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Nutritional Quality and Toxicological Safety of Leaves and Fruit Fractions of *Xylopia aethiopica*

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ABSTRACT

Plants are a major source of nutrients for healthy living and medicines for humans. They are important for growth and development as well as in the prevention of diseases. The body needs a balanced diet in appropriate amounts for optimal functioning of its organs. The plant *Xylopia aethiopica* (Negro pepper), is one of such commonly used plants not only as a condiment but also as an ingredient in foods. It is in the public's interest to scientifically verify the toxicity and health benefits of this plant. This study aimed to investigate the nutritional qualities and toxicological safety of the leaves and fruits (with and without seeds) of *X. aethiopica*. Hydro-ethanolic extracts of the leaves and fruits (with and without seeds) were obtained using the Soxhlet extraction technique. Toxicological safety of the extracts was determined by acute toxicity test. Phytochemical constituents were screened qualitatively. Proximate and nutritional analyses were performed using AOAC (Association of Official Analytical Chemists) International methods, while elemental analyses were conducted using atomic absorption spectroscopy (AAS). Results revealed that, Carbohydrate, protein, fat, vitamin, and moisture were present in the leaves and fruits of *X. aethiopica,* though in varying amounts. Toxic heavy metals such as, lead, nickel, and chromium were within acceptable limits, and cadmium was not detected. *X. aethiopica* is safe for consumption even above 5g/Kg body weight per day..In conclusion, the findings of this study show that, *Xylopia aethiopica* is a 'value-added ingredient.'

Keywords: *Xylopia aethiopica*, Leaf, Fruit, Hydro-ethanolic, Extract, Heavy Metals.

Introduction

Xylopia aethiopica, widely recognized as African Guinea pepper or "Uda," is an aromatic evergreen tree that belongs to the Annonaceae family. Found abundantly in the rainforests and savanna woodlands of Sub-Saharan Africa, this plant plays a key role in traditional remedies and African cuisine (Iwu *et al.,* 2022). It is particularly valued for its use in postpartum soups and herbal preparations. In addition to these applications, there is growing interest in exploring the plant's broader dietary benefits, particularly those derived from its leaves and fruits (Ahereza 2025).

In many developing regions, native plants serve as crucial sources of essential nutrients (Ezenobi *et al.,* 2022). X. *aethiopica* holds promise far beyond its traditional use as a spice. Initial findings indicate that the plant contains noteworthy levels of Carbohydrates, Proteins, essential fats, and various vitamins (Ifesan *et al.,* 2020; Fategbe *et al*., 2021; Iwu *et al*., 2022). However, the distribution of nutrients can vary significantly between the plant's different parts, such as its leaves and fruits. The common practices of consuming the fruit either whole or deseeded introduced further variables, raising questions about the nutritional yield from these fractions. Investigating these aspects is vital to optimizing the plant's potential as a nutritive food source.

Although *X. aethiopica* has been used traditionally for centuries, its safety profile is still a subject of scientific inquiry. As plants often absorb elements from the soil, they can sometimes accumulate heavy metals like lead (Pb), nickel (Ni), chromium (Cr), and cadmium (Cd) (Angon *et al*., 2024). When present in concentrations exceeding permissible limits established by international health standards, these elements pose serious health risks.

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To certify a plant for use as a safe food source or supplement, it must undergo thorough scrutiny to ensure its elemental purity.

In addition to assessing heavy metal contamination, it is crucial to evaluate the acute toxicity of *X. aethiopica's* extracts (Ehigiator and Adikwu, 2020. While traditional usage may suggest general safety, empirical data particularly an established lethal dose (LD50) is necessary for scientific validation. Demonstrations of safety even at high consumption levels, surpassing 5 g/kg of body weight, are essential milestones in transitioning this plant from a culturally significant remedy to a standardized nutritional resource.

This research aims to provide a comprehensive assessment of the nutritional profile and safety of *X. aethiopica's* leaves and fruit fractions. By analyzing their proximate composition, conducting heavy metal assessments, and investigating the acute toxicity profile, this study seeks to validate the plant's potential both as an effective nutritional resource and as a safe addition to the human diet. The findings will contribute to ensuring its widespread use remains not only beneficial but also free from health risks.

Materials and Methods

This research was carried out in accordance with the ethical approval obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria, with reference number EC/FP/018/19 issued on 04 April, 2018.

Plant Collection, Authentication, and Processing

The leaves and fruits of mature *X. aethiopica* were harvested during the daytime from cultivated trees growing in Uhe village in Ovia North-East Local Government area of Edo state, Nigeria. A plant taxonomist of the Plant Biology and Biotechnology Department, Faculty of Life Sciences, University of Benin, Benin City, authenticated them and assigned them a voucher number of UBH_X 348.

The harvested leaves and fruits of *X. aethiopica* were sorted so as to remove unwanted materials. Each plant part was then packed into different sacks and transported to the laboratory.

In the laboratory, they were spread out on very clean benches for two weeks at room temperature (25 - 27ºC) to allow for proper air drying. The leaves were further dried in a thermostatically regulated oven at 60 °C for 30 min, while the fruits were further dried at 60 °C for 1 hr before milling with an electric miller (Kenwood, UK). The powdered samples were packed into air - tight amber coloured glass bottles and stored until required for the work.

Extraction Process

Hydro -ethanol extracts of the leaves and fruits (with and without seeds) of *X. aethiopica* were obtained by the Soxhlet extraction method. A measured quantity of 2.5 kg of the powdered leaves and fruits (with and without seeds) was extracted separately with 7.5L of hydro-ethanol (20% ethanol in distilled water). Each of the extracts obtained was concentrated separately in a vacuum using a rotary evaporator and reduced to dryness in a thermostatically regulated electric oven maintained at a temperature of 20°C (Emokpae and Emokpae, 2024). They were packed separately into screw-capped bottles, labelled and preserved in the refrigerator at 4°C for biological evaluations.

Acute toxicity test

The acute toxicity test was done using Miller and Tainter (1944) method. Thirty male Wistar rats and thirty female Wistar rats were used. Prior to the experiment, the animals were fasted over-night but given only water *ad libitum*. Each of the sexes was divided into five (5) groups (A - E) of 6 rats per group. Group A which served as the control received 10 ml/kg of 2% Tween-80 solution while groups B, C, D and E were served 0.5, 1, 2 and 5 g/Kg of the hydro-ethanol extract of the leaves and fruits (with and without seeds) in 2% Tween-80 respectively orally by oral gastric administration (Mekuneen *et al.,* 2020).

Adverse side effects like weakness, aggressiveness, refusal to eat, diarrhoea, eye discharge, ear discharge, noisy breathing, morbidity and mortality due to the administration of the plant extracts to the Wistar rats were monitored first for 24 hours (immediate effects) after dosing and subsequently for two weeks (14 days), for delayed effects post drug administration (Uwumarongie and Oviana, 2017).

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Phytochemical Tests

Phytochemical tests were carried out on the hydro-ethanol extracts of the powdered leaves and fruits (with and without seeds) of *X. aethiopica* for the presence/absence of glycosides, saponins, cyanogenetic glycosides, cardiac glycosides, flavonoids, anthraquinones, tannins and alkaloids, by using standard phytochemical procedures (Harborne, 1973; Sofowora, 2008; Evans, 2009). Each of the tests was done in triplicate. The colour change, turbidity or precipitation was noted and recorded.

Proximate Analysis

Determination of moisture content

Three grams (3g) of the powdered *X. aethiopica* leaves or fruits (with or without seeds), were accurately weighed into clean crucibles. It was then heated for one hour at 105 °C and cooled in a desiccator. It was then weighed and noted. The procedure was repeated until there was no further loss in weight. The average percentage weight loss in relation to the air-dried powdered drug was determined for three replicates.

Total ash content

Three grams (3g) of the powdered sample (leaves or fruits (with and without seeds) was accurately weighed into a previously ignited and tarred crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 550 °C until it was white, indicating the absence of carbon. It was cooled in a desiccator and weighed (WHO, 1998). The total ash was calculated in the following equation:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of original powder}} \times 100$$

Crude fibre

About 2 g of the plant sample (leaves or fruits) was boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25 g of H_2SO_4 per 100 mL of solution. The solution was filtered through linen on a fluted funnel. The residue was washed with boiling water until the washings were no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes with 200 mL of a solution containing 1.25 g of carbonate free NaOH per 100 mL.

The final residue was filtered through a thin but close pass of washed and ignited asbestos in a Gooch crucible. The residue was then incinerated, cooled and weighed (Onwuka, 2005). This was then determined for three replicates.

% Crude fiber = loss in weight after incineration × 100

Crude fat

Clean 250 mL boiling flasks were dried in an oven at 105°C for 30 min. They were transferred into a desiccator and allowed to cool. Two grams (2 g) of the powdered sample (leaves or fruits) was weighed accurately into labelled thimbles. Another set of correspondingly cooled boiling flasks was weighed and filled with 300 mL of petroleum ether. The extraction thimble was plugged lightly with cotton wool. The Soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimble was removed with care, and petroleum ether was drained off into a container for reuse. When the flask was almost empty of petroleum ether, it was removed and dried at 105°C for 1 hr. The flask was transferred from the oven into a desiccator, allowed to cool and was weighed (Onwuka, 2005). This was then determined for three replicates.

$$\% \text{ Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Crude protein

Determination of the crude protein content was done for three replicates by first digesting the protein and then, distilling the protein as described below (Onwuka, 2005).

Two grams (2 g) of the plant sample was weighed into a Kjeldahl flask and five grams (5 g) of anhydrous sodium was added. One gram (1g) of copper sulphate and a speck of selenium were added. Then, 25mL of concentrated sulphuric acid and 5 glass beads were introduced into the mixture. The mixture was heated very gently at first in the fume cupboard and the heat was increased with occasional shaking till the solution assumed a green colour. Black particles at the mouth and neck of the flask were cooled and washed down with distilled water. Mixture was reheated at first until the green colour disappeared and was allowed to cool.

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The digest with several washings were transferred after cooling into a 250 mL volumetric flask and it was made up to the mark with distilled water. The digest was then distilled using the Markham distillation apparatus.

Protein Distillation

The Markham distillation apparatus was steamed through for about 15 minutes. A 100 mL conical flask containing 5 mL of boric indicator was placed under the condenser such that the condenser tip was under the liquid. 5 mL of the digest was then pipetted into the body of the apparatus via the small funnel aperture and was weighed down with distilled water, followed by 5 mL of 60 % NaOH solution. The apparatus was steamed through for about 5 minutes to collect enough ammonium sulphate. The receiving flask was removed and washed down the tip of the condenser into the flask and the condenser water was then removed. The solution was titrated into the receiving flask using 0.01N hydrochloric acid and the nitrogen content and protein content of the sample was calculated with the formula:

% Crude protein = %N × Conversion factor
Conversion factor is usually 6.25 and %N represents nitrogen content.

$$\%N = \frac{(Vs - Vb) \times Nacid \times 0.01401}{\text{Weight of original powder (g)}} \times 100$$

(Onwuka, 2005)

Determination of carbohydrate

The carbohydrate content was obtained by subtracting the summed values of total ash, crude protein, fibre, and fat from 100% (Onwuka, 2005).

That is, the % of available carbohydrate in the plant part = 100 – (% Ash + % Protein + % Fibre + % Fat)

Elemental analysis

In the elemental analysis, Sodium (Na) and Potassium (K) were analyzed using a flame photometer while Magnesium (Mg), Calcium (Ca), Copper (Cu), Zinc (Zn), Cadmium (Cd), Nickel (Ni), Lead (Pb), Manganese (Mn), Chromium (Cr) and Iron (Fe) were analyzed using Atomic Absorption Spectroscopy (AAS) (Onwuka, 2005).

Results

The results of the nutritional and vitamin C content of the leaves and fruits with seeds and fruits without seeds of *X. aethiopica* are shown in Table 1. They, however, were found to have vitamin C, carbohydrate, crude protein, crude fat, crude fibre, moisture, and produce ash in varying amounts.

Table 1: Results of the nutritional and vitamin C contents of the leaves and fruits (with and without seeds) of *X. aethiopica*

Parameter	Leaves	Fruits with seeds	Fruits without seeds
Moisture content (%)	11.54	8.50	8.42
Total ash content (%)	0.79	1.97	1.91
Crude fibre (%)	55.12	62.18	60.03
Crude fat (%)	12.73	12.27	12.13
Crude protein (%)	21.56	10.11	10.08
Carbohydrate (%)	9.80	13.47	15.85
Vitamin C (mg/kg)	352.37	89.11	85.07

Table 2 shows the elemental contents of the leaves, fruits with seeds and fruits without seeds. Copper, Iron, Magnesium, Sodium, Potassium, Calcium, Manganese, Lead, Chromium, Zinc and Nickel were detected. Cadmium was not detected in any of the three plant parts used.

Table 2: Elemental contents of the leaves and fruits (with and without seeds) of *X. aethiopica*

Parameters (mg/kg)	Leaves	Fruits with seeds	Fruits without seeds
Copper	0.16	0.07	0.06
Iron	2.43	2.10	2.00
Magnesium	1.65	1.64	1.63
Sodium	1.20	0.70	0.60
Potassium	94.60	31.96	31.40
Calcium	13.30	60.60	60.60
Manganese	0.07	0.32	0.30
Lead	0.01	0.02	0.01
Chromium	0.01	0.08	0.08
Zinc	0.15	0.12	0.11
Cadmium	ND	ND	ND
Nickel	0.01	0.03	0.02

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Discussion

The relatively high fibre contents of the leaves and fruits align with the study done by Adesuyi *et al*., (2012). Dietary fibre aids bowel movement and absorption of trace elements. It increases gastric empting time and reduces lipid and glucose absorption from the gut. Moisture content in a plant is also very important for the storage of the plant parts. It is worthy of note that high moisture content, easily degrades plants. The presence of carbohydrate, protein, fat, vitamin and moisture in the leaves and fruits of *X. aethiopica* though in varying amount, indicates its' nutritional potential. This is the most likely reason why it is commonly referred to as "VALUE ADDED" ingredient, whenever it is used. These observations are consistent with previous studies (Osabor *et al*., 2015; Obiloma *et al*., 2020). The presence of Vitamin C is of great importance due to it's antioxidant property. It however, contributes to the anti-oxidant property of the plant parts studied. Vitamin C was observed to be more in the leaves than the fruits (with seeds and without seeds).

A proteomics study that was done on *X. aethiopica* from Nigeria identified 608 proteins while 260 proteins were identified in that from Ghana. This further probably explains why the *X. aethiopica* collected from Nigeria has better nutritive potentials than that from Ghana. However, of all the proteins identified in both countries, only 114 proteins were common between the samples from them (Yin *et al.,* 2019). Bioinformatics and functional analyses showed that the differential levels of the proteins were mainly due to pathways involved in amino acids metabolism and biosynthesis (Yin *et al*., 2019).

Trace metals or minerals are very important for healthy living. They are useful in making enzymes and hormones in the body. They contribute to ensuring that the bones, muscles, heart and brain are working properly. An elemental analysis of the leaves and fruits (with and without seeds) of *X. aethiopica* used in this study revealed that they each have eleven trace metals.

In the leaves, potassium had the highest amount while calcium was highest in the fruits (with or without seeds). Lead, cadmium and chromium are none nutritive heavy metals which are toxic upon consumption at no matter how small the amount over time (Rafique *et al*., 2021).

They were however found to be present in parts of *X. aethiopica*, though their contents were within acceptable limits. Most interestingly is the non-detection of cadmium in this study.

Potassium is useful for proper electrolyte balancing of fluids in the body, nerve-impulse transmission as well as control of the activity of the heart muscle. Calcium is known to play a key role in the health of teethe and bone, nerve function, relaxation and contraction of muscle. Iron, is used by the body to make haemoglobin which is a protein in red blood cells that is responsible for transporting oxygen in the body, serves as co-factor for some enzymes and important for immunity (Obeagu, 2025). This could be the reason why when the leaves and fruits are used traditionally to treat rheumatism and boast post partum mothers; there are positive responses health wise. Magnesium is important for bone health, nerve transmission, and muscle contraction and involved in protein synthesis. Sodium, like potassium is useful for proper electrolyte balancing of fluids in the body, heart functioning, aids nerve transmission and muscle contraction. Manganese is a constituent of many enzymes in the body. It is a reactive oxygen species scarvenger. It is involved in bone formation. Copper is a constituent of many enzymes in the body, plays an important role in iron metabolism, blood clotting, red and white blood cell maturation (Pavelkova *et al.,* 2026). Zinc, is also a constituent of many enzymes in the body. It partakes in protein and genetic material synthesis. It is also important for sperm production, normal growth, sex maturation as well as taste perception. Nickel, is usually needed by some enzymes, to function (Patil *et al*.., 2023).

However the results of the elemental analysis of the three plant parts used in this study, differ in elemental content and amount from that of John-Dewole *et al*. (2012); Obiloma *et al*. (2020) and Dodo *et al*. (2020). Abolaji *et al*., (2007) observed same elemental content with variation in amount. Cadmium and lead were not seen.

Conclusion

The *Xylopia aethiopica* used in this study may be superior to those found and used elsewhere. The parts are relatively safe for use (consumption) up to the dose of 5g/Kg body weight per day, as used in this study.

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The nutritional potentials exhibited by the presence of dietary fibre, carbohydrate, protein, fat, vitamin and moisture as well as the presence of metals or minerals that are very important for healthy living, could be the most likely reason why *Xylopia aethiopica* is commonly referred to as "VALUE ADDED INGREDIENT". The leaves and fruits (without seeds and with seeds) contain different specific chemical compounds that possess useful medicinal and nutritive properties that may find application in industries (skincare products).

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