethiopica is a common edible spice over central and western Africa with antioxidant, antimicrobial and antibacterial properties and as such can be used as a food preservative. In Congo, the infusion of the tree's bark into palm wine is used in the treatment of asthmatic attacks, stomach aches and rheumatism while the dried root powder is used as mouthwash for toothache and pyorrhea in Senegal [5]. In Senegal, the fruit is also used to flavor *café touba* which is the country's spiritual beverage and the traditional drink of the Mouride brotherhood. The fruits make up key ingredients of "Nah poh" and "Nkui", the two ethno-dietary soups from West region of Cameroon and a whole lot of varied delicacies in different parts of Africa. Due to the presumed high nutritive value, the fruits of *X. aethiopica* are used by herbalists as components of herbal medicine for the treatment of cough, fever and female sterility [6]. The fruits are also used as analgesics and painkillers, and in the treatment of skin infections, dysentery and bronchitis [7]. Nwaichi *et al*., [8] also reported that these spices are particularly very important in the diets of post-partum women as an aid to the contraction of the uterus. Ezekwesili *et al.,* [9] noted that it is part of the diet usually given to post-partum women not only to relieve post-delivery pains but also to promote healing and lactation. The fruits of *X. aethiopica* are used in folk medicine for treating cardiovascular and diabetic diseases [10].

Several studies have evaluated the chemical constituents and bioactivities of *X. aethiopica* fruit and its extracts. Adaramoye *et al.,* [2] demonstrated the anti-proliferative effect of *X. aethiopica* fruit extract on human cervical cancer cells and it was discovered that the fruit extract led to a dose-dependent growth inhibition in most cell lines, with selective cytotoxicity towards cancer cells and particularly the human cervical cancer cell line C-33A. Similarly, Choumessi *et al.* [11] showed that ethanol extract of *X. aethiopica* possesses anti-proliferative activity against a panel of cancer cell lines. They discovered that the main cytotoxic and DNA-damaging compound in ethanol extracts of *X. aethiopica* is ent-15-oxokaur-16-en-19-oic acid (EOKA) which exhibited sufficient cytotoxic activity against pancreatic and leukemia cells sufficient for the plant to be considered a potential source of cytotoxic compounds. EOKA triggers DNA damage and accumulation of the cells in the G1 phase of the cell cycle, followed by apoptosis. Adefegha *et al.,* [12] reported that the diet supplemented with 2-4% of *X. aethiopica* could enhance some *in vivo* antioxidant status, maintain membrane integrity and protect the liver against oxidative stress. Xylopic acid, one of the components of *X. aethiopica* has been shown to possess prophylactic, curative, antimalarial and antipyretic properties, making the compound a safe one for the treatment of diseases where selective toxicity towards the parasite is highly needed which makes it an ideal antimalarial agent [13]. Etoundi *et al.,* [14] also ascertained the anti-amylase, anti-lipase and antioxidant effects of aqueous extracts of *X. aethiopica* from Cameroon. Okpashi *et al.,* [15] showed that *X. aethiopica* extracts possess antidiabetic effects on alloxan-induced diabetic male Wistar albino rats. John-Dewole *et al.,* [5] rationalized how the phytochemical constituents of *X. aethiopica* fruit extract can contribute to its anti-microbial and medicinal effects; cardiac glycoside, saponin and saponin glycoside, tannin, volatile oil and balsam were detected in aqueous extract of *X. aethiopica*. We have also shown that methanol extract of the plant contain about 58 bioactive constituents [16]. Considering high level of nutritional deficiencies facing Africa and other low income countries, there are needs to search for locally available source of nutrition. *Xylopia aethiopica* forms part of diets in Africa and hence, there are needs to validate the nutritive and anti-nutritive contents. The present study was also designed to evaluate the effects of methanol extraction on some nutritive and anti-nutritive contents of *X. aethiopica* fruits.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The fruits of *Xylopia aethiopica* were sourced from a farm in Orba, Udenu Local Government Area, Enugu State, Nigeria and were authenticated at the Herbarium of Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were air-dried and divided into two portions: one portion for preparation of crude powdered sample and the other for preparation of methanol extract.

2.2 Extraction of Plant Materials

Air-dried fruits of *X. aethiopica* (1.0 Kg) was ground into coarse powder, macerated in 4 litres of methanol (JHD, China), stirred and allowed to stand for 48 hours in an air-tight container with occasional stirring. The suspension was filtered using Whatman No. 42 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40^{o}C. The concentrate was stored in a labeled sterile container at 8^{0}C.

2.3 Determination of Mineral Contents of METHANOL EXTRACT of Xylopia aethiopica Fruits

This was done using the atomic absorption spectrophotometric (AAS) method. Methanol extract of *X. aethiopica* fruit was digested (wet digestion method using aqua regia). The residue remaining was dissolved in hydrochloric acid. The atomic absorption spectrophotometer (AAS) was set up to measure the absorption from hollow cathode lamp of any of the cations using acetylene/air flame. The appropriate wavelength for each element corresponding to the lamps was set. The working standard solution of any of the cation being measured was nebulized and the controls were adjusted until steady zero and suitable maximum readings were obtained. The intermediate standards were nebulized and a graph relating galvanometer reading to µg/ml of cation in all the standard solutions was constructed. The concentration of each cation was read from the standard graph equivalent to the galvanometer readings of solutions and blank solutions.

2.4 Determination of Amino Acid Profile of Methanol Extract of Xylopia aethiopica Fruits

The amino acid profile of *X. aethiopica* sample was determined using a high performance liquid chromatographic (HPLC) machine. Extract (3.0 mg) was hydrolyzed with 6M HCl at 150°C for 6 hours. After hydrolysis, the acid was removed by rotary evaporation (RE500 Yamato, Scientific America Inc.). Sample was resuspended in sodium citrate buffer (2 ml), pH 2.2. Sample derivation was achieved by adding phthalaldehyde (OPA) (7.5 mM) to the sample on citrate buffer (OPA reagent contains β-mercaptoethanol and Brij 35). The precision and accuracy of the HPLC method were evaluated using external and internal standards. The amino acid reference standard consisted of fifteen amino acids (0.05 µmole/l of each amino acid) and was used to determine the retention times for each amino acid. Also, internal standard α-aminobutyric (0.05 µmoles/ml) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content. A gradient mobile phase of sodium acetate (0.1 M, pH 7.2) and methanol (9:1) elute sample for amino acid separation trough C18 column reversed-phase octadecyl dymethylsilane particles (100 x 4.6 mm x 1/4" Microsorbe 100-3 C18). Fluorescence detection was realized using an excitation-emission wavelength of 360 and 455 nm, respectively. Star chromatography work station (Varian version 5.51) software was used to achieve amino acid peak integration.

2.5 Determination of Fatty Acid Profile of Xylopia aethiopica Fruits Oil Extract

Fatty acid profile of *X. aethiopica* fruits oil extract was analyzed using a Hewlett Packard gas chromatography-mass spectrometry (GC-MS) system. The fused silica HP-20 M polyethylene glycol column (50 m x 0.2 mm, 0.2 µm thickness) was directly coupled to the mass spectrometer. The carrier gas was helium (1 mL/min). The program used was 4 min isothermal. The injection port and detector temperatures were 250^o C and 280^o C, respectively. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatograms was Turbomass version 5.2.0.

2.6 Determination of Phytochemical, Proximate, Vitamin and Anti-Nutrient Contents of Xylopia aethiopica Fruits

The phytochemical constituents of crude (coarse powdered sample) and methanol extract of *X. aethiopica* fruit were determined according to the methods of Harbone [17] and Trease and Evans [18]. The proximate, vitamin and anti-nutrient contents of crude and methanol extract of *X. aethiopica* fruits were determined using the method of AOAC [19].

2.7 Statistical Analysis

Data obtained from the laboratory were analyzed using Student T-test to compare the means between the crude and extract samples in IBM Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as mean ± standard deviation (SD) and presented in tables. Mean values with $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Mineral Composition of Methanol Extract of Xylopia aethiopica Fruits

The results of the mineral composition of the methanol extract of *X. aethiopica* fruits are shown in Table 1. Calcium is the most abundant element in the fruit of plant with a concentration of 1259.26 mg/kg. Other mineral elements such as iron (332.85 mg/kg), magnesium (112.46 mg/kg) and potassium (164.37 mg/kg) were detected in higher concentration when compared to sodium (5.46 mg/kg), zinc (5.05 mg/kg) and manganese (4.05 mg/kg). Elements like silicon, lead, nickel and chromium were not detected. Abolaji *et al*. [20] analyzed the mineral contents of *X. aethiopica* sourced from Lagos, Western Nigeria and found lower iron (18 mg/kg) calcium (190 mg/kg) and manganese (3 mg/kg) contents but higher potassium (510 mg/kg), sodium (53 mg/kg), zinc (20 mg/kg) and magnesium (2240 mg/kg) contents when compared with the results of the present study. Also, John-Dewole *et al.*, [5] analyzed fruits of *X. aethiopica* from Ibadan, Western Nigeria and found very lower zinc (10.2 mg/kg) and manganese (3.70 mg/kg) contents when compared to the results from the present study. John-Dewole and colleagues also reported the presence of 11.0 mg/kg content of nickel which was not detected in the presence study. In another study, Udofa and Alozie [21] analyzed fruits of *X. aethiopica* from Akwa-Ibom and found lower calcium (480 mg/kg) and sodium (9.7 mg/kg) contents but higher magnesium (230 mg/kg) and potassium (300 mg/kg) contents when compared to the findings of the present study. Similarly, Bouba *et al.*, [22] analyzed the mineral contents of the plant from Cameroun and found lower calcium (228 mg/kg), iron (34.7 mg/kg) and zinc (3.8 mg/kg) contents as well as higher magnesium (172 mg/kg), sodium (17.1 mg/kg), potassium (27.7 mg/kg) and manganese (11 mg/kg) contents. Variations in these results could be attributed to differences in the locations from where the plant materials were sourced. Minerals are beneficial to health and their deficiencies have been linked to many ailments [23]. Calcium helps in blood clotting and building of strong bones and teeth and might suggest why the plant is used to stop bleeding in postpartum mothers [24]. The daily reference intake of calcium, magnesium, potassium and sodium are 700, 285, 3500 and 1600 mg/day, respectively, suggesting that fruits of *X. aethiopica* provide appreciable mineral to suite the required quantities for growth and normal body functions. However, sodium content was found to be very low when compared with the daily requirements. Also, the contents of zinc (5.05 mg/kg), manganese (4.05 mg/kg) and iron (332.85 mg/kg) were close to the required daily values of 5.5, 5.5 and 6.7 mg/day, respectively [25]. Na/K ratio (0.03 as compared to reference level of 0.1) of the extract showed that consumption of the extract is capable of lowering the rate of oxidation, thereby enhancing the body's antioxidant protection [26]. *X. aethiopica* fruit has high magnesium content which serves as a co-factor for hexokinase, the first enzyme in glycolytic pathway for the breakdown of glucose to provide energy in the form of adenosine triphosphate (ATP) [27]. Magnesium is important in the metabolism of glucose and insulin, mainly by influencing tyrosine kinase activity of the insulin receptor, by transferring the phosphate from ATP to protein. Magnesium also affects the activity of phosphorylase b kinase by releasing glucose-1-phosphate from glycogen [28]. Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction making it important for normal digestive and muscular function [29]. The presence of these minerals in appreciable amounts makes the fruits of *X. aethiopica* nutritionally important.

Table 1: Mineral composition of *Xylopia aethiopica* fruits

Minerals	Concentration (mg/kg)	Concentration (%)	Daily reference intake (mg/day)
Calcium (Ca)	1259.26	1.26	700
Iron (Fe)	332.85	0.33	6.7
Potassium (K)	164.37	0.16	3500
Magnesium (Mg)	112.46	0.11	285
Sodium (Na)	5.46	0.05	1600
Zinc (Zn)	5.05	0.05	5.5
Manganese (Mn)	4.05	0.04	5.5
Lead (Pb)	0.00	0.00	0.00
Silicon (Si)	0.00	0.00	0.00
Nickel (Ni)	0.00	0.00	0.00
Chromium (Cr)	0.00	0.00	0.00
Na/K	0.03		0.1

3.2 Fatty Acid Profiles of Xylopia aethiopica Fruits Oil Extract

The result of fatty acid profile of methanol extract of *X. aethiopica* fruits indicated that arachidonic acid (31.41%), dihomo ϒ-linolenic acid (20.63%), palmitic acid (11.98%), caproic acid (10.52%), and linoleic acid (9.83%) were detected in high amount when compared to palmitoleic acid (5.91%), stearic acid (4.93%), myristic acid (3.51%), and myristoleic acid (1.04%) which were detected in moderate amount, whereas oleic acid (0.30%), vaccenic acid (0.23%), α-linolenic acid (0.19%) and erucic acid (0.16%) were detected in low amount (Table 2). Elhassan *et al*., [30] reported that oleic acid is the most abundant (69.37%) fatty acid, followed by palmitic acid (15.66%) and stearic acid (9.47%) in *X. aethiopica* fruits from Sudan. Ezekwesili *et al*., [9] analyzed the fatty acid profile of chloroform extract of *X. aethiopica* fruits from Eastern Nigeria and found that the most abundant fatty acid is oleic acid (39.12%), followed by linoleic acid (25.98%), palmitic acid (19.21%), stearic acid (4.54%), linolenic acid (1.10%) and palmitoleic acid (0.81%). The variation in the fatty acid profile may be due to differences in procedures for processing the extract, solvent of extraction as well as differences in geographical locations where the plant was sourced from [31]. The high concentrations of monounsaturated fatty acids (myristoleic and palmitoleic acids) present in the extract have been reported to be beneficial to the heart; they have been found to inhibit the heart-damaging oxidation of low density lipoprotein (LDL) cholesterol [32]. The extract contains high level of ω-6 fatty acids (arachidonic and dihomoϒ-linolenic acids) which are important not just in lowering the triacylglycerol level but are essential precursors for the biological synthesis of the prostaglandins in a reaction catalyzed by prostaglandin synthetase [33]. Prostaglandins possess a variety of physiological and pharmacological properties which include smooth muscle contraction, a biochemical event implicated in the expulsion of the placental debris after delivery in women and healing of wounds [10]. Some polyunsaturated fatty acids such as linoleic acid and arachidonic acid are required for the growth and protection of the skin; their deficiency would result in a skin condition such as alopecia and oedema [34]. The presence of arachidonic and eicosatetraenoic acids as well as dihomoϒ-linolenic and eicosatrienoic acids from the same precursor fatty acid (C20:4) and (C20:3), respectively present in the extract is an indication that there is a balance of ω-6 and ω-3 fatty acids in the extract. Nutritionally, it is highly recommended that there should be equilibrium between ω-6 and ω-3 fatty acids as it has been reported that an overabundance of one precursor can drive down production of the products of another [35]. The presence of appreciable quantity of these fatty acids in *X. aethiopica* fruits may therefore be responsible for its use in folk medicine for arresting bleeding among postpartum mothers as well as treating cardiovascular diseases and diabetes [2,10] and cancer [36].

Table 2: Fatty acid profile of *Xylopia aethiopica* Fruits Oil Extract

Number of carbon	Common names	Amount (%)
Saturated fatty acids		
C6:0	Caproic Acid	10.52
C14:0	Myristic	3.51
C16:0	Palmitic	11.98
C18:0	Stearic	4.93
Total		**30.94**
Monounsaturated fatty acids		
C22:1	Erucic	0.16
C14:1	Myristoleic	1.04
C16:1	Palmitoleic	5.91
C20:1	Eicosenoic	0.17
C18:1 (9)	Oleic	0.30
C18:1 (11)	Vaccenic	0.23
Total		**7.81**
Diunsaturated fatty acids (unsaturated fatty acids with two double bonds)		
C18:2	Linoleic	9.83
Triunsaturated fatty acids (unsaturated fatty acids with three double bonds)		
C18:3	α-Linoleic	0.19
C20:3	Dihomo ϒ-Linolenic	20.63
Total		**20.82**
Tetraunsaturated fatty acids (unsaturated fatty acids with four double bonds)		
C20:4	Arachidonic	31.41

3.3 Amino Acid Profile of Methanol Extract of Xylopia aethiopica Fruits

Results of the amino acid constituents of methanol extract of *X. aethiopica* fruits as shown in Table 3 indicated that proline (57.60%) and alanine (20.85%) were detected in high amount when compared to the percentages of norleucine (5.99%), and isoleucine (4.18%). Glycine (3.34%), threonine (3.28%), cysteine (3.12%), methionine (2.48%), tryptophan (2.28%), lysine (1.68%), tyrosine (1.57%) and glutamine (1.54%) were detected in moderate amount while asparagine (1.22%), phenylalanine (1.09%), arginine (0.90%), and histidine (0.71%) were detected in low amount. Amino acids play innumerable roles in human health and disease [37,38]. Proline is an important component in certain medical wound dressings that use collagen fragments to stimulate wound healing [39]. The high amount of proline in the extract may be part of the reason why *X. aethiopica* fruits is used as an antiseptic in women who had just delivered to arrest bleeding [40]. Alanine stimulates lymphocyte production and hence, useful for immocompromised individuals. It strengthens the immune system by producing antibodies [41]. Threonine possesses immunostimulatory effects while methionine and cysteine exhibit antioxidant activities. Cysteine, as a precursor of glutathione, plays important roles in antioxidant defence and regulation of cellular events [42]. Also, glutamine, lysine and methionine have immunomodulatory effects and are beneficial for people with immunodeficiency [38]. L-arginine and L-cysteine can significantly improve sperm quality and therefore male fertility [43]. The presence of L-arginine and L-cysteine in the extract suggests that the extract may be useful to men who have low sperm count.

Table 3: Amino acid profile of methanol extract of *Xylopia aethiopica* fruits

Amino acids	**Amount (%)**
Methionine	2.48
Threonine	3.28
Phenylalanine	1.09
Cysteine	3.12
Asparagine	1.22
Lysine	1.68
Alanine	20.85
Glycine	3.34
Isoleucine	4.18
Norleucin	5.99
Proline	57.60
Glutamine	1.54
Arginine	0.90
Histidine	0.71
Tyrosine	1.57
Tryptophan	2.28

3.4 Phytochemical Constituents of Crude and Methanol Extract of Xylopia aethiopica Fruits

Preliminary phytochemical analysis of the crude sample indicated that flavonoids, steroids and terpenoids were in high concentration while alkaloids, anthocyanin, carotenoids, and saponins were in moderate concentration. Phenols, glycosides and tannins were detected in low concentration. Anthraquinones was not detected. Flavonoids (18.02 ± 1.73), steroids (19.15 ± 2.18), anthocyanin (5.50 ± 0.49) and terpenoids (25.82 ± 4.22) levels were significantly ($p < 0.05$) higher in crude sample when compared with methanol extract (14.71 ± 2.98), (14.05 ± 1.87), (2.00 ± 0.09) and (17.22 ± 1.26), respectively. They were the most abundant phytochemicals in the crude and methanol extract of *X. aethiopica* fruits. The levels of carotenoid (2.40 ± 0.17) and glycosides (0.05 ± 0.01) were significantly ($p < 0.05$) higher in the methanol extract when compared with crude sample (1.03 ± 0.08) and (0.08 ± 0.01), respectively (Table 4). These results agreed with the report of Fetse et al. [44], that phytochemicals such as alkaloids, saponins, tannins, reducing sugar, phlobatannins, anthraquinones, steroids, flavonoids, and glycosides were present in the fruit, seed, stem and bark. Tanwar and Modgil [45] detected the presences of steroids, saponins, carbohydrates, glycosides, mucilage, acidic compounds, tannins, balsams, cardiac glycosides, volatile aromatic oils, phenols, alkaloids, and fixed oils. However, there were variations in the amount of phytochemicals detected in this study when compared with the previous studies. The different between the concentration and types of phytochemicals found in the earlier studies and the present work could be attributed to variations in part of the plant used and solvent of extraction. For instance, anthraquinones were not detected in the fruit of *X. aethiopica* in the present study, but were found in the stem [45].

Flavonoids constitute 18.02 ± 1.73% of the crude and 14.71 ± 2.98% of methanol extract of fruit of *X. aethiopica* as observed in the present study. Flavonoids are the largest group of plant phenols and provide much of flavor and colour to fruits and vegetables [45]. This may be responsible for the strong flavour and deep colour of *X. aethiopica*. Flavonoids and phenols constitute a wide range of substances that play important roles in protecting biological systems, serve as potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage, lower the risk of heart diseases and have strong anticancer activity [46]. Hence, the fruit of *X. aethiopica* possess some biologically-active compounds which could serve as potential drugs to man and animals. Alkaloids contents of raw and methanol extract of *X. aethiopica* fruit as observed in the present study were 5.94 ± 0.48% and 4.00 ± 0.18% respectively. Some alkaloids are considered toxic but others are often used medicinally [47]. Atropine for instance is used to stimulate the central nervous system and to dilate the pupils of the eyes [48]. Anonecaine, an alkaloids constituent of *X. aethiopica*, is known to have anti-pyretic effect. They are powerful antibiotics [49] and valuable medicine against malaria as well as their application in local anesthesia as pain relief [50].

Saponins, another phytochemical component of *X. aethiopica* (4.97 ± 0.18% in crude sample and 5.03 ± 1.13% in methanol extract) have wide range of biological properties; they are used to recover homeostasis. They also have anti-inflammatory and anticancer actions. Saponins cause reduction of blood cholesterol by preventing its re-absorption. They have antitumor and anti-mutagenic activities and can lower the risk of human cancers by preventing cancer cells from growing apart from their biocidal effects against pathogens [47]. Tannins are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms and protect the tree. They can be classified into three broad groups - hydrolysable tannins, condensed tannins and pseudotannins. They are often known as anti-nutrients and interfere with iron absorption through a complex formation with iron in the gastro-intestinal lumen which decreases the bioavailability of iron [51]. They also help to prevent urinary tract infections. Many plants produce volatile terpenes in order to attract specific insects for polCossignanition or repel certain animals using these plants as food [52]. Our study found that terpernoids constitute 25.82 ± 4.22% of crude sample and 17.22 ± 1.26% of methanol extract of *X. aethiopica* fruit. Terpenoids have medicinal properties such as anti-carcinogenic, antimalarial, antiulcer, hepaticidal, antimicrobial and diuretic activities [53]. Aguoru et al. [54] detected the presence of alkaloids, saponins, steroids and flavonoids in fruits of *X. aethiopica* obtained from Benue State, North-central Nigeria while Omeh et al. [55] detected the presence of tannins, flavonoids, alkaloids and saponins, and Enemchukwu et al. [56] detected tannins, flavonoids and saponins only from the fruits of the plant procured from Abia State and Ebonyi State, all in Southeastern Nigeria. These results showed that geographical locations have effects on the phytochemical constituents of *X. aethiopica* fruits.

The roles of active principles of plant origin in drug synthesis cannot be over emphasized. Therefore, the findings of this work showed that *X. aethiopica* fruit could be of great medicinal importance and may justify its wide use in folkloric medicine. Our results showed that flavonoids, steroids, anthocyanin and terpenoids levels were significantly ($p < 0.05$) higher in crude sample when compared with methanol extract. These phytochemicals were the most abundant in both the crude and methanol extract of the fruits. Carotenoids and glycosides levels were significantly ($p< 0.05$) higher in methanol extract when compared with crude *X. aethiopica* fruit sample. The high amount of flavonoid in the stem bark, petiole and seed supports the anti-inflammatory, antimicrobial and antitumor activities of *X. aethiopica* reported by Fleischer [49]. Also, high amount of saponins and alkaloids observed in this study therefore accounts for their pepperish and bitter taste and confirms their numerous therapeutic functions. Reports on the medicinal uses of *X. aethiopica* by many authors across the globe fully supported the findings of this work [48,49]. This is because the plant contains significant amounts of major active principles of therapeutic benefits. The correlations among the quantified phytochemicals therefore suggest the multifaceted uses of this plant in curing many types of diseases and ailments. A good solvent is characterized by its optimal extraction and its capacity in conserving the stability of the chemical structure of desired compounds [57]. Liu et al. [26] used different solvents (water, methanol, absolute acetone and aqueous acetone 80%) for extraction of the plant and they evaluated the effects on the phytochemical components and antioxidant activities. Their result revealed that polar solvent extracted the highest amount of bioactive compounds. In the extraction of phytochemical components of *X. aethiopica* fruits in this work, methanol extract was used because it has better efficiency of solvation as a result of interactions (hydrogen bonds) between the polar sites and nonpolar one [58].

Table 4: Quantitative phytochemical constituents of crude and methanol extract of *Xylopia aethiopica* fruits

Phytochemicals	**Composition (%) in crude**	**Composition (%) in extract**
Flavonoids	18.02 ± 1.73^b	14.71 ± 2.98^a
Alkaloids	5.94 ± 0.48^b	4.00 ± 0.18^b
Steroids	19.15 ± 2.18^b	14.05 ± 1.87^a
Terpenoids	25.82 ± 4.22^b	17.22 ± 1.26^a
Anthocyanin	5.50 ± 0.49^b	2.00 ± 0.09^a
Carotenoids	1.03 ± 0.08^a	2.40 ± 0.17^b
Phenols	0.59 ± 0.05^b	0.28 ± 0.03^a
Saponins	4.97 ± 0.18^a	5.03 ± 1.13^a
Tannins	0.38 ± 0.05^a	0.55 ± 0.01^b
Glycosides	0.08 ± 0.01^a	0.05 ± 0.01^a

Values are mean ± SD (n = 3). Values with different superscripts in a row are significant at $p < 0.05$

3.5 Proximate Composition of Crude and Methanol Extract of Xylopia aethiopica Fruits

The moisture (8.67± 1.58%) and crude ash contents (1.30± 0.20%) were reduced in the methanol extract when compared to the raw sample (14.50 ± 2.45% and 22.71 ± 2.10% respectively). The crude fibre content (0.13± 0.09%) was also reduced to a very minute concentration. Conversely, the crude total lipid (20.01± 3.93%), crude total protein (14.05± 3.12%) and carbohydrate (55.84± 3.72%) levels increased in the methanol extract when compared to the crude sample (Table 5). Bouba et al. [22] found the following; moisture content of (9.6%), ash (9.5%), protein (7.9%), total fat (33.7%) and carbohydrates (40%) in *X. aethiopica* fruits from Cameroun. Similarly, Abolaji et al. [20] found moisture contents of 16.04%, ash (4.37%), protein (2.1%), fat (9.55%), fibre (12.4%) and carbohydrate (55.80%) in *X. aethiopica* fruits from Lagos, Western Nigeria. In the same vein, Udofa and Alozie [21] found moisture content (10.74%), ash (3.77%), protein (10.5%), fat (14.27%), fibre (2.56%) and carbohydrate (58.57%) from Akwa-Ibom State, Southern Nigeria. Variation between these findings and that of the crude extract may have resulted in variation in geographical locations in which plant was collected. The low moisture content (8.67 ± 1.58%) of the methanol extract of *X. aethiopica* fruits indicates that the plant fruits possess high shelf life and would be less susceptible to microbial damages [59]. Also, the percentage composition of ash content of methanol extract (1.30 ± 0.20%) was lower compared to the crude extract (5.00 ± 0.52%).

In a study, Shirley and Parsons [60] noticed that an increase in ash content causes a decrease in protein quality of meat and bone meal and this is due to a decrease in analyzed amino acid per unit of crude protein. Therefore, low ash content may suggest higher protein quality of the plant fruits. Crude fibre was highly present in the crude sample (22.71 ± 2.10%) than in the methanol extract (0.13± 0.09%). The higher concentration in the crude extract confers so many characteristic features like increased heart rate and good bowel movement which may be lost in the methanol extract. Carbohydrate content of the methanol extract (55.84 ± 3.72%) was higher compared to that of the crude sample (42.47 ± 4.52%) implying that there is a calorific increase as a result of methanol extraction. Crude total protein and crude total lipid contents of the methanol extract (14.05± 3.12% and 20.01± 3.9%) is significantly higher than that of the crude extract (7.36 ± 1.22% and 7.96 ± 0.65%) respectively. The increase in these energy sources indicates that consumption of the methanol will increase the production of clotting factors and hormones, which may include prolactin that induces milk production in postpartum mothers [61,62].

Table 5: Proximate composition of crude and methanol extract of *Xylopia aethiopica* fruits

Proximate	Composition (%) in crude	Composition (%) in methanol extract
Crude total protein	7.36 ± 1.22^{a}	14.05 ± 3.12^{b}
Cude ash	5.00 ± 0.52^{b}	1.30 ± 0.20^{a}
Crude total lipid	7.96 ± 0.65^{a}	20.01 ± 3.93^{b}
Moisture	14.50 ± 2.45^{b}	8.67 ± 1.58^{a}
Crude fibre	22.71 ± 2.10^{b}	0.13 ± 0.09^{a}
Total carbohydrate	42.47 ± 4.52^{a}	55.84 ± 3.72^{b}

Data are mean ± SD (n = 3). Values with different superscripts in a row are significant at $p <0.05$

3.6 Vitamin Contents of Crude and Methanol Extract of Xylopia aethiopica Fruits

Table 6 shows that both the crude and methanol extract of *X. aethiopica* fruits were rich in vitamins. Vitamins B1, B3, B6, B9, B12, C and E contents were significantly ($p < 0.05$) higher in crude extract when compared with methanol extract. Vitamin A content was significantly ($p < 0.05$) higher in methanol extract when compared with crude extract. Vitamins are known to have positive effects on health. Epidemiological studies have shown that high intakes of phenols, vitamins C and E and carotenoid-rich vegetables is associated with decreased incidence of some cancers and cardiovascular diseases [63]. High vitamin contents were obtained in both the crude sample and methanol extract in this study, which agrees with the report of Faiza et al. [64] that fruits of *X. aethiopica* are good sources of vitamins. Vitamins play vital role in the function of the nervous system, aid in the formation of red blood cells and help to build tissues [65]. The vitamin C content of crude sample of *X. aethiopica* fruit was 34.40 ± 3.18 mg/100g. Vitamin C chelates iron to enhance iron absorption in the body [66]. The vitamin A contents of the crude sample and extract were 185.53 ± 11.48 and 673.76 ± 12.81 mg/100g, respectively. Vitamin A, stored in the liver as retinyl ester mainly in special cells called stellate cells, functions in vision (night, day and colour), immune response, haematopoiesis, cell differentiation, skeletal growth, reproduction or fertility (male and female) and embryogenesis [67]. Vitamins A, C and E are known as antioxidant vitamins because they possess free radical scavenging potential. This suggests that they may play important roles in curbing the incidence of oxidative stress in humans and animals [64]. The vitamin E contents of crude and methanol extract of *X. aethiopica* fruits were 29.26 ± 5.51 and 7.38 ± 1.19 mg/100 g respectively. Research has shown that vitamin E is required for maintaining normal immune function and also may be important in preventing muscular degeneration and cognitive decline [65].

The result of this study showed that both the crude and methanol extract of *X. aethiopica* fruits are rich in B-vitamins. Vitamin B1 occurs primarily in populations that rely exclusively on polished rice. Symptoms of mild thiamin deficiency include loss of appetite, constipation, nausea, mental depression, peripheral neuropathy, irritability and fatigue [68]. Pyridoxine is important in the production of hormones such as serotonin, dopamine, and melatonin, as well as for processing amino acids. Its richest sources are meat, vegetable, whole grain cereals and egg yolk [69]. Folate is unstable, not fully bioavailable and is not found in great density in most foods except the liver, which is not a large part of most diets. The best sources of folate are vegetables, others sources are yeast, liver, milk, egg-white, fish, kidney, and cereal products. There is an increased requirement for folate during pregnancy and lactation to aid in building blood for both the mother and foetus. The deficiency of it causes sideroblastic microcytic anaemia, irritability, nervousness and depression [69]. The results of the present study showed that both the crude and methanol extract of *X. aethiopica* fruits were rich in vitamins. Vitamins B1, B3, B6, B9, B12, C and E contents were significantly ($p < 0.05$) higher in crude sample when compared with methanol extract. However, vitamin A content was significantly ($p < 0.05$) higher in methanol extract when compared with crude sample. This agrees with the opinion of Faiza et al. [64] that the total vitamin contents with regards to different solvents used for extraction were as follows: water > methanol > aqueous acetone > absolute acetone.

Therefore, the type of extraction solvent and its polarity have a significant impact on the level of extracted vitamins. This could be responsible for the sharp increase of vitamin A content in the methanol extract when compared to crude sample as seen in the present study.

Table 6: Vitamin contents of crude and methanol extract of *Xylopia aethiopica* fruits

Vitamins	**Vitamin content (%) in crude**	**Vitamin content (%) in extract**
Vitamin A	185.53 ± 11.48^{a}	673.76 ± 12.81^{b}
Vitamin B1	34.56 ± 6.48^{b}	14.53 ± 3.81^{a}
Vitamin B2	3.76 ± 0.12^{b}	1.21 ± 0.11^{a}
Vitamin B3	17.80 ± 3.29^{a}	15.56 ± 4.16^{a}
Vitamin B6	1.37 ± 0.09^{b}	0.46 ± 0.09^{a}
Vitamin B9	4.41 ± 1.08^{b}	1.52 ± 0.17^{a}
Vitamin B12	276.85 ± 28.15^{b}	61.23 ± 10.03^{a}
Vitamin C	34.40 ± 3.18^{b}	1.05 ± 0.03^{a}
Vitamin E	29.26 ± 5.51^{b}	7.38 ± 1.19^{a}

Values are mean ± SD (n = 3). Values with different superscripts in a row are significant at $p < 0.05$

3.7 Anti-nutrient Composition of Crude and Methanol Extract of Xylopia aethiopica Fruits

The result of the anti-nutrient composition of crude and methanol extract of *X. aethiopica* is show in Table 7. The presence of oxalate (1.10%), phytate (1.70%), tannins (0.83%), haemaglutinin (50.34 IU/mg) and hydrogen cyanide (133.89 ppm) were detected in the crude sample. The concentrations of these anti-nutrients reduced considerably in the methanol extract such as oxalate (0.23%), phytate (0.47%), tannin (0.83%), haemaglutinin (9.33 IU/mg) and hydrogen cyanide (66.64 ppm). The presence of trypsin inhibitors was not detected in both the crude and methanol extract of the plant sample. The results indicate that methanol extraction has a reductive effect on the anti-nutrient concentration of *X. aethiopica* fruits. One might be quick to dismiss anti-nutrients as purely detrimental due to their ability to make nutrients bio-unavailable to the body but this is not always so. As observed by Bora [70], anti-nutritional factors affect gastrointestinal tract and micro-flora count of the intestine by promoting the growth of beneficial bacteria. Habatamu et al. [71] also noted that some anti-nutrients may exert beneficial health effects at low concentrations. For example, phytate, lectins, tannins, saponins, amylase inhibitors and protease inhibitors have been shown to reduce the availability of nutrients and cause growth inhibition. In addition, phytate, tannins, saponins, protease inhibitors and oxalates have been related to reduced cancer risks [71]. Fereidoon [72] observed that when used at low levels, phytate, lectins and phenolic compounds as well as enzyme inhibitors and saponins reduce the blood glucose and/or plasma cholesterol and triacylglycerol levels and hence, the risk of cancers. Also, the hypoglycemic property of phytate might stem from its intrinsic ability to influence the rate of starch digestion by binding directly with the amylase, thus inactivating it. Indirectly, phytate and tannins may also bind with calcium which is required for stabilization of amylase activity or possibly with starch in order to affect its gelatinization or accessibility to digestive enzymes [72]. Phytate has also been implicated as having a significant effect on reducing plasma cholesterol and triacylglycerol levels. The effect is thought to be related to the ability of phytate to bind to zinc and thus lower the ratio of plasma zinc to copper which is known to dispose humans to cardiovascular disease. The effect may also be related to the ability of phytate to reduce the plasma glucose and insulin concentrations which may, in turn, lead to reduced stimulus for hepatic lipid synthesis [72]. Also, Young [73] noted the beneficial outcomes in hypercholesterolemia after intake of heat treated chickpea in rats. This implies that anti-nutrients are not always harmful and that the balance between beneficial and hazardous effects of plant bioactives and anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components [71].

The abundance of anti-nutritional factors and toxic influences in plants used as human foods and animal feeds certainly calls for concern. Therefore, ways and means of eliminating or reducing their levels to the barest minimum is a call for research [74]. It is widely accepted that simple and inexpensive processing techniques are effective methods of achieving desirable changes in the composition of plant materials [75]. It has been observed that processing significantly improved macronutrients and energy content with significant reduction in anti-nutrient concentration of products making them suitable for consumption [76]. Bora [70] and Fereidoon [71] reported that processing method like fermentation and thermal treatment are very effective in inactivating some anti-nutrients; due to their particular protein nature, protease inhibitors may be easily denatured by heat processing thereby effectively inactivating them, which is associated with growth inhibition and pancreatic hypertrophy. In this srudy, there was a significant ($p < 0.05$) decline in the composition of oxalate (from 1.10% to 0.23%), phytate (from 1.70% to 0.47%) and tannin (from 0.83 to 0.66%) upon methanol extraction. Significant decrease ($p < 0.05$) was also observed in haemaglutinin composition upon methanol extraction (Table 7). Hydrogen cyanide composition also conforms to this trend with its composition reducing significantly from 133.89ppm in crude sample to 66.64 ppm in methanol extract. The reason for this reduction may be due to the increased solubility of these compounds in methanol [58]. This is also in line with findings of Tadele [74] that chemical techniques like treating with alkalis, organic solvents and precipitants have a reductive effect on anti-nutrient concentration. There are many techniques to recover potent bioactive contents from plants such as Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction [77]. However, extraction yield not only depend on the extraction method but also on the solvent used for extraction as differences in the structure of plant compounds also determine their solubility in solvents of different polarity [58]. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols [77].

Table 7: Anti-nutrient composition of crude and methanol extract of *Xylopia aethiopica* fruits

Anti-nutrient	**Composition of crude sample**	**Composition of methanol extract**
Oxalate (%)	1.10 ± 0.04^{b}	0.23 ± 0.02^{a}
Phytate (%)	1.70 ± 0.08^{b}	0.47 ± 0.06^{a}
Tannin (%)	0.83 ± 0.11^{b}	0.66 ± 0.09^{a}
Haemaglutinin (IU/mg)	50.34 ± 2.34^{b}	9.33 ± 2.40^{a}
Hydrogen cyanide (ppm)	133.89 ± 10.09^{b}	66.64 ± 13.04^{a}
Trypsin inhibitors	ND	ND

Data are mean ± SD (n = 3); ND = Not detected. Values with different superscripts in a row are significant at $p < 0.05$

3.8 Bioavailability Prediction Indices of Crude and Methanol Extract of X. aethiopica Fruits

To predict the effect of phytate on the bioavailability of zinc and iron in the crude sample, phytate to nutrients ratios were calculated. The calculated molar ratios of [Phytate]/[Zn], [Phytate]/[Fe], [Phytate]/[Ca] and [Ca][Phytate]/[Zn] were above the critical level 10, 0.4, 0.2 and 0.5, and this indicate poor bioavailability of calcium and iron in the crude sample due to phytate [78]. To predict the effect of oxalate on the bioavailability of Ca and Mg, the oxalate to minerals ratios were calculated. The calculated molar ratios of [oxalate]/[Ca] and [oxalate]/[Ca + Mg] were below the critical level of 2.5 which indicates that the minerals are not rendered unavailable by oxalate [79]. The bioavailability indices of methanol extract showed that the solvent extraction procedure increased the bioavailability of the minerals when compared with the crude extract (Table 8).

Table 8: Bioavailability prediction indices of crude and methanol extract of *X. aethiopica* fruits

Bioavailability prediction indices	**Crude *X. aethiopica***	**Methanol extract of *X. aethiopica***	**Critical level**
[Oxalate]/[Ca]	0.87	0.18	2.5
[Oxalate]/[Ca + Mg]	0.84	0.36	2.5
[Ca][Phytate]/[Zn]	59.2	11.84	0.5
[Phytate]/[Zn]	1.35	0.37	0.2
[Phytate]/[Fe]	5.15	1.42	0.4
[Phytate]/[Zn]	34	9.4	10

4. CONCLUSION

Findings from this study showed that *Xylopia aethiopica* fruits from Nsukka are rich in nutritive factors but also contain some amounts of anti-nutrients. It was also observed that extraction with methanol affected the nutritional contents and reduced the anti-nutrient contents, hence, increasing bioavailability of the inherent nutrient.

Conflict of interest: The authors declare none.

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