

Comparative Biochemical Evaluation of the Proximate, Mineral, and Phytochemical Constituents of *Xylopia aethiopica* Whole Fruit, Seed, and Pericarp

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ABSTRACT: This study evaluated the relative abundance of proximate, mineral, and phytochemical constituents of the anatomical parts of *Xylopia aethiopica* (XA) fruit using standard analytical procedures. The results showed that whole fruits (WF) have higher contents of crude protein, crude fiber, fat, ash, and moisture than the seeds (S) and pericarps (P). However, highest contents of crude carbohydrate and nitrogen free extracts were found in the P, followed by the S. The content of minerals (sodium, potassium, calcium, phosphorus, iron, zinc, magnesium, and copper) and phytochemicals were present in the following order of abundance: WF>S>P. Furthermore, the phytochemical constituents in each XA parts were present in the following order of relative abundance: total flavonoids>tannins>total phenolics>cardiac glycoside>alkaloids>steroids. Vitamin A was abundant in all three parts, with the abundance highest in WF [4.83±0.06 g vitamin A equivalent (vit A eq)/100 g] and lowest in P (1.64±0.02 g vit A eq/100 g). This preliminary study indicates XA fruits are rich in minerals, anti-nutrients, and phytochemicals. Therefore, these data could represent a biochemical rationale for inclusion of XA as a spice or functional ingredient in many Nigerian local soups to help prevent ailments.

Keywords: functional foods, minerals, phytochemicals, proximate composition, *Xylopia aethiopica*

INTRODUCTION

Phytochemicals are naturally occurring chemical compounds found in plants. They are regarded as antinutrients and possess a variety of nutritional, biological, and pharmacological properties (Okwu, 2004; Saxena et al., 2013; Anigboro et al., 2019). Phytochemicals have many ecological and physiological roles, and are present in plants in diverse chemical forms, including alkaloids, saponins, tannins, steroids, phenols, and flavonoids (Ndukwe et al., 2013; Anigboro et al., 2014; Anigboro et al., 2021). In the human body, phytochemicals can induce a range of physiological effects, including inducing antioxidant activity, mimicking hormones, and suppressing development of disease (Whitney et al., 2002; Nwanna et al., 2016; Anigboro et al., 2021).

Minerals in spices and food products are essential for both human health (Darby, 1976; Seth et al., 2014) and for maintaining certain physicochemical processes (Dibaba et al., 2014; Perez and Chang, 2014). Mineral deficiencies or dysregulation may result in a variety of diseases (Fang

et al., 2016). Indeed, the human body requires specific quantities of both metallic and non-metallic elements to function optimally (Lenntech, 2020). Many elements play important roles in metabolic processes and in maintaining overall well-being; however, some can be toxic due to their capabilities to stimulate oxidative stress in cells (Aganbi et al., 2015). Antioxidant vitamins such as vitamin C, vitamin A, and vitamin E are potent scavengers of free radicals (reactive oxygen species; ROS) that induce cellular oxidative damage. However, humans only need to consume low quantities of these vitamins (organic compounds) in order to maintain normal integrity and homeostatic function (Christakos et al., 2019; Price and Preedy, 2020; Otuechere et al., 2021).

Assessing the proximate and nutrient contents of edible fruit and vegetables is important to determine their nutritional significance (Pandey et al., 2006; Mbibong et al., 2019). Therefore, it is important to analyze the proximate and mineral content of plants capable of treating illnesses to improve understanding of nutritional health benefits.

Xylopia aethiopica (XA) is an aromatic tree that belongs

Received 18 December 2020; Revised 12 March 2021; Accepted 15 March 2021; Published online 30 June 2021

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to the Annonaceae family. XA is indigenous to lowland rainforest and damp forest in the savannah zones of Africa, and grows up to 15~30 m in height (Mbibong et al., 2019). Fruits of XA represent small, twisted bean-pods; they are dark brown in color, cylindrical in shape, 2.5~5.0 cm in length, and 4~6 mm in thickness (Fig. 1). Each pod contains 5~8 kidney-shaped seeds, each of which are approximately 5 mm in length (Mbibong et al., 2019). In Nigeria, XA fruits and seeds are used to prevent fever, cough, and postpartum bleeding, and to facilitate postnatal recovery (Iwueke and Chukwu, 2020; Imo et al., 2021). Previous studies have reported antioxidant, hypolipidemic, antifungal, and antibacterial effects of whole XA fruits (Tatsadjieu et al., 2003; Nwozo et al., 2011), as well as their preventive effects against dysentery and male/female fertility challenges (Tatsadjieu et al., 2003; Imo et al., 2021). However, the volume of information on the relative abundances of proximate, mineral, and phytochemical constituents in the different anatomical parts of XA fruits remains limited.

In the present study, we examined the relative abundances of proximate, mineral, and phytochemical constituents in XA whole fruit (WF), seeds (S), and pericarp (P) using standard analytical procedures. The results of this study may provide biochemical validation for inclusion of the XA spices as functional ingredients in Nigerian beverages and soups.

MATERIALS AND METHODS

Sample collection

Fresh XA fruits were obtained from a farm in Fiditi, Oyo State, Nigeria. Fruits were air-dried and separated into two portions: WF and a portion to be separated manually into S and P. WF, S, and P were grinded and kept



Fig. 1. *Xylopia aethiopica*.

in labeled containers.

Chemicals/reagents

All chemicals used in this study, including oxalic acid, concentrated hydrochloric acid, ammonium thiocyanate, iron (III) chloride, phytic acid, potassium hexacyanoferrate (III), acetone, glacial acetic acid, tannic acid, ethanol (80%), chloroform, methanol, zinc acetate, potassium ferrocyanide, petroleum ether, and lead acetate, were of analytical grades.

Determination of proximate contents

The chemical compositions of the WF, S, and P were examined using AOAC methods. Moisture content (952.08, AOAC, 1961), total ash (923.03, AOAC, 2000), crude fiber (985.29, AOAC, 2000), total fat (948.15, AOAC, 2000), and crude total protein (991.20, AOAC, 2000) were assayed, and carbohydrate content was obtained by calculating differences. Proximate values were expressed in percentages (Okwu and Morah, 2004; Gonçalves et al., 2010).

Determination of mineral contents

AOAC (1990) methods (990.05, 973.51, 974.27, 968.31, 960.40, 986.24, and 984.27) were used to determine the mineral compositions using standard solutions for each element. Sodium and potassium contents were determined by flame photometer (Model 2655-00, Cole-Parmer Instrument Company, Chicago, IL, USA), whereas calcium, iron, manganese, magnesium, zinc, copper, cobalt, chromium, cadmium, and lead were determined using atomic absorption spectroscopy, and phosphorus content was determined by the spectrophotometric Molybdovanadate method, as described by AOAC method no. 986.24 (1990).

Determination of phytochemical contents

Total polyphenol: The total polyphenolic content was determined following the method described by Harborne (1998). Briefly, 1 g of samples was weighed into 250 mL conical flasks. Each sample was soaked in 20 mL distilled water for 4 days, filtered, and made up to 100 mL with distilled water in volumetric flasks. Filtered samples (1 mL) were pipetted into labeled test tube, and 3 mL of each 0.008 N potassium hexacyanoferrate (III) and FeCl_3 (0.01 N) were added. The absorbance of each mixture was measured at 760 nm after 10 min. Gallic acid was used as the standard phenolic compound, analyzed following the assay procedure. The total phenolic content of each sample was expressed in mg of gallic acid equivalent (mg GAE) per g of sample.

Flavonoids: The flavonoid content of each sample was quantified following the method of Harborne (1973), with slight modification. Briefly, 1 g of samples was soaked in

20 mL of ethyl ether and filtered using filter paper, and 5 mL of each filtrate was measured into test tubes. Dilute ammonia (5 mL) was added and then each test tube was shaken. The upper layers were removed, and the absorbance measured at 490 nm. Rutin was used as a standard, analyzed following the assay protocol. The flavonoid content of each sample was expressed in mg of rutin equivalent (mg RE) per g of sample.

Tannic acid (tannin): The tannin content of each sample was quantified using the Folin-Dennis colorimetric method (Harborne, 1993). Briefly, 5.0 g of samples were mixed 1:10 (w/v) with distilled water, shaken for 30 min at 25°C, and filtered to obtain extracts. A standard tannic acid solution was also prepared. Two milliliters of each experimental sample, the standard solution and distilled water (the standard and blank, respectively) were placed separately into 50 mL volumetric flasks. Samples were mixed with 35 mL of distilled water, and 1 mL of Folin-Dennis reagent followed by 2.5 mL saturated Na₂CO₃ solution. Distilled water was then added to each flask up to a total volume of 50 mL, and solutions and incubated for 90 min at 25°C.

The absorbance of each sample was measured at 760 nm using a colorimeter, relative to the reagent blank at zero. The tannin content was calculated as indicated below:

$$\% \text{ Tannin} = \frac{100}{w} \times \frac{au}{as} \times \frac{c}{1,000} \times \frac{vt}{va}$$

where *w* is weight of the sample, *au* is absorbance of the test sample, *as* is absorbance of the standard tannin solution, *c* is concentration of the standard tannin solution, *vt* is total volume of the extract, and *va* is volume of the analyzed extract.

Cardiac glycosides: Cardiac glycoside content was determined following the method of Harborne (1973). Lead acetate (15%; 2.5 mL) was added to 1 g of sample and the solution was filtered. Chloroform (2 mL) was then added to the filtrate, the filtrate was shaken vigorously, and then the lower layer was collected and dried through evaporation. The dried samples were mixed with 3 mL of glacial acetic acid followed by 0.1 mL of 5% FeCl₃ and 0.25 mL of concentrated H₂SO₄. The mixture was then shaken and, following incubation in the dark for 2 h, the absorbance was measured at 530 nm. Digoxin was used as a standard, analyzed following the assay protocol. The cardiac glycoside content of each sample was expressed in mg of digoxin equivalent (mg DE) per g of sample.

Total alkaloids: Total alkaloid content was determined following the method of Harborne (1973), with slight modification. Briefly, 1 g of each sample was added to 20 mL of 20% H₂SO₄ in ethanol (1:1, v/v). Solutions were filtered, and 1 mL was mixed thoroughly with 5 mL of 40%

H₂SO₄ in test tubes. The mixtures were incubated for 3 h at room temperature and the absorbance measured at 568 nm. Boldine, a standard alkaloid, was used as the standard, analyzed following the assay protocol. The total alkaloid content of each sample was expressed in mg of boldine equivalent (mg BE) per g of sample.

Terpenes: Terpene content was determined following the method described by Soladoye and Chukwuma (2012). Briefly, 0.5 g of samples was added into conical flasks with 20 mL of chloroform and 10 mL of methanol. Mixtures were shaken, incubated at 25°C for 15 min, and then centrifuged at 3,000 rpm. Supernatants were removed, and samples were washed with 20 mL chloroform and 10 mL methanol, and centrifuged. Sodium dodecyl sulphate (10%; 40 mL) was added to dissolve the precipitate, followed by 1 mL of 0.01 M ferric chloride at 30 s intervals. Mixtures were shaken, incubated for 30 min, and the absorbances measured at 510 nm. Terpene (linalool, 0~5 mg/mL) was prepared as the standard from a freshly prepared 100 mg/mL terpene stock solution (linalool, Cat. No. L2602-5G, Sigma-Aldrich Chemicals, St. Louis, MO, USA), analyzed following the assay procedure. The terpene content of each sample was calculated using the following formula:

$$\% \text{ Terpenes} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

Oxalate: Oxalate content was determined following the method described by Onwuka (2005). Briefly, 2 g of extracts were suspended in 190 mL distilled water in 250 mL volumetric flasks. Then, 10 mL of 6 M HCl was added and solutions were incubated at 100°C for 1 h. Digested samples were cooled and distilled water was added to a total volume of 250 mL. Solutions were filtered and 125 mL of filtrates were added into beakers in triplicate with three drops of methyl red indicator. Concentrated NH₄OH solution was added until test solutions changed colour from salmon pink to a faint yellow. Solutions were heated to 90°C, and 10 mL of 5% CaCl₂ solution were added with continuous stirring and cooled overnight at 5°C. Solutions were then centrifuged at 2,500 rpm for 5 min, supernatants removed, and precipitates dissolved in 10 mL of 20% H₂SO₄ solution and diluted to a total volume of 300 mL. Twenty-five mL of each filtrate was heated until close to boiling point, then titrated against 0.05 M standardized KMnO₄ until a faint pink colour developed and remained for 30 s. Oxalic acid content was calculated using the following formula:

$$\text{Oxalate (mg/g)} = \frac{T \times (V_{me})(D_f) \times 10^5}{M_E \times M_f}$$

V_{me} = volume – mass equivalent
 (1 mL of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid)

where T is volume of $KMnO_4$, D_f is dilution factor, M_E is molar equivalent of $KMnO_4$ in oxalic acid ($KMnO_4$ redox reaction is 5), and M_f is mass of sample.

Saponin: Saponin content was determined following the method of Harborne (1973), with slight modification. Briefly, 1 g of samples were soaked with 10 mL of petroleum ether and suspensions (without sediment) were gently poured into beakers with 10 mL of petroleum ether. Supernatants were removed again and mixed with the first supernatants. Mixtures were then evaporated to remove moisture and 6 mL of ethanol was added. Approximately 2 mL of mixtures were pipetted into test tubes, incubated for 30 min, and absorbances were measured at 550 nm. Saponin (aescin) was used as the standard, analyzed following the same assay conditions. Saponin content of each sample was expressed as mg of aescin equivalent (mg AE) per g of sample.

Steroids: Steroid content was determined following the method of Harborne (1973), with slight modification. Briefly, 1 g of each sample was soaked with 20 mL of ethanol. Mixtures were filtered using filter paper and 2 mL of each filtrate was pipetted into test tubes with 2 mL of colored reagent. Solutions were incubated for 30 min and the absorbance was measured at 550 nm. Cholesterol was used as a standard steroid, analyzed following the same assay conditions. Total steroids content of each sample was expressed as mg cholesterol equivalents (mg CSE) per g of sample.

Phytic acid (phytate): Phytic acid content was determined following the method of Lolas and Markakis (1975). Briefly, 2 g of samples were soaked in 100 mL of 2% concentrated HCl for 3 h in 250 mL conical flasks. Solutions were filtered using a double layer of hardened filter paper, and 50 mL of each filtrate was poured into 250 mL beakers with 107 mL of distilled water and 10 mL of 0.3% ammonium thiocyanate solution as an indicator. Solutions were then titrated with standard $FeCl_3$ solution containing 0.00195 g of iron per mL, with the endpoint considered met when a slightly brownish-yellow color persisted for 5 min. The phytic acid content was calculated as follows:

$$\begin{aligned} \% \text{ Phytic acid} &= y \times 1.19 \times 100 \\ y &= \text{titre value} \times 0.00195 \end{aligned}$$

Trypsin inhibitor activity (TIA): TIA was determined using the procedure of Kakade et al. (1974). Briefly, 1 g of each sample was stirred with 100 mL of 0.009 M HCl, shaken at an ambient temperature for 2 h and centrifuged at 10,000 g for 20 min. The clear supernatant was used to

estimate TIA. Diluted extract (1 mL) and 1 mL of distilled water (for the trypsin standard) were added to separate test tubes, followed by 1 mL of trypsin solution. Test tubes were placed in a water bath at 37°C for 10 min, then 2.5 mL of pre-warmed N- α -benzoyl-D-L-arginine-p-nitroanilide (the substrate) was added for 10 min at 37°C before the reaction was stopped using 0.5 mL of acetic acid (30%, v/v). This procedure was also used to prepare sample blanks, whereby trypsin solution was added to samples after reactions were terminated with acetic acid. The absorbance was measured at 410 nm against the sample blank using the spectrophotometer. TIA was calculated using the following equation derived by Hamerstrand et al. (1981):

$$\text{TIA (mg/g)} = \frac{A_{\text{standard}} - A_{\text{sample}}}{0.019 \times \text{Sample wt (g)}} \times \frac{\text{Dilution factor}}{1,000 \times \text{Sample size (mL)}}$$

where A_{standard} is absorbance of standard and A_{sample} is absorbance of test sample.

Anthraquinone: Anthraquinone content was determined following the method of Soladoye and Chukwuma (2012). Dried samples (0.05 g) were added to 50 mL of distilled water and shaken for 16 h. Solutions were incubated at 70°C, and then 50 mL of 50% methanol was added, solutions were filtered, and absorbances were measured at 450 nm. Calibration standards were prepared using alizarin and purpurin at a concentration of 0.01 mg per 1 mL. **Vitamin C:** Vitamin C content was determined by ultraviolet-spectrophotometry, as described by Rahman et al. (2007). Briefly, 1 g of sample, 1 mM ascorbic acid stock solution (standard), and 1 mM trichloroacetic acid (TCA) solution (blank) were added into separate test tubes. Then, 10 mL of TCA solution was added to each test tube, followed by 1 mM of dinitrophenyl hydrazine-thiourea-copper sulphate reagent. Test tubes were capped, incubated in a water bath at 37°C for 3 h, and chilled for 10 min in an ice bath with slow shaking. Two milliliters of cold 12 M H_2SO_4 was then added to all solutions. The spectrophotometer was adjusted to zero absorbance against the blank at 520 nm. The absorbances of standard and test samples were measured 520 nm, and vitamin C content was calculated as follows:

$$\text{Vitamin C (mg/100 g)} = \frac{A_{\text{sample}} \times \text{Conc}_{\text{standard}}}{A_{\text{standard}} \times \text{Sample wt}}$$

where A_{standard} is absorbance of standard solution, A_{sample} is absorbance of test sample, and $\text{Conc}_{\text{standard}}$ is concentration of standard.

Vitamin E: Vitamin E content was determined following the method of Pearson (1976). Briefly, 1 g of samples, 10 mL of absolute alcohol, and 20 mL of alcoholic tetraoxo-

sulphate (VI) acid (H_2SO_4) were added into 100 mL flasks. Ten-milliliter aliquots of the clear solutions were pipetted into test tubes, heated in a water bath at 90°C for 30 min, and allowed to cool. The absorbances of the solutions were measured using spectrophotometer at 470 nm. Blank and standard vitamin E solutions were also prepared, and vitamin E content was calculated as follows:

$$\text{Vitamin E (mg/100 g)} = \frac{(A_{\text{sample}} - A_{\text{standard}}) \times \text{Conc}_{\text{standard}}}{(A_{\text{blank}} - A_{\text{standard}}) \times \text{Sample wt}}$$

where A_{sample} is absorbance of test sample, A_{standard} is absorbance of the standard solution, A_{blank} is absorbance of the blank, and $\text{Conc}_{\text{standard}}$ is concentration of standard in mg/100 g.

Vitamin A: Vitamin A content was determined following the method of Pearson (1976), with slight modification. Ground sample (1 g) was macerated with 20 mL of petroleum ether, decanted into test tubes, and then evaporated until dry. Chloroform-acetic anhydride (0.2 mL) was then added to the residue (1:1, v/v), followed by 2 mL of TCA-chloroform (1:1, v/v), and absorbances were measured at 620 nm. Vitamin A standards were prepared following the same assay procedures and the absorbances measured at 620 nm. The concentration of vitamin A in the sample was extrapolated from standard curves.

Statistical analysis

Data were expressed as mean \pm standard of three experimental repeats. Mean values were analyzed using one-way analysis of variance (ANOVA) with the aid of GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA), followed by Tukey's post-hoc test used for multiple comparisons. Differences were considered significant at P -values < 0.05 .

RESULTS

Proximate composition of XA

Proximate composition of WF, S, and P of XA are shown

in Table 1. WF contained the highest crude protein, crude fibre, fat, ash, and moisture contents, whereas P contained the highest content of nitrogen free extracts (NFE). The crude protein and fat compositions of the WF and S significantly differed ($P < 0.05$) from those of P, and the crude fibre, ash, and moisture contents of WF and P significantly differed ($P < 0.05$) from those of S. In addition, the carbohydrate composition (NFE) of S and P significantly differed ($P < 0.05$) from WF.

P had the highest NFE content ($56.77 \pm 0.24\%$) followed by S ($54.52 \pm 0.02\%$) and WF ($30.39 \pm 0.47\%$). However, WF had the highest fat and crude protein contents ($32.74 \pm 0.11\%$ and $7.80 \pm 0.02\%$, respectively), followed by S ($28.60 \pm 0.05\%$ and $5.67 \pm 0.06\%$, respectively) and P ($18.50 \pm 0.08\%$ and $3.45 \pm 0.03\%$, respectively). The crude protein content in WF was relatively lower than that reported by Effiong et al. (2009) (13.08%), which may be attributed to differences in varieties of spice used or geographical locations where they were cultivated. Furthermore, crude fibre, moisture, and ash contents were highest in WF ($18.39 \pm 0.39\%$, $11.33 \pm 0.10\%$, and $10.68 \pm 0.02\%$, respectively) followed by P ($12.59 \pm 0.08\%$, $10.79 \pm 0.04\%$, and $8.70 \pm 0.05\%$, respectively) and S ($4.61 \pm 0.04\%$, $6.60 \pm 0.04\%$, and $9.70 \pm 0.05\%$, respectively).

Vitamin content of XA

Vitamins C, A, and E content in the WF, S, and P of XA are shown in Table 2. Vitamin C was measured in mg/100 g, vitamin A in g equivalent of vitamin A (vit A eq) per 100 g, and vitamin E in g equivalent of α -tocopherol per 100 g. Results showed vitamin A was more abundant than vitamins C and E, with higher contents in WF (4.83 ± 0.06 g vit A eq/100 g) than S and P (3.50 ± 0.06 g vit A eq/100 g and 1.64 ± 0.02 g vit A eq/100 g, respectively). Vitamin C was also found in samples at concentrations (WF: 1.83 ± 0.03 mg/100 g; S: 0.61 ± 0.01 mg/100 g; P: 0.40 ± 0.01 mg/100 g) lower than the recommended dietary allowance (RDA) value for adults (60 mg/100 g) (Seeley et al., 1996). These data support use of XA as a condiment and as ingredients in herbal concoctions.

Table 1. Proximate composition of whole fruit, seed, and pericarp of *Xylopi* *aethi* *opica*

(unit: %)

Parameters	Whole fruit	Seed	Pericarp
Crude protein	7.80 ± 0.02^a	5.67 ± 0.06^b	3.45 ± 0.03^c
Crude fibre	18.39 ± 0.39^a	4.61 ± 0.04^c	12.59 ± 0.08^b
Fat	32.74 ± 0.11^a	28.60 ± 0.05^b	18.50 ± 0.08^c
Ash	10.68 ± 0.02^a	6.60 ± 0.04^c	8.70 ± 0.05^b
Moisture content	11.33 ± 0.10^a	9.70 ± 0.05^c	10.79 ± 0.04^b
Carbohydrate (NFE)	30.39 ± 0.47^c	54.52 ± 0.02^b	56.77 ± 0.24^a

Mean \pm SD (n=3).

Means with different letters (a-c) within the same row are significantly different ($P < 0.05$).

NFE, nitrogen free extracts.

Table 2. Vitamin composition of the whole fruit, seed, and pericarp of *Xylopia aethiopica*

Parameters	Whole fruit	Seed	Pericarp
Vitamin C (mg/100 g)	1.83±0.03 ^a	0.61±0.01 ^b	0.40±0.01 ^c
Vitamin A (g vitamin A eq/100 g)	4.83±0.06 ^a	3.50±0.06 ^b	1.64±0.02 ^c
Vitamin E (g α-tocopherol eq/100 g)	0.62±0.02 ^a	0.26±0.02 ^b	0.10±0.01 ^c

Mean±SD (n=3).

Means with different letters (a-c) within the same row are significantly different ($P<0.05$).

Mineral element composition of XA

The relative mineral compositions WF, S, and P of XA are shown in Table 3. Mineral analysis revealed the presence of macro elements (sodium, potassium, calcium, phosphorus, and magnesium) and micro elements (iron, zinc, manganese, copper, and chromium) in WF, S, and P, with highest concentrations present in WF. However, mineral contents in both WF and S were significantly higher than P ($P<0.05$). Potassium, calcium, and magnesium were present in higher concentrations than the other mineral elements analyzed in this study. Indeed, potassium, calcium, and magnesium were all more abundant in WF (288.69±0.09 mg/100 g, 236.42±0.96 mg/100 g, and 175.29±1.21 mg/100 g) followed by S (246.90±0.56 mg/100 g, 212.09±1.22 mg/100 g, and 168.21±0.67 mg/100 g, respectively) then P (138.06±0.82 mg/100 g, 187.68±1.10 mg/100 g, and 141.42±0.58 mg/100 g, respectively). Heavy metals such as chromium and cobalt were absent in the investigated samples and minute quantities of lead and cadmium were detected, suggesting XA is still safe for consumption.

Phytochemical content and TIA

Phytochemical content and anti-nutrient of WF, S, and P of XA are shown in Table 4. WF contained the highest

total phenolic, total flavonoids, tannin, saponin, cardiac glycoside, terpenoid, steroid, phytate, oxalate, alkaloid, and anthraquinone contents, and demonstrated the highest TIA, followed by S and then P. The phytochemical contents of WF and S were significantly higher ($P<0.05$) than those of P. Furthermore, flavonoids, tannin, phenol, and cardiac glycoside were present at higher relative abundances than the other phytochemicals. Of note, flavonoid content ranged from 18.68±0.16 mg/g (WF) to 9.85±0.31 mg/g (P), tannin content ranged from 16.46±0.50 mg/g (WF) to 9.60±0.09 mg/g (P), phenol content ranged from 14.42±0.59 (WF) to 8.70±0.05 mg/g (P), and cardiac glycoside content ranged from 11.38±0.55 (WF) to 6.40±0.02 mg/g (P). Phytate and TIA were relatively lower than those of the other anti-nutrients examined.

DISCUSSION

Proximate composition

The crude protein content in WF, S, and P ranged from 3.45 to 7.80. The quantity of crude protein in WF was lower than that described by Effiong et al. (2009) (13.08%) but higher than that described by Freiburghaus et al.

Table 3. Mineral composition of the whole fruit, seed, and pericarp of *Xylopia aethiopica*

(unit: mg/100 g)

Mineral elements	Whole fruit	Seed	Pericarp
Sodium (Na)	15.77±0.08 ^a	13.60±0.04 ^b	10.41±0.10 ^c
Potassium (K)	288.69±0.09 ^a	246.90±0.56 ^b	138.06±0.82 ^c
Calcium (Ca)	236.42±0.96 ^a	212.09±1.22 ^b	187.68±1.10 ^c
Phosphorus	62.75±0.05 ^a	59.01±1.07 ^b	47.70±0.52 ^c
Iron	32.50±0.48 ^a	29.04±0.36 ^b	21.16±0.89 ^c
Zinc	4.14±0.04 ^a	2.78±0.61 ^b	1.89±0.04 ^c
Magnesium (Mg)	175.29±1.21 ^a	168.21±0.67 ^b	141.42±0.58 ^c
Manganese	12.46±0.06 ^a	9.60±0.04 ^b	6.57±0.32 ^c
Copper	1.55±0.02 ^a	1.32±0.02 ^b	0.61±0.01 ^c
Lead	0.0033±0.0006 ^a	0.0010±0.0000 ^b	0.0010±0.0000 ^b
Cadmium	0.002±0.000 ^a	0.001±0.000 ^b	0.001±0.000 ^b
Cobalt	ND	ND	ND
Chromium	ND	ND	ND
Na/K ratio	0.055 ^b	0.055 ^b	0.075 ^a
Ca/Mg ratio	1.35 ^a	1.26 ^c	1.33 ^b
K/Na ratio	18.31 ^a	18.15 ^b	13.26 ^c

Mean±SD (n=3).

Means with different letters (a-c) within the same row are significantly different ($P<0.05$).

ND, not detected.

Table 4. Phytochemical constituents, anti-nutrients, and trypsin inhibitory effects of *Xylopi* *aethi* *opica* whole fruit, seed, and pericarp (unit: mg/g)

Parameters	Whole fruit	Seed	Pericarp
Total phenolics	14.42±0.59 ^a	11.71±0.04 ^b	8.70±0.05 ^c
Total flavonoids	18.68±0.16 ^a	15.57±0.20 ^b	9.85±0.31 ^c
Tannin	16.46±0.50 ^a	14.34±0.54 ^b	9.60±0.09 ^c
Saponin	4.65±0.09 ^a	2.94±0.04 ^b	0.81±0.02 ^c
Trypsin inhibitors	2.45±0.07 ^a	1.82±0.04 ^b	0.72±0.03 ^c
Cardiac glycoside	11.38±0.55 ^a	9.43±0.03 ^b	6.40±0.02 ^c
Terpenes	3.72±0.03 ^a	1.92±0.03 ^b	0.82±0.03 ^c
Steroid	5.33±0.07 ^a	3.85±0.03 ^b	1.96±0.02 ^c
Phytate	2.73±0.14 ^a	1.82±0.02 ^b	0.62±0.02 ^c
Oxalate	3.16±0.07 ^a	2.11±0.01 ^b	1.12±0.02 ^c
Alkaloid	6.81±0.03 ^a	4.78±0.08 ^b	2.19±0.04 ^c
Anthraquinone	4.72±0.03 ^a	2.80±0.04 ^b	2.22±0.32 ^c

Mean±SD (n=3).

Means with different letters (a-c) within the same row are significantly different ($P<0.05$).

(1996) (1.8~3.6%). The high protein content of WF may supplement protein needed for daily function, and could be responsible for its anti-malarial effects (Isong and Essien, 1996). Plants containing approximately 12% of their calorific values from protein are considered a rich source of protein (Effiong et al., 2009; Aberoumand, 2010). The crude fat contents of WF (32.74%) and S (28.6%) were significantly higher ($P<0.05$) than P (18.50%). The crude fat contents of WF and P fall within the range described by acceptable macronutrient distribution ranges (AMDRs) for adults (20~35%) (Institute of Medicine, 2005). Furthermore, ash content, regarded as an indicator of mineral content in living organisms, was significantly ($P<0.05$) higher in both WF (10.68%) and P (8.70%) than S (6.6%). Therefore, WF of XA could be a rich source of mineral elements.

Values for NFE or available carbohydrates for WF, S, and P ranged from moderate (for WF) to high (for P) (Table 1). NFE values for P and S was significantly higher than for WF ($P<0.05$), both residing within the range for adult provided by AMDRs (45~60%) (Institute of Medicine, 2005). Carbohydrates supply energy to cells in the body, especially the brain, the only organ that depends solely on carbohydrates. Slavin (2013) stated that the Institute of Medicine acknowledged dietary fibre consists of indigestible carbohydrates and lignin, which is intrinsic and complete in plants.

The moisture contents of WF and P were significantly higher than those of S. The low moisture content of S allows it to be stored for a longer duration than WF and P without spoiling; higher moisture contents lead to food spoilage through increasing microbial activity (Embaby and Mokhtar, 2011).

The crude fibre contents of both WF (18.39%) and P (12.59%) were significantly higher than that of S (4.61%) (Table 1). The crude fibre content of WF was within the RDA for children of 19~20% (National Research Coun-

cil, 1989). In the human body, crude fibre contributes to weight, bulk, and softness of fecal matter, and helps prevent constipation and lessen the incidence of coronary and breast cancer (Lintas, 1992).

Dietary fibre cleanses the digestive tract by removing potential carcinogens from the body, therefore preventing absorption of excess cholesterol (Gunniss and Gidley, 2010; Soliman, 2019). In addition, fibre adds calories to food and lessens intake of excess starchy food, a common feature of diets in Nigeria (Tonukari et al., 2013), thereby helping to prevent metabolic conditions such as hypertension and diabetes mellitus (Nwanna et al., 2016; Avwioroko et al., 2020).

Phytochemicals

Phytochemicals do not provide nourishment but have shielding properties (Ifesan et al., 2013). Phytochemicals are secondary metabolites or plant products that possess pharmacological, medicinal, and nutritive characteristics in terms of flavor and color attributes. Flavonoids and tannins in fruits are important sources of naturally occurring antioxidants and are preferred over man-made variants due to lower toxicity (Ifesan et al., 2013). Flavonoids scavenge free radicals produced by ROS, thereby helping to prevent diseases caused by oxidative stress (Oyagbemi et al., 2016; Atanu et al., 2019). Flavonoids followed by tannin were the most abundant phytochemicals in WF, S, and P, showing that the three samples can scavenge free radicals. Saponins exhibit hypocholesterolemic properties by forming insoluble complexes with cholesterol, resulting in slower absorption (Aletor, 1993).

Mineral constituents and anti-nutrients

Minerals are inorganic (i.e., not organic nutrients). Most minerals are essential in very small quantity ranging from <1 to 2,500 mg per day (Dibaba et al., 2014; Seth et al., 2014) and are essential for proper functioning of the hu-

man immune system. Minerals are usually consumed from plant materials such as spices. Phosphorus is an essential component of adenosine triphosphate (ATP) and nucleic acids and is important for the acid-base balance and formation of bone and tooth (Tang et al., 2020). Iron is essential in haemoglobin (the pigment that carries oxygen) for normal red blood cell function and in cytochromes functioning in cellular respiration (Bahadir et al., 2018).

Magnesium, copper, zinc, iron, and manganese are major co-factors present in the structure of enzymes vital for numerous metabolic pathways (Ribeiro et al., 2020). In addition, sodium, potassium, and chlorine are critical for maintaining osmotic balance between cells and interstitial fluid (Alcázar Arroyo, 2008). Aside from XA, other fruits and spices (e.g., *Piper guineense*, *Monodora myristica*, *Aframomum melegueta*, and *Parkia biglobosa*) have also been reported to contain high amounts of potassium (Borquaye et al., 2017). Potassium was the most abundant mineral contained within WF, S, and P of XA.

Of the XA parts measured, WF had the highest mineral content followed by S then P. In human cells, potassium is accumulated by the action of the sodium/potassium-ATPase and it is an activator of several enzymes, including being a cofactor involved in normal growth and muscle function (Birch and Padgham, 1994).

Minerals were present in XA in the following order of abundances: potassium>calcium>magnesium>phosphorus>iron>sodium>manganese>zinc>copper. Our data show that WF of XA is a reliable source of minerals. Copper has several important functions in human, including roles in producing erythrocytes and leucocytes and in triggering liberation of iron to form haemoglobin (Idris et al., 2010; Jimoh et al., 2010).

Sodium and potassium are vital for intercellular activities, such as maintaining the osmotic balance of body fluid, protecting against excessive fluid loss, contraction of muscle cells, and conduction of impulses along nerve fibers (Rankin and Hildreth, 1976). Sodium/potassium ratios also help prevent high blood pressure, with sodium/potassium ratios of 0.6 recommended for hypertensive patients (Nieman et al., 1992). In this study, sodium/potassium ratios of WF, S, and P were all lower than 0.6 (0.055, 0.055, and 0.075, respectively), indicating that they may help suppress high blood pressure when consumed. Furthermore, potassium and sodium regulate muscle contraction and nerve impulse transmission, with high potassium/sodium ratios potentially playing roles in excess water and salt excretion (Ashurst and Arthey, 2001). In the present study, the sodium contents of WF, S, and P were lower than those of potassium, indicating high potassium/sodium ratios (18.31, 18.15, and 13.26, respectively). In addition, potassium and sodium help in maintaining the normal equilibrium between

acids and alkalis and osmotic pressure (Alcázar Arroyo, 2008). Magnesium and phosphorus are also vital for growth and maintenance of bones, teeth, and muscles, both of which are prevalent in the WF, S, and P of XA (Baj et al., 2020; Salau et al., 2020).

Phytates react by chelating different cations, thereby reducing bioavailability of minerals in the S or WF. However, small quantities of phytates lower blood glucose levels and show protective effects against colon cancer due to their antioxidant effect. Furthermore, phytates can serve as prebiotics due to their capacity to bind enzymes such as amylases, causing portion of the starch to enter the intestine undigested (Harland and Morris, 1995). It has also been reported that certain other spices (e.g., *P. biglobosa*, *P. guineense*, and *M. myristica*) contain moderate amounts of phytate (Borquaye et al., 2017).

Tannins are important for enhancing wound healing (Okwu and Josiah, 2006) and may exhibit anti-diabetic properties (Iwu, 1983). At high concentrations, tannin may also be considered anti-nutrients due to their abilities to bind and precipitate proteins and other organic compounds such as alkaloids (Van Buren and Robinson, 1969).

Cardiac glycosides exert potent and direct action on the heart, helping to support its strength and rate of contraction when it is failing (Malik and Siddiqui, 1981). Furthermore, anthraquinones possess laxative, anti-malarial, and anti-carcinogenic effects (Aelami et al., 2020; Eom et al., 2020).

Munro and Bassir (1969) stated that oxalates form insoluble complexes with calcium, magnesium, zinc, and iron, thereby inhibiting the effects of these minerals. Indeed, oxalates can have harmful effects on human nutrition and health, such as by reducing calcium absorption and aiding creation of kidney stones (Fekadu et al., 2013). Most urinary stones formed in humans are calcium oxalate stones; therefore, oxalate ingestion should not exceed 60 mg/d (Massey et al., 2001). Oxalates and phytates are often regarded as anti-nutritional factors because of their strong binding affinity to important minerals such as calcium, iron, and zinc at elevated concentrations, but they are often destroyed or condensed to non-toxic levels by extensive slow heating (Munro and Bassir, 1969; Dendougui and Schwedt, 2004; Coe et al., 2005). However, oxalates possess certain health benefits when present at low concentrations, such as in maintaining levels of certain minerals. The anti-nutritional activity of phytates can also be beneficial in adults, especially menopausal women, who often have high levels of iron that can cause biological stress due to its strong oxidant properties. Although trypsin inhibitors decrease the rate of protein digestion (Sá et al., 2020), they are also involved in appetite regulation and maintaining energy balance involving satiety hormones, and serve as anticarci-

nogenic and radioprotective agents (de Lima et al., 2019).

Data reported on the WF, S, and P in the present study indicate that these parts of XA are rich in phytochemicals, minerals, and certain moderate anti-nutrients. These components may be accountable for the role of XA in traditional medicine for treatment of microbial infections and fever, and for boosting appetite and blood levels in sick individuals, especially in rural areas.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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