AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

A Comparative Study of Nutritional and Electrolyte Qualities of *Xylopia Aethiopica* in Novel Hydro-Alcoholic Formulations

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ABSTRACT

In the southern and southwestern parts of Nigeria, *xylopia aethiopica* is much acclaimed to possess nutritional properties for the control of diverse or definite physiological processes and were evaluated and compared based on the folklore methods of the finished medicinal products and practices. Result showed variability and statistically significant differences in the percent composition of the detected bio-active phyto-mineral elements and suggested that the novel hydro-alcoholic formulations, specifically, hydro-methanolic, potentiated the stimulation of the phyto-minerals and ranked: hydro-methanolic (60.8%), > hydro-ethanolic (21.3%), > methanolic (11.81%) > ethanolic (6.15%). Evident in hydro-methanolic formulation, it can be a good source of the following micro-minerals: iron (32.7%), magnesium (10.2%), manganese (8.9%), calcium (7.6%), potassium (0.69%), zinc (0.36%), sodium (0.28%), phosphorus (0.08%) and lead (0.001%). The percent proximate composition ranged: carbohydrate 72.6 to 77.7, protein 12 to 14.7, fiber 2.99 to 6.0, ash value 2.5 to 3.9, lipid/fat 1.47 to 2.3, and moisture content 0.98 to 1.96. While the anti-nutrients detected were: tannin 87%, oxalate 12.03%, hydrogen cyanide 0.93%, and phytate 0.08%. The findings of this study provided importantly that *xylopia aethiopica* can ameliorate natural healing and scientific credence on the rationale of the folklore hydro-alcoholic methods, particularly, hydro-methanolic formulations in processing and dispensing the finished bio-active products from which our understanding of the safety, effectiveness and quality of finished nutritional or medicinal products and practices may emerge.

Keywords: *Xylopia aethiopica*, proximate composition, anti-nutrients, hydro-alcoholic solvent, electrolyte profile.

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Received 29 August 2012, Accepted 17 September 2012

Please cite this article in press as: Chinwe ND *et al.*, A Comparative Study of Nutritional and Electrolyte Qualities of *Xylopia Aethiopica* in Novel Hydro-Alcoholic Formulations. American Journal of PharmTech Research 2012.

INTRODUCTION

Traditionally, *xylopia aethiopica* is acclaimed to possess medicinal and nutritional properties with diverse or definite physiological functions [1-3]. It is employed singularly or in combination along with other medicinal plants to treat various ailments included: as post partum tonic to women, who have newly given birth, used as abortifacient and in the control of fertility, employed in the control and regulation of blood pressure, diabetes traditionally. World Health Organization (WHO) specified the necessities to knowing the composition of phyto-mineral elements of biologically active botanical substances considered for usage for nutritional and medicinal purposes. Accordingly, such scientific evidence will ensure the use of safe, effective and quality products and practices[4].Chemical studies have demonstrated that finished natural products prepared by local methods of processing and dispensing show variability and statistically significant differences in the percentage composition of the detected phyto-medicinal forms of *xylopia aethiopica*[5]. However, scientific evidence from tests done to evaluate the safety, effectiveness and quality of nutritional agents - phyto-mineral elements, proximate composition and anti-nutritional factors by local methods of processing and dispensing the finished nutritional products is limited. It is recognized that counterfeit, poor quality or adulterated herbal products in international markets are serious patient safety threats[4]. This study therefore was a comparative assessment of novel innovative *xylopia aethiopica* formulations based on the folklore methods of processing and dispensing therapeutic finished natural medicinal and nutritional products and practices from which our understanding of the physiological activities of the bio-active phyto-mineral elements of medicinal importance may emerge.

MATERIALS AND METHODS

Plant materials and authentication:

Whole dried fruits of *Xylopia aethiopica* (Dunal) A.Rich were purchased from Ariaria International market Aba Abia State, Nigeria. The botanical identification and authentification was confirmed by Dr. (Mrs.) Essiet a taxonomist in the Department of Plant Science & Biotechnology, Faculty of Science, University of Uyo Akwa Ibom State. A herbarium specimen with Voucher No UUH 1819 has been deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria. The choice of this acclaimed nutritional and medicinal plant was necessitate by its frequent local usage by both pregnant and non-pregnant women, men, young and old in the southern and Southwestern parts of Nigeria for their primary health care.

The carpals of *xylopia aethiopica* were removed from their strands, as previously described[5], washed, sun dried (except for moisture content), cut into smaller pieces and further oven dried for some days at 40˚C. After which, 2kg was pulverized with an electric grinding machine into a fine powdery form which 100g was soaked in 300ml of either hydro-methanolic (1:4, v/v), hydro-ethanolic (1:4, v/v), methanolic, ethanolic or aqueous solution at 28^{oC} and shaken intermittently. The mixture was kept for 72 hours after which there were filtered with Whatman No.1 filter paper. The obtained samples were concentrated (dried) using a rotary evaporator at 45^{oC} and then collected and kept at 4^{oC} until analysis. Samples employed for the study were freshly prepared unless otherwise stated [5].

Proximate Compositions, mineral elements and Anti-Nutritional Analysis:

The proximate compositions (crude protein, lipid, moisture content, crude fiber, ash content and carbohydrate), mineral elements (phosphorus, calcium, iron, magnesium, manganese, sodium, potassium, zinc and lead) and toxic anti-nutritional factors of the sample (phytates, tannins, hydrogen cyanide, total oxalates) were determined according to the standard methods as recommended by the Association of Official Analytical Chemists[6] in the Department of Pharmacognosy and Natural Medicine University of Uyo, Akwa Ibom State, Nigeria. Briefly:

Determination of moisture content:

Each dried sample was milled with a Thomas Wiley Milling machine and sieved with 1.00 mm sieve and stored. Two crucibles were properly washed and allowed to dry in an air Fisher Isotemp Oven (Model 175) oven at 110ºC for 10 min to a constant weight. The crucibles were allowed to cooled in a desiccators for 30 min, then labeled A and B and weighed (W_1). 2.0 g of each sample was accurately weighed into the previously labeled crucibles and reweighed (W_2). The crucibles containing the samples were placed in the oven maintained at 103ºC for 14 h. They were removed and transferred to desiccators to cooled, finally weighed (W_3).

The loss in weight represented the moisture content of the sample. The percentage moisture content was calculated.

$$\text{Total moisture content} = (W_2 - W_3)$$

$$\% \text{ moisture} = \frac{\text{Loss in weight } (W_2 - W_3) \times 100}{\text{Weight of sample used}}$$

Determination of ash content:

Muffle Furnace Ignition method was used. Two porcelain crucibles were washed and dried in an oven to a constant weight at 100ºC for 10 min. They were allowed to cool in a desiccators, then labeled A and B and weighed (W_1). 2.0 g (W_0) of each sample were weighed into each of the

previously weighed porcelain crucibles and reweighed (W_2). The crucibles containing the samples were transferred into a Muffle Furnace Ignition furnace, which was set at 550ºC for 18 hrs to ensure proper ashing. They were then removed and allowed to cool in the desiccators for about 1hr then finally weighed (W_3). The percentage ash content was calculated.[7]

Weight os samples before ignition= (W_0)

Weight of sample after ignition (ash)= (W_3)

$$\% \text{ of ash} = \frac{\text{Weight of ash } (W_2\text{-}W_3) \text{ X } 100}{\text{Weight of sample used}}$$

$$\% \text{ of organic matter} = \frac{\text{Weight of organic matter } (W_0\text{-}W_3) \text{ X } 100}{\text{Weight of sample used}}$$

Determination of crude fiber:

The Weende method was used. 2.0 g of fat free sample A and B were weighed into two separate round bottom flasks labeled A and B, respectively. 100 ml of 0.25 M Sulphuric acid solutions was added to each sample in the flask, and the mixtures were boiled under reflux for 30 min. The hot solutions were quickly filtered under suction through silk material (cloth) in a buchner funnel. The residues were thoroughly washed with hot water and after each cooling acidity test carried out until acid free. Each residue after acid digestion was quantitatively transferred into the labeled flasks and 100 ml of hot 0.3 M sodium hydroxide solutions was added and the mixtures were boiled again under reflux for 30 min and filtered quickly under suction. Each insoluble residue was washed with hot water until it was base free. Finally the residue was washed twice with 95% methanl and quantitatively transferred into a porcelain crucible. They were dried to a constant weight in an oven at 100ºC for 2hours, cooled in desiccators and weighed (C_1). The weighed samples were then incinerated, and reweighed (C_2). Percentage crude fibre content was calculated.The crude fiber content was determined from the loss in weight of crucible and its content after ignition.[6-8]

Determination of crude protein:

Micro Kjeldahl method described was used. Briefly, 0.5 g of sample A and B were weighed and placed on each nitrogen free filter paper, then folded and dropped into a Kjeldahl digestion tubes labeled A and B, respectively. 3.0 g of digesting mercury tablet mixed catalyst ($CuSO_4$ + Na_2SO_4) and 25 ml of Conc. Na_2SO_4 were added to each sample in the digestion tube. The mixtures in the digestion tubes were transferred to the Kjeldahl digestion apparatus; the heater was regulated at a temperature below the boiling point of the acid for 1hr to avoid vigorous frothing until frothing ceased. The flask and its content was then heated strongly for 6-8 hrs. The

mixtures boil vigorously as temperature was increased, until clear (light) green color was obtained by occasionally rotating the flask. The digests were allowed to cooled then transferred into 100cm^3 volumetric flasks each labeled A and B and diluted with distilled water to make up 100 cm3.10ml aliquot of each digest was introduced into the distillation jacket of the micro steam distillation apparatus that was connected to the main, as the water in the distiller flask boils. 20 ml of 40% NaOH was added to each digest in the distillation jacket. The ammonia liberated was steam distilled into a cornical flask with 50 ml of 40% boric acid measured into two 250 ml conical flasks labeled A and B, respectively, four (4) drops of methyl red double indicator was added each. The conical flasks containing the mixture were placed onto the distillation apparatus with the outlet tubes inserted into each conical flask and NH_3 was collected through the condenser. The distillation continued until 25 ml of the distillate were trapped into the boric acid solution and color changes from red to yellow. The distillates were then titrated with 0.02 M HCL until a purple-pink colouration was obtained and the titer values were recorded. A blank was set up using water instead of the sample. Percentage nitrogen and crude protein was calculated [6].

Nitrogen content= a x K of nitrogen.

% Protein= a*K*K_2/ W

Where

a=amount of 0.1NHCL neutralized.

K= 0.014g of nitrogen (N-constant)

K_2= 6.25 Protein factor

W= weight of sample used for digestion.

Determination of crude lipid content:

The method described was used. Fat was determined by the continuous solvent extraction method using Sohxlet apparatus. 5.0 g of sample A and B were placed in two different extraction thimbles respectively then covered with cotton wool. The extraction thimbles containing the samples were placed in the extraction jacket. Two clean dried 500 mL round bottom flasks containing few anti-bumping granules was weighed (W_1) and 300 mL of petroleum either was poured into each flask fitted with sohxlet extraction units. The round bottom flasks and the condenser were connected to the sohxlet extractor, the extraction apparatus was set up with the flask sitting in the water ,heating mantle connected to a reflux condenser and cold-water circulation was put on. The heating mantle was switched on the heating rate was adjusted until the solvents were refluxing at a steady rate. Extraction was carried out for 6 h. As the ether

evaporated, it condensed and dropped into the thimble extracting the ether soluble content into the round bottom flask. The solvent containing the extracted lipid was recovered, distilled off using a water bath. The lipid extracted was left in the flask. The oil was dried in the oven at 70ºC for 1 h. The round bottom flask and oil was cooled and then weighed (W_2). The lipid content was calculated. The amount of lipid extracted was obtained from the difference between the weight of the flask and the solvents extracted. [8]

Determination of carbohydrate:

The total carbohydrate content was determined by difference method. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fiber was subtracted from 100% Carbohydrate = 100 - (% moisture + % ash + % protein + % lipids + % fiber).[6]

Determination of the mineral content:

This method described was adopted. calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), iron (Fe), and manganese (Mn), phosphorus and lead were analyzed from the triple acid digestion (wet digestion method). Exactly 1 g of sample A and B were weighed each into a 150 mL beaker, and 10 ml of conc. HNO_3 was added to each sample in the beaker and allowed to soak thoroughly. 3 ml of 60% $HClO_4$ and tetra-oxosulphate (vi) acids (1:1:1) was added and the mixtures were heated slowly at first until frothing ceases. Heating was continued until HNO_3 evaporated; the heating was stopped as charring occurred. 10 ml cone HNO_3 was added and heating continued until white fumes were observed. The digests were allowed to cool and 10 ml conc. HCL was added and transferred to 50 ml volumetric flask. The volume of the solutions was made up to the mark with distilled water, and then transferred to a bigger flask. The solutions were further diluted to 100 ml with distilled water.

Calcium and Mg were determined by the Versenate Complexometric titration method using EDTA (ethylene diamine tetra acetic acid) as indicator with NaOH while K and Na were measured by Flame photometer analyzer.

Phosphorus (P) was determined calorimetrically with Vanado Molybdate-Yellow procedure. Micronutrients (Fe, Mn and Zn) concentrations in the digest were measured using atomic absorption spectrophotometer (AAS – unicam 939/959 model).[6]

Determination of Phytate:

The phytic acid was determined using the procedure described by (9). 2.0 g of each sample (A and B) were weighed into 250 ml conical flask. 100 mL of 2% concentrated HCL acid was used to soak each sample in the conical flask for 3 h and then filtered through a double layer of hardened filter papers. 50 ml of each filtrate was placed in 250 mL beaker and 100 mL of

distilled water was added to each to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. Each solution was titrated with standard iron chloride solution, which contained 0.00195g iron per ml. The end point color was slightly brownish - yellow which persisted for 5 min. The percentage phytic acid was calculated.[9-10]

Determination of tannins:

The method described by (9-10) was adopted. Briefly, 400 mg of sample A and B were placed into two conical flasks each and 40 ml diethyl ether containing 1% acetic acid (v/v) was added, then the mixtures were properly mixed to removed the pigment materials. Each supernatant was carefully discarded after 5 min and 20 ml of 70% aqueous acetone was added and the flasks were sealed with cotton plug covered with aluminum foil, then kept in electrical shaker for 2 h for extraction. Each content in the flasks was filtered through Whatman filter paper and samples (filtrates) were used for analyzing. 50 ml of tannins extract from each sample was taken into test tubes and the volume of each was made up to 1.0 ml with distilled water. 0.5 ml Folic ciocalteu reagent was added to each and mixed properly. Then 2.5 ml of 20% sodium carbonate solution was added and mixed. The mixtures were kept for 40 min at room temperature, after which absorbance was taken using spectrophotometer and concentration was estimated from the tannic acid standard curve.

Determination of oxalate:

Oxalate was determined[10]. 1.0g of sample A and B were placed each in a 250 ml volumetric flask, 190 ml of distilled water and 10 ml of 6 m HCL were added. Each mixture was warmed on a water bath at 90ºC for 4 h and the digested samples were centrifuged at a speed of 2,000 rpm for 5 min. The supernatant were then diluted to 250 ml. Three (3) 50 ml aliquots of each supernatant were evaporated to 25 m L, and then the brown precipitate was filtered off and washed. The combined solution and washings were titrated with concentrated ammonia solution in drops until Salmon pink color of methyl orange changed to faint yellow. The solutions were heated on a water bath to 90ºC and the oxalate was precipitated with 10 ml of 5% calcium chloride (CaCl2) solution. The solutions were allowed to stand overnight then centrifuged. Each precipitate was washed into a beaker with hot 25% H2SO4, diluted to 125 ml with distilled water and after warming to 90ºC it was titrated against 0.05 m KMnO4.

Mineral elements compositions determination:

The mineral compositions were determined on aliquots of the solutions of the ash by established atomic absorption/emission spectrophotometer model 200-A produced by Buck Scientific. Phosphorus was determined by calorimetric means using the Vanadomolybdate -yellow method[6].

Statistical Analysis.

Analysis of variance and comparison of mean ± SEM were carried out on all data of proximate composition using statistical analysis system (SAS). Differences between means were assessed for significance at $p < 0.05$ by Duncan's Multiple range test (DMRT).

RESULTS AND DISCUSSION:

Normally, mineral elements are usually difficult for the body to absorb because they sometimes bind with other molecules that the body cannot break down. But plants, on the other hand, naturally contain phyto-minerals which are the bio-available forms the body can easily use. However, knowledge of the interplay between the potentiating stimulating effect of solvent extractability and bio-availability of natural substances considered for medicinal and nutritional purposes is limited. Thus the result of the nutritional and electrolyte qualities of *xylopia aethiopica* -phyto-mineral elements in novel hydro-alcoholic formulations- hydro-methanolic 1:4v/v, and hydro-ethanolic 1:4v/v, respectively were compared in table1.

Table 1: Comparison of micromineral element composition of *xylopia aethiopica* in different formulations.

Mineral elements (%)	Hydro-methanol (mg/100g)	Hydro-ethanol(mg/100g)	Methanol (mg/100g)	Ethanol (mg/100g)
Iron (Fe)	129.00 ±0.00	0.11 ± 0.00*	4.00 ±0.00*	2.00 ± 0.00*
Magnesium (Mg)	40.11 ± 0.00	32.11 ± 0.00*	17.11 ±0.00*	10.00 ± 0.00*
Manganese (Mn)	35.00 ± 0.00	26.32 ± 0.00*	20.11 ± 0.00*	10.02 ± 0.00*
Calcium(Ca)	30.10 ± 0.00	23.10 ± 0.00*	5.00 ± 0.00*	2.00 ± 0.00*
Potassium(K)	2.70 ± 0.00	0.41 ± 0.00*	0.11 ± 0.00*	0.21 ± 0.00*
Zinc (Zn)	1.40 ± 0.00	0.61 ± 0.00*	0.12 ± 0.00*	0.00 ± 0.00 *
Sodium(Na)	1.12 ± 0.00	0.91 ± 0.00*	0.00 ± 0.00*	0.01 ± 0.00 *
Phosphorus(P)	0.31 ± 0.00	0.20 ± 0.00*	0.00 ± 0.00*	0.02 ± 0.00*
Lead(Ld)	0.004 ± 0.00	0.01 ± 0.00*	0.11 ± 0.00*	0.01 ± 0.00*

Mean±S.EM, n = 4, *p < 0.05, R^2=0.323, 0.420 and 0.418 for hydro-methanolic

Upon comparing our results of novel innovative processes of *xylopia aethiopica* based on the folklore technique[5] it showed that the hydro-alcoholic formulations, specifically, hydro-methanolic, potentiated the stimulation of the phyto-mineral elements compared with the conventional methods. It also revealed variability and statistically significant differences in the percent content of the mineral supplements and ranked: hydro-methanolic (60.8%) > hydro-ethanolic (21.3%) > methanolic (11.8%)> ethanolic (6,2%) giving a ratio of 10:3:2:1 suggesting the suitability of hydro-methanolic formulations in the potentiating, isolation and characterization of natural substances of medicinal and nutritional importance consistent with the findings in the studies of phyto-medicine analysis [5]. The essential phyto-mineral elements with

the exception of lead are predominantly high in the hydro-methanolic formulations (Table1).

The result of the hydro-methanolic formulation analysis over other preparations also revealed that *xylopia aethiopica* may be a major source of mineral supplements of iron needed for good health. Of the 60.8% phyto-mineral element content, iron accounted as high as 32.7% (Table 1) which nutritionally is beneficial as it could explain its local use as post partum tonic in women who have given birth newly. This probably may aid in energy production, or improve oxygen delivery to muscles, boost the immune system and cell functions as well as increase haematocrit (% of blood volume that is red cell mass) and the total red cell mass in the body. The high iron content could also significantly contribute to reduction of anaemia caused by iron deficiency and correspondingly lead to the production of high oxygen blood level and transport [11-16]. On the other hand, excess amounts of iron can result in toxicity and even death [17]. Interestingly, iron is the only nutrient for which women have a higher daily requirement than men. The U.S. Recommended Daily Allowance (RDA) of iron for men is 10 milligrams or less and 15-18 milligrams for women and the average child while pregnant and nursing women need about 50–60 mg per day [11]. Additionally, the result also showed that *xylopia aethiopica* can be a good source of the following phyto-minerals evident in hydro-methanolic formulation in relation to other formulations (Table 1) and ranked: iron (32.7%), magnesium (10.2%), manganese (8.9%), calcium (7.6%), potassium (0.69%), zinc (0.36%), sodium (0.28%), phosphorus (0.08%) and lead (0.001%) all naturally fashioned clinically to perform specific or diverse biochemical and/or physiological functions in the body included: in the control and regulation of blood pressure, diabetes and wide spectrum of biological activities of physiological significant.

Table 2: Proximate composition of *xylopia aethiopica* in hydro-methanolic formulation compared with aqueous medium.

Nutrients/Parameters(%)	Aqueous	Hydro-methanol (1:4, v/v)	Difference (%)
Moisture content	2.00 ± 0.06	1.00 ± 0.06	50.00
Fiber	3.05 ± 0.02	6.09 ± 0.00	49.92
Ash value	4.00 ± 0.06	2.50 ± 0.05	37.50
Lipid/fat	1.50 ±0.04	2.38 ± 0.01	36.97
Protein	12.25 ± 0.09	15.00 ± 0.60	18.33
Carbohydrate	79.25 ± 0.06	74.03 ± 0.00	7.05

Mean±SEM, n = 2 Correlation of 0.997, p< 0.05. R^2=0.511 for aqueous against hydro-methanolic formulation.

The present result also showed significant differences ($p < 0.05$) in proximate analysis with high protein, lipid/fat and fiber concentrations in hydro-methanolic formulations compared with the conventional aqueous model (Table 2).

It revealed importantly that proximate composition ranged: carbohydrate 72.5 - 77.7%, protein 12 - 14.7%, fiber 2.99 - 5.97%, ash value 2.45 - 3.92%, lipid/fat 1.47 - 2.33% and moisture content 0.98 - 1.96% respectively suggesting it could provide the basic micronutrients needed for the daily requirements of the body functions as in the supply of the daily protein which is essential for the synthesis of body tissues and regulatory substances such as enzymes and hormones; as well as a dietary supplement for the daily requirement of the body. The result of the low fat content may imply that it is without any risk of obesity [18] and the low moisture content probably may not encourage microbial growth and enzyme activities [19] The high content of carbohydrate may confer good source of energy and building block for plant and animal life. However, the ash values and the carbohydrate content which was slightly higher in the present study are in line with previous studies [10].

The present result also revealed the presence of anti-micro nutrients (Table 3) and traces of the heavy metals like lead (Table 1) in *xylopia aethiopica* in contrast to some studies [10], and ranked the anti-nutrient minerals: tannin 86.96%, oxalate 12.03%, hydrogen cyanide 0.93% and phytates 0.08% which under certain conditions may form complexes either with nutritionally important phyto-minerals/electrolytes and proximate (Tables 1 and 2) or phytomedicines [5] to provide therapeutic, nutritional, or toxic effects in the body. There were statistically no detectable differences in the anti-nutritional composition in hydro-alcoholic formulations.

Table 3. Anti-nutritional factors of *xylopia aethiopica* .

Anti-nutrients (g/100g)	Hydro-methanol(80%) (mg/100g)	Hydro-ethanolic (80%) (mg/100g)
Tannin	274.71 ± 0.13	274.71 ± 0.01
Oxalate	38.00 ± 1.79	38.00 ± 1.79
HCN(hydrogen cyanide)	2.95 ± 0.01	2.94 ± 0.00
Phytates	0.25 ± 0.00	0.23 ± 0.00

Mean± SEM n = 2 Correlation of 1.00 p=0.05. R^2=0.709, 0.420 and 0.418 for hydro-alcoholic formulations.

However, the presence of the detected traces of anti-nutrient factors and heavy metal elements in this study may provide evidence contrary to the popular believe that because medicines are herbal (natural) or traditional they are safe (or carry no risk for harm). It has been recognized however, that traditional medicines and practices can cause harmful, adverse reactions if the product or therapy is of poor quality [4]. Aside, the stock or liquid which the natural food is cooked in, the phyto- mineral cyanide (2.95mg) may be volatilized as hydrogen cyanide (HCN) during cooking which is safe for consumption. This is evident in its wide consumption in special local preparations named “pepper soup” in the Southwestern and Southern parts of Nigeria. Beside,

the intake of *xylopia aethiopica* may control fertility [20-23]

On the basis of the proximate analysis**,** the phyto-mineral contents and anti-nutritional factors as approximate indices of nutritional and electrolyte qualities, scientifically, it has been shown that x*ylopia aethiopica* holds tremendous promise in providing mineral supplements supply that could enhance the maintenance of good health and curative process of ill health. The results provide additional information to nutritionists, health officers, industrialists, homestead and distant farmers for both raw material productions as well as for local consumptions. Additionally, the finding of this study provides importantly scientific evidence that hydro-alcoholic formulations, specifically, the hydro-methanolic formulation is more effective in stimulating the isolation of the finished bio-active nutritional or medicinal products than the conventional means. It therefore gives credence to folklore methods of hydro-alcoholic processing and dispensing the finished mineral supplement products from which our understanding of the safety, effectiveness and quality or the diverse or definite physiological, pharmacological and/or pathophysiological activities of the finished nutritional and/or medicinal products and practices may emerge.

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