Anti-nutritional Composition, Heavy Metal Content and Mineral Bioavailability of Red Tree Vine (Leea guineensis G. Don) Fruits

M. B. Olaniyi*, S. O. Rufai

Biomedicinal Research Centre, Forestry Research Institute of Nigeria, PMB 5054, Jericho Hills, Ibadan, Nigeria

* Corresponding author email: musbay2012@gmail.com

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ABSTRACT

Leea guineensis G. Don is an evergreen shrub that has been reported to be important in Nigeria due to its medicinal and nutritional uses. However, there is paucity of documented information on the anti-nutritional composition, heavy metal content and mineral bioavailability of L. guineensis whole fruits. Hence, this study investigated the essential minerals, heavy metals and anti-nutritional compositions of L. guineensis whole fruits with a view to assess the quantity, bioavailability of the mineral elements and ultimately the safety of the fruits using standard analytical procedures. The results of phytochemical screening confirmed presence of secondary metabolites in varying quantities. The anti-nutrient contents (mg/100g) were: phytates (29530); oxalates (510); saponins (10333); alkaloids (30533) and tannins (53.3). The results of mineral analysis revealed that L. guineensis fruits were rich in essential minerals (mg/100g) like, potassium (493); calcium (200); magnesium (103.3); phosphorus (9.9); zinc (8.5); manganese (5.5) and iron (5.0). The heavy metals concentrations were in the order lead>chromium>nickel>cobalt>cadmium. The values obtained were within the WHO permissible limits for heavy metals in medicinal plants. Meanwhile, the results of molar ratio phytates: Ca (8.97); oxalates: Ca (0.7978) and Ca:phytates:Zn (0.64) were below the suggested critical values indicating the bioavailability of calcium to be high. While phytates: Fe (501.06) and phytates: Zn (348.22) were higher than the critical values indicating poor bioavailability of Fe and Zn. The study therefore suggests that L. guineensis fruits are a good source of phyto-minerals which can be harnessed for nutritional purpose. Also, the anti-nutrient contents in L. guineensis whole fruits should be reduced to a safe level through processing methods (soaking, de-pulping or fermentation) in order to achieve its optimum nutritional or medicinal use.

Keywords: Anti-nutrients, Bioavailability, Heavy metal, Mineral composition, Leea guineensis, wild fruits

1 Introduction

Fruits are the vital part of human diet owing to the enormous health benefits they render including disease prevention and improving overall wellbeing. They contain array of nutrients including vitamins, minerals, bioactive compounds and useful phytochemicals especially antioxidants which help in lowering risk of chronic diseases like coronary heart diseases, hypertension. Fruits are naturally rich in dietary fibre, ascorbic acid, potassium, iron and low in sodium, calories and fat [1]. Wild fruits are potential source of antioxidants, vitamins and minerals which also act as an important source of nutrient to the rural dwellers [2]. In most developing nations, several types of edible wild plants are utilised as sources of food to provide alternative nourishment to the local inhabitants. In recent years, there has been a growing interest to evaluate various wild edible plants for their nutritional characteristics in order to promote their utilization and acceptability as an alternative source of essential nutrients (minerals) and means of enhancing livelihood [3].
Meanwhile, the main functions of minerals which can be derived from wild fruits are confirmed by Anita et al. [4] who reported that minerals are necessary for normal cellular functions as they provide additional protection to the human body and act as secondary players in some biochemical cascade mechanisms. Also, Sanchez-castillo and James [5] inferred that optimal intake of minerals such as sodium, potassium, magnesium, calcium, manganese, copper and zinc could potentially reduce risk factors associated with cardiovascular and other degenerative diseases. Though, it has been reported that some fruits especially the wild edible fruits contain some levels of anti-nutrients (phytates, oxalates, tannins, alkaloids, saponins and many more) which diminish useful nutrient bioavailability specifically when they are found in high levels [6]. As a result, the anti-nutrient factors must be determined to ensure human and animal nutritional security. The nutritional value and anti-nutrient content of wild edible fruits have not been given much attention they deserved such that massive volume of these wild fruits are often wasted or eaten by wild animals. However, a possible way of achieving nutrition security is through careful exploitation and utilization of available, cheap nourishing wild edible fruits like *Leea guineensis* [7]. Anti-nutrients have been described as substances that impair or inhibit important metabolic pathways especially digestion of some essential nutrients. For instance, phytates possess a high density of negative charges due to the presence of phosphate groups which make phytates form stable complexes with calcium, manganese, iron, copper and zinc resulting in precipitation and subsequent unavailability for intestinal uptake [8]. According to Hallberg and Rossander [9], the ascorbic acid helps iron absorption by making iron less vulnerable to phytate complexation thereby increasing its bioavailability. Park [10] submitted that the free oxalates released from food after digestion result insoluble calcium oxalates formation which then reduces the amount of calcium available for absorption in the intestinal lumen. It was also confirmed that oxalates remove calcium from the blood in the form of Ca-oxalates which may cause gallstone leading to kidney damage [11].

*Leea guineensis* Linn commonly called red tree vine is an evergreen shrub or small tree belonging to the family *Leeaceae* with single, trifoliate or feathered leaves. It is locally called Alugbokita in Yoruba and Okatakoi in Twi Language [12]. *L. guineensis* is native to tropical Africa countries which include Cote d’Ivoire, Liberia, Sierra Leone, Ghana, Cameroon and Nigeria. The fruits of *L. guineensis* are usually rounded, yellow when immature and pink or brown when matured [12]. *L. guineensis* has been reported to be important in Nigeria due to its medicinal and nutritional uses. Neji et al. [13] reported that *L. guineensis* is commonly used in the treatment of several ailments such as toothache, gonorrhoea, general weakness, skin lesions, rashes, ulcer, diarrhoea, dysentery, boils, herpes, paralysis, epilepsy and spasm, convulsions as a diuretic, pain-killer and purgative. *L. guineensis* fruits contain appreciable amounts of vitamin A, C, D and E [12]. They also possess bioactive constituents and exhibit antibacterial and antifungal properties.

In the developing countries like Nigeria where mineral deficiencies are common among rural dwellers, increasing the bioavailability of mineral in the food is of utmost concern. *L. guineensis* fruits could provide a cheap source of nutrients which can improve the nutritional status, reduce the prevalence of malnutrition especially among resource-constrained households and also serve as a means of dietary diversification. The presence of anti-nutrients is one of the major setbacks limiting the nutritional qualities of the food [14]. Wider utilization and acceptability of wild fruits is limited, due to the presence of anti-nutritional compositions which are associated with irritation, inflammation and gastrointestinal disturbances and may have negative effects on bioavailability of useful minerals [15]. Many reports on some underutilized wild seeds and fruits indicate that they could be good sources of nutrient for both man and animals [16]. However, *L. guineensis* has been considered as a wild fruit and there is limited information on its anti-nutritional composition and mineral bioavailability. This study therefore evaluated the essential minerals and heavy metal concentrations of *L. guineensis* whole fruits in order to know their quality and safety as well as their
anti-nutrients to determine bioavailability of minerals.

2 Materials and Methods

2.1 Sample collection and preparation

Fresh, mature fruits of *L. guineensis* were collected from the Forestry Research Institute of Nigeria Herbal garden, Ibadan, Oyo state, Nigeria. The specimens were identified at the Forestry Herbarium Ibadan, Nigeria where a voucher specimen (FHI002019) was deposited. The whole fruits were cleaned, sorted and oven-dried at 55 °C for 3 days in an electric oven at the Biomedicinal Research Centre, Forestry Research Institute of Nigeria, Ibadan to reduce the moisture content of the fresh fruits. The dried fruits were cooled to ambient temperature and subsequently ground to powder using household blender and the powdered samples were stored in air-tight containers. The samples were coded for chemical analysis and all the laboratory determinations were carried out in triplicates.

2.2 Qualitative screening of phytochemical constituents of *Leea guineensis* fruits

*Leea guineensis* plant is shown in Figure 1. Phytochemical screenings were carried out on the powdered sample using standard analytical procedures to confirm the presence of constituents (alkaloids, anthraquinones, flavonoids, saponins, tannins, steroids, cardiac glycosides and total phenolic compounds) as described by [17], [18] and [19].

Test for saponins was done where 1 g of powdered sample was boiled with 10 mL of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Frothing test was done where 2.5 mL of filtrate was diluted to 10 mL with distilled water and shaken vigorously for 2 minutes. Appearance of frothing indicated the presence of saponins in the filtrate. Also, emulsifying properties was tested on the sample where 2 drops of olive oil were added to the solution obtained from diluting 2.5 mL of filtrate to 10 mL with distilled water (as above), then shaken vigorously for a few minutes. The formation of persistent foam was evidence of the presence of saponins. Test for alkaloids was carried out by stirring 1 g of sample in 10 mL of concentrated HCl on a steam bath followed by filtration. Filtrate (1 mL) was mixed with two drops of Wagner’s reagent, then two drops of Dragendorff’s reagent were added to another 1 mL of the filtrate in a different test tube, and the two mixtures were then observed for turbidity. Presence of tannins was done where powdered sample (1 g) was boiled with 20 mL of distilled water in a water bath and was filtered while hot. Then, cooled filtrate (1 mL) was made up to 5 mL with distilled water and 3 drops of 10% ferric chloride were added and observed for any formation of precipitates and any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins. Flavonoids were tested for by boiling 1 g of sample with 10 mL of ethanol and 2 drops of ferric chloride were added to 5 mL of the extract. The mixture was observed for a dusty green colouration as positive result. Test for anthraquinones was carried out where sample (0.5 g) was shaken with 5 mL of chloroform for 10 min, filtered and 5 mL of 10% ammonium solution was added to the filtrate. The mixture was then shaken and the presence of a pink, red or violet colour in the ammonia phase confirmed the presence of free anthraquinones. Test for cardiac glycosides was done where 1g of sample was extracted with 10 mL of 80% ethanol for 5 min on a water bath. The extract was filtered, diluted with equal volume of distilled water and two drops of lead acetate solution were added, then shaken and filtered after standing for 10 minutes. The filtrate was then extracted with
Aliquots of chloroform and the extract was dissolved in 2 mL of glacial acetic acid containing one drop of FeCl₃ solution in a clean test tube. Concentrated H₂SO₄ acid (2 mL) was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a reddish brown or brown ring at the interface and a green colour in the acetic layer was taken for a positive result. Test for phenolic compounds was done by soaking sample (1 g) in 25 mL of 2% of HCl for 1 hour then, filtered through a 10 cm Whatman No. 1 filter paper. The extract (5 mL) was mixed with 1 mL of 0.30% ammonium thiocyanate solution with 3 drops of ferric chloride solution. A brownish-yellow colour indicated the presence of phenols.

2.3 Determination of anti-nutrients

The anti-nutrient contents of L. guineensis whole fruits were determined using various standard analytical procedures. Aluminium chloride method was adopted for the determination of total flavonoids content of the sample according to Quettier-deleu et al. [20] while, alkaloids determination was done according to the quantification method as described by Harborne [21]. Total saponins were determined according to the method of Obadoni and Ochuko [22] with slight modifications. Estimation of tannins was carried out using the modified vanillin–HCl method [23]. Total phenolic content of the sample was estimated according to the method described by Lister and Wilson [24] with slight modification. Oxalates content was determined according to the method described by [25]. Phytates content was carried by an indirect colorimetric method of Wheeler and Ferrel [26]. Glycosides were determined according to the method described by Sofowora [27].

2.4 Determination of mineral contents

Minerals and heavy metal content of L. guineensis whole fruits were determined according to standard analytical procedures of [25]. The mineral sodium and potassium were determined by flame photometry, while other trace elements such as iron and zinc and the heavy metals such as nickel, cobalt, chromium and lead were determined by acid digestion method with a mixture of nitric and perchloric acid (6:2) at 100°C. The digested sample was used for measurement of above minerals using an Atomic Absorption Spectrophotometer (AAS, SP9 model). Phosphorus was determined using potassium phosphate as the standard in a spectrophotometer (Spectronic 20 model, Gallenkamp UK).

2.5 Determination of Mineral bioavailability

In this study, the bioavailability of minerals (calcium, magnesium, iron and zinc) was calculated as reported by Woldegiorgis et al. [28]. The molar ratio between anti-nutrients (phytates and oxalates) and minerals (Ca or Fe or Zn) was obtained after dividing the mole of anti-nutrient with the mole of mineral. The mole of phytates (phytic acid) was calculated as the measured value of phytic acid divided by the molecular weight of phytic acid while the mole of various minerals (Ca or Fe or Zn) was calculated as the measured value of the mineral divided by the individual mineral molecular weight.

2.6 Statistical analysis

Data were analysed using the descriptive statistical analyses where means and standard deviation (SD) were obtained using Microsoft Excel 2013 version. Results were expressed as mean ± SD

3 Results

The result of phytochemical screening of L. guineensis whole fruits confirmed the presence of various phyto-constituents including anti-nutrients in the sample in varying degrees from slightly present to abundant (Table 1).

Table 1: Qualitative phytochemical screening of L. guineensis fruits (dry basis)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

+ means present
Alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, steroids tannins were found to be in abundance while saponins and terpenoids were slightly present. The result of anti-nutritional content of *L. guineensis* whole fruits revealed the presence of various anti-nutrients in varying concentrations (alkaloids > phytates > flavonoids > saponins > oxalates > tannins) most especially the main anti-nutrients; phytates, oxalates and saponins were 29530, 10330 and 516.67 (mg/100g) respectively as presented (Table 2).

**Table 2: Anti-nutritional content of *L. guineensis* fruits (dry basis)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Composition (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>30533 ± 0.05</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>10900 ± 0.01</td>
</tr>
<tr>
<td>Oxalates</td>
<td>516.67 ± 0.01</td>
</tr>
<tr>
<td>Phytates</td>
<td>29530 ± 0.18</td>
</tr>
<tr>
<td>Saponins</td>
<td>10330 ± 0.06</td>
</tr>
<tr>
<td>Tannins</td>
<td>53.30 ± 0.01</td>
</tr>
</tbody>
</table>

Results are presented as mean ±SD of triplicate measurements

Table 3: Mineral composition and Heavy metal content of *L. guineensis* fruits (dry basis)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Composition (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macro elements</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>200 ± 0.000</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1033 ± 0.001</td>
</tr>
<tr>
<td>Potassium</td>
<td>492.66 ± 0.001</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>9.90 ± 0.000</td>
</tr>
<tr>
<td>Sodium</td>
<td>218.33 ± 0.001</td>
</tr>
<tr>
<td><strong>Trace elements</strong></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>5.00 ± 0.00</td>
</tr>
<tr>
<td>Manganese</td>
<td>5.50 ± 0.00</td>
</tr>
<tr>
<td>Copper</td>
<td>5.46 ± 0.00</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.43 ± 0.00</td>
</tr>
<tr>
<td><strong>Heavy metals</strong></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.008 ± 0.000</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.927 ± 0.000</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.050 ± 0.000</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.750 ± 0.000</td>
</tr>
<tr>
<td>Lead</td>
<td>3.67 ± 0.000</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation of triplicate measurements

Table 4: Calculated molar ratios of anti-nutrients to mineral elements of *L. guineensis* fruits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calculated molar ratio</th>
<th>Critical molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytates:Ca</td>
<td>8.97</td>
<td>0.24</td>
</tr>
<tr>
<td>Phytates:Fe</td>
<td>501.06</td>
<td>1.0</td>
</tr>
<tr>
<td>Phytates:Zn</td>
<td>348.22</td>
<td>15.0</td>
</tr>
<tr>
<td>Oxalates:Ca</td>
<td>0.79</td>
<td>2.5</td>
</tr>
<tr>
<td>[Phytates (Ca)]/[Zn]</td>
<td>0.64</td>
<td>1.0</td>
</tr>
</tbody>
</table>

4 Discussion

4.1 Phytochemical composition

The presence of various phytochemical constituents such as alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, tannins in the *L. guineensis* fruits reported in this study confirms their therapeutic uses and in conformity with the medicinal properties of secondary plant metabolites [29]. The result obtained in this study was similar to the reports of Awotedu et al. [30]. Alkaloids have been reported as the most potent and therapeutically important of all substances isolated from plants which may explain its use against stomach ache, dysentery, diarrhoea, vomiting, constipation and intestinal worms [31]. The detection of flavonoids and saponins in this study is in line with the results obtained for *L. guineensis* stem bark and leaves respectively by Neji et al. [13] and Fagbohun et al. [32]. According to the report of Lewis and Elvis-Lewis [33], saponins have been reported to have anti-inflammatory and cardiac depressant properties and restrain growth of carcinogenic cells, without necessary destroying the normal cells in the process. Phenol possesses anti-bactericidal and antimicrobial properties which have shown to exert preventive activity macro/micro minerals (magnesium, potassium, sodium, calcium, phosphorus, zinc, magnesium, copper, iron) in varying amounts while and heavy metals (lead, chromium, nickel, cobalt, cadmium) were in minute quantities as evidenced in the concentrations obtained. Results obtained from the calculated molar ratios of anti-nutrients (phytates and oxalates) to mineral elements (calcium, iron, zinc) were in the order phytates: Fe > phytates: Zn > phytates: Ca > oxalates: Ca > phytates: Ca/Zn as shown (Table 4).
against infectious and degenerative diseases, inflammation and allergies through antioxidant, antimicrobial and proteins [30]. Steroids are of great importance in the pharmaceutical industry due to its function as sex hormones [34]. Olaleye et al. [35] reported cardiac glycosides reported as a potent remedy against cardiac arrest or renal failure. Tannins are known to have antiviral, antifungal, antibacterial and anti-tumour properties [27]; hence, the presence of tannins in L. guineensis fruits strongly supports its use in wound treatments, tumours, malaria and vaginal discharge [36].

### 4.2 Anti-nutritional components of Leea guineensis fruits

The main anti-nutrient contents (alkaloids, phytates, oxalates, tannin and saponins) found in in varying quantities in the L. guineensis whole fruits are presented (Table 2). Anti-nutrients reduce the maximum utilization of nutrients especially proteins, vitamins and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value [37]. Phytate impedes the absorption of phosphorus, calcium, iron, zinc and magnesium by forming complexes with them and reduces amino acid digestibility. This makes these minerals readily unavailable to the body [38]. However, the amount of phytates (29530 mg/100g) obtained in this study was far higher than the 0.0006; 0.0064; 0.0027 and 0.0182 mg/100g for Gardenia erubescens, Sclerocarya birrea, Diospyros mespiliformis and Balanites aegyptiaca fruits respectively [39]. In contrast, the value obtained in this study is lower than 1950, 1370, 490 and 880mg/100g reported for Mochis sylvestris, Citrullus lanatus, Carica papaya and Ananas comosus fruits respectively [41]. The value 53.30mg/100g obtained is far below 560 mg/100g recommended daily limit of tannic acid for man. Therefore, the toxicity effects of the tannin may not be significant. Fekadu et al. [37] stated that oxalates can exert a harmful effect on human nutrition and health especially by reducing calcium absorption and aiding the formation of kidney stones. Gemedede and Ratta [45] also reported that high- oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people. Oxalate value 516.67 mg/100g was higher than 0.0025; 0.0011; 0.0014 and 0.0038 mg/100g obtained for Gardenia erubescens, Sclerocarya birrea, Diospyros mespiliformis and Balanites aegyptiaca fruits respectively [37] and 0.09, 0.052. 0.55 and 0.079mg/100g obtained for Mochis sylvestris, Citrullus lanatus, Carica papaya and Ananas comosus fruits respectively [41]. Oxalate is known to inhibit renal calcium absorption, especially at concentrations of about 45000 mg/100g [46] therefore, the value obtained in this study is far less than the value that is postulated to be harmful. This suggests that the fruits may not pose any mineral absorption problems if consumed.

### 4.3 Mineral composition of Leea guineensis fruits

Fruits are commonly regarded as good sources of minerals with the major minerals, calcium,
magnesium and potassium in high abundance. The micro minerals such as zinc, manganese and copper were present in trace amounts. Minerals are good for maintaining proper body function and good health. Though, its deficiency leads to increased susceptibility to infectious diseases due to weakened immune systems. Micro elements are associated with decreased DNA damage, maintenance of immune functions, reduced lipid peroxidation and inhibition of malignant cell transformation but their absence leads to numerous metabolic disorders [47]. In general, the levels of minerals obtained in this study were higher than the values reported for closely related edible, medicinal plants like Chrysophyllum albium, Spondias mombin and Cola milleni fruits [48]. On the other hand, the obtained values were lower than the values reported for Gymnobotryum umbellata fruits except for Mg, Na, Cu, Fe, and Zn [41]. Kermanshah et al [49] and Mlitan [50] reported that iron is an essential trace element for haemoglobin formation, normal functioning of central nervous system and in the oxidation of carbohydrates protein and fats. It also plays an active role in oxygen transfer in the body and low iron content causes gastrointestinal infection, nose bleeding myocardial infection [51]. According to Longo and Camaschella [52], iron deficiency results in anaemic condition when there is decreased level of red blood cells as a result of significant reduction of iron content in the human body. This condition can present a severe complication with adverse consequences for expectant mothers [53]. The concentration of iron (5.0 mg/100g) obtained in this present study is higher than 0.85; 1.92 and 2.17 mg/100g obtained for Cola milleni, Irvingia gabonensis and Chrysophyllum albium respectively but lower than 14.59 mg/100g obtained for Spondias mombin [45]. Calcium is the major component of bone and assists in teeth development, essential for blood coagulation and the integrity of intracellular cement substances [54]. From the report of Prasad [55], deficiency of calcium also known as hypocalcaemia leads to diseases such as osteoporosis and osteopenia. The quantity of calcium (200 mg/100g) obtained for L. guineensis fruits was higher than the values (6.48; 14.95; 8.81 and 10.58 mg/100g) obtained for Gardenia erubescens, Sclerocarya birrea, Diospyros mespiliformis and Balanites aegyptiaca fruits [39]. This confirmed L. guineensis fruit as a good source of dietary calcium. Zinc is an essential trace element which plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing [51]. A deficiency in zinc results in increased rate of diarrhoea, delayed wound healing process, impaired immune function and some psychological disorders [56]. In this study, quantity of zinc obtained (8.43 mg/100g) was higher than 2.71; 1.52; 2.60; 1.73 and 1.25 mg/100g obtained for related edible fruits of Styelma spinosa, Detarium microcarpa, Diospyrus mespiliformis, Dialium guineensis and Gardenia tenfifolia respectively. The heavy metals concentrations obtained in this study were 0.75, 0.927, 0.05 and 0.008mg/100g for lead, nickel, chromium, cobalt and cadmium respectively. These values obtained are harmless due to the fact they were within the WHO permissible or safe limits for heavy metals in medicinal plants.

### 4.4 Molar ratios and bioavailability of minerals of Leea guineensis fruits

The molar ratios for calcium, zinc, iron and phytate, oxalate were calculated to evaluate the effects of high levels of phytate and oxalate in the bioavailability of dietary minerals. Mineral bioavailability can be defined as the ability of the body to digest and absorb the mineral in the food consumed [37]. The calculated Phy:Ca, Phy: Zn, Phy: Fe, Ox: Ca and Ca Phy/Zn molar ratios of L. guineensis fruits in comparison with critical values are shown in Table 4. Woldergiorgis et al [28] reported that the phy:Ca molar ratio has been proposed as an indicator of Ca bioavailability and critical molar ratio of less than 0.24 indicating good calcium bioavailability. The value of phy:Ca 8.97 obtained in this study was higher than the reported critical molar ratio 0.24 indicating that absorption of calcium may be slightly affected by phytate content in the samples. Meanwhile, Hurrell et al. [57] confirmed that phytate starts to lose its inhibitory effect on iron absorption when phytate:iron molar ratios are less than 1.0 although even ratios as low as 0.2 exert some negative effect. The result 501.16 obtained for L. guineensis...
fruits indicates that the phytate:iron molar ratios of the sample is far higher than the critical value 1.0 which implies poor absorption of iron in the sample. In addition, zinc is another important dietary mineral of foodstuffs which depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Bhandari and Kawabata [58] stated that phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH level which depends on relative amounts of both zinc and phytates. Hence, the phytate: Zn molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone [28]. The values (348.22) obtained in this study was far higher than the critical molar ratios of Phy:Zn, 15.0. This therefore indicates poor zinc availability in the sample according to the submissions of Morris and Ellis [59]. Furthermore, oxalic acid can pose harmful effects on human diet and health particularly by decreasing calcium absorption and helping the formation of kidney stones [58]. The function of oxalate contents in plant product in limiting total dietary Ca availability is of significance only when the ratio of Ox:Ca is greater than one [60]. In this study, the value of Ox:Ca was 0.79 which is lower than the reported critical value (1.0). This implies that a low level of oxalate could have no adverse effects on bioavailability of dietary calcium in this sample. However, the potential effect of calcium on zinc absorption in the presence of high phytate intakes has led to the suggestion that the [Ca][Phy]/[Zn] molar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone [61] therefore, high calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca][phytate]/[Zn] value exceeds 1.0 [62]. The value of [Ca][Phy]/[Zn] ratio in this study was 0.64 which is less than the critical level which then implies good zinc bioavailability in the sample studied. The bioavailability of Fe, Ca and Zn may be affected by the phytate content which can be overcome by mild processing of fruit prior to consumption.

5 Conclusion

The study revealed that *L. guineensis* whole fruits were found to be a good source of dietary macro/micro elements with traces of heavy metal that is less than the quantity that can be harmful to health therefore these fruits can be recommended as a remedy to alleviate mineral deficiency in the country. The fruits of *L. guineensis* is edible and can therefore be integrated into human food and animal feed. In order to maximally tap the mineral content of *L. guineensis* fruits for better mineral bioavailability, the anti-nutrient contents need to be drastically reduced to safe level through processing methods such as soaking in water, depulping, seed removal, prior to consumption.

6 Declarations

6.1 Acknowledgement

Authors appreciate Dr. (Mrs) E.C. Odozie, the Head of Central Laboratory, Forestry Institute of Nigeria Jericho Hills, Ibadan, for her immense contribution during mineral analysis.

6.2 Competing Interests

Authors declare no conflict of interest on this publication.

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References


