JFBE 5(2): 123-128, 2022

Journal of Food and Bioprocess Engineering

Journal homepage: https://jfabe.ut.ac.ir

Original research

Comparative studies of the proximate composition, and anti-nutritional factors of *Garcinia kola*

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A B S T R A C T

The study was undertaken to determine the comparative studies on the proximate composition and anti-nutritional factors of *Garcinia kola*. The proximate composition and anti-nutritional factors were performed on the seed kernel, fruit pulp, and the fruit pod of *Garcinia kola*. Analyses for nutritional (moisture content, ash, protein content, crude fibre, fat, carbohydrate) and anti-nutritional (phytate, oxalate tannin, alkaloid, saponin, flavonoid) composition were conducted. The fruit pod had higher ash content than the fruit pulp and seed kernel. The fruit pulp had the highest moisture content, followed by the fruit pod and lastly by the seed kernel. The protein and fat contents of the seed kernel and fruit pod were similar but higher than those of the fruit pulp. Carbohydrate content was higher in seed kernel than in the fruit pulp and fruit pod. The results of anti-nutritional analyses indicated that the seed kernel and fruit pulp of *Garcinia kola* did not have a high concentration of anti-nutrients. Thus, the consumption of the species is not detrimental to health and can be used as food supplements in food manufacturing.

Keywords: *Garcinia kola*; Anti-nutrient; Proximate analysis; Rainforest

Received 13 June 2022; Revised 19 August 2022; Accepted 27 August 2022

1. Introduction

Non-Timber Forest Products (NTFPs) play an important role in rural communities around the world. These products may be sold, thus providing cash income and economic empowerment. The extraction of NTFPs is often considered to be a mean of improving rural people's living standard as well as a suitable approach for forest conservation. They have medicinal values and are very numerous in the forest (Maňourová et al., 2019).

These NTFPs are highly valued by rural people and form part of their daily diets (Ilo et al., 2021). They are used not only for household consumption, but also for trade. They could be essential sources of proteins, vitamins, minerals, and amino acids, thus making them nutritionally important (Nyadjeu et al., 2019).

Garcinia kola (Bitter kola) seeds are smooth elliptically shaped, with yellow pulp, and brown seed coat. *G. kola* has economic value across West African countries where the seeds are commonly chewed and used for traditional ceremonies. The seeds are also used in folk medicines. Many herbal formulations have potential therapeutic benefits due largely to the activity of their flavonoid and other bioactive compounds (Oluwatosin et al., 2014). The potential utilization of *Garcinia kola* has been reported to possess some therapeutic benefits, especially in reducing the Cytokine in SARS-CoV-2, and some other acute coronavirus infections (Olajide et al., 2021).

The nutritional, social, economic, medicinal, and traditional importance of some indigenous forest food tree species to rural communities in developing countries cannot be over-emphasised (Ijarotimi et al., 2015) . These species provide food necessary for survival, rural employment, and increase the economic empowerment of the people living in the rural areas, and alleviating rural poverty (Dah-Nouvlessounon et al., 2015).

The medicinal uses include purgative, antiparasitic, and antimicrobial properties (Akinpelu et al., 2008) . The seeds are used in the treatment of bronchitis and throat infections. They are also used to prevent and relieve colic, cure head or chest colds, and relieve coughs. The plant is also used for the treatment of liver

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https://doi.org/10.22059/jfabe.2022.344416.1118

disorders and as a chewing stick. The constituents include bioflavonoid, xanthene, and benzophenones (Ilo et al., 2021). The antimicrobial properties of this plant are attributed to benzophenone and flavanones. This plant has shown anti-inflammatory, antimicrobial, and antiviral properties (Durand et al., 2015). In addition, the plant possesses antidiabetic and antihepatotoxic activities. Bitter Kola, better known as *Garcinia kola*, has been identified as a potential antibiotic.

As a result of the usefulness of these important NTFPs, it is very important to analyze and evaluate their proximate composition and other anti-nutrient content. Anti-nutritional factors are substances that inhibits metabolic pathway, which are known to reduce the bioavailability of many important nutrients such as protein, vitamins, and minerals (Durand et al., 2015).

The novelty of this research involved measurements of proximate analysis of important NTFPs known as *Garcinia kola*, and determination of the nutritional and ant-nutritional factors of the three main parts such as the seed kernel, fruit pulp and pods. This research examined the nutrient availability in *Garcinia kola* by measuring these constituents in the seed kernel, fruit pulp, and the fruit pod with a view to quantifying the nutrients availability, and to lay to rest the widely believe that some parts of the *Garcinia kola* are nutrient deficient and should not be consumed.

2. Material and Methods

Fig. 1 shows the pictures of the Garcinia kola with whole trees, the whole fruits, fruit pulp, seed kernel and fruit pod. The fruit pulp, seed kernel and fruit pod were exclusively studied for the purpose of proximate measurement in them.

2.1. The research area

The fruits of *Garcinia kola* were collected from trees of the species in agroforestry farms in the rainforest ecosystem of Ondo State, Nigeria. The state, which has a total of 18 local government areas and covers about 14,793km^2 of land, is located between longitudes 4° 30´and 6° East of the Greenwich Meridian, 5° 45" and 8^0 15" North of the Equator. This means that the state lies entirely in the tropics, so its vegetation is entirely tropical. The fruits were rinsed with distilled water to remove any attached dirt and were dried using tissue paper. The fruit pulp and seed of *G. kola* were cleaned and weighed using the electronic weighing balance.

2.2. Sampling procedure

The chemical used includes 2% Boric acid, Kjeldahl catalyst tablets, concentrated Tetraoxosulphate (vi) acid, 0.1% Methylene blue indicator, 0.1 Methyl red indicator, 40% sodium hydroxide solution (w/v),0.1M Hydrochloric acid, 10% Hydrochloric acid (v/v), 1.25% Tetraoxosulphate (vi) acid (v/v), 1.25% Sodium Hydroxide(w/v), 5M Hydrochloric acid, Petroleum ether (b.p. 40-60°C), absolute ethanol, distilled water, deionized water.

The chemical balance, oven, water bath distillation apparatus, shaker calorimeter, Soxhlet apparatus, and different glassware are some of the apparatuses used.

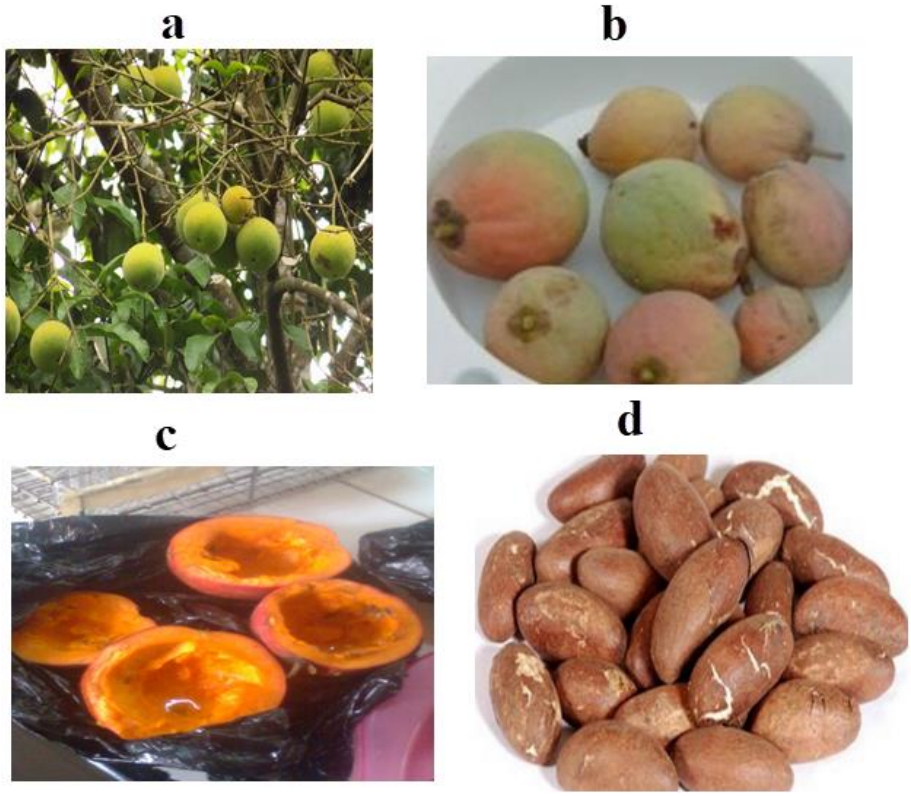

Fig. 1. Pictures of *Garcinia kola*: (a) Tree; (b) the whole fruits; (c) the fruit pods and (d) the whole seeds.

Table 1. Nutritional composition of *Garcinia kola*.

	Ash (%)	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	CHO (%)
Seed	0.33±0.03[a]	71.99±0.00[a]	1.74±0.00[b]	0.95±0.12[b]	3.22±0.19[c]	21.79±0.36[b]
Pulp	0.26±0.06[a]	92.62±1.36[c]	1±0.18[a]	0.38±0.08[a]	0.53±0.09[a]	5.81±1.54[a]
Pod	0.81±0.00[b]	87.68±0.19[b]	1.68±0.03[b]	0.83±0.03[b]	2.23±0.08[b]	7.60±1.02[a]

Each value is a mean of two replicates ± standard error. Means within the same column followed by the same letter are not significantly different ($p \geq 0.05$).

The Fruit pod of *Garcinia kola* was split open as shown in the Fig. 1. The seed was extracted, washed thoroughly with distilled water, and dried using tissue paper. The seeds were then weighed using an electronic weighing balance, and the seed coat was peeled off and the resulting white seed kernel (mesocarp) was taken to the oven.

2.3. Analytical method

The routine analysis of food is termed as "proximate analysis" or "Wende analysis". *Garcinia kola* seeds were tested for moisture content, ash content, fat, crude fiber, carbohydrate, crude protein, while the determination of tannin, saponin, alkaloid, flavonoid, phytin phosphorus, phytatesky, and oxalate was done for anti-nutritional contents.

2.3.1. Analysis of nutritional properties

2.3.1.1. Moisture content determination

The moisture content gives an idea of the amount of water in each food. The moisture content was determined by using the oven-drying method, which is based on weight loss of water due to evaporation (FAO, 1983). Clean and dry foil was weighed using a Wiggen Hauser electronic balance and the weight was recorded (W_1). About 5 g of the sample was weighed into the foil (W_2). The fruits were weighed whole using a Wiggen Hauser electronic balance and their pulp was weighed and transferred into the oven after wrapping with Aluminum foil for drying at a controlled temperature of about 70-80°C to a constant weight. The drying method took about 1 week due to the controlled temperature to avoid nutrient denaturing. After drying, they were transferred to the desiccator to cool and then weighed. This process was continued until a constant weight (W_3) was obtained (A.O.A.C, 1990). The percentage moisture content was then calculated using the equation:

$$Moisture\ content\ (\%) = \frac{loss\ in\ weight\ due\ to\ drying}{weight\ of\ sample\ taken} \times 100 \quad (1)$$

$$Dry\ MC\ (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (2)$$

where W_1 is the weight of foil, W_2 is the weight of foil + sample, and W_3 is the weight of moisture content loss.

2.3.1.2. Crude protein determination

0.5 g of the samples was weighed into the Micro Kjeldahl digestion flask and one tablet of selenium catalyst was added. The mixture was digested on an electro thermal heater until the mixture turned pale green. The flask was allowed to cool, after which the solution was diluted with distilled water to 50 ml, and 5 ml of this was transferred into the distillation apparatus. It was later distilled with 5 ml of 2% Boric acid and three drops of indicator were treated. 10ml of 40% NaOH was poured into the receiving flask and distilled until the receiving flask measured 50 ml. The residue was then titrated with mineral acid H_2SO_4 until it turned sharp pink. The resulting solution in the conical flask was then titrated with 0.1M HCl (Pearson, 1976). Protein content was then computed using the equation below:

$$Crude\ protein = \frac{Titre\ value \times 0.1\ HCl \times 0.014 \times 100 \times \frac{50}{5}}{Original\ weight\ of\ sample} \quad (3)$$

2.3.1.3. Crude fibre determination

This is the residue that remains after the food sample has been treated under standardized conditions with petroleum spirit, boiling dilute NaOH, boiling dilute H_2SO_4, dilute HCl, and alcohol, 150 ml of 1.25% of H_2SO_4, which was added to 1 g of the dried fruit samples and was boiled for 30 min, and filtered through a white handkerchief, and rinsed with water. The filtrates were then boiled again with 1.25% of NaOH and allowed to boil gently for 30 min followed by a rinse with 10% alcohol. (A.O.A.C, 1990). The filtrate was then dried and ashed and the fibre content was determined. The fibre was calculated using the equation below:

$$Crude\ fibre\ (\%) = \frac{W_2 - W_3}{W_1} \times 100 \quad (4)$$

where W_1 is the weight of the sample used, W_2 is the weight of the crucible + dried sample, and W_3 is the weight of the oven dried sample.

2.3.1.4. Ash contents determination

The ash of biological materials is an analytical term. The inorganic residue that remains after the organic matter has been burnt away is termed the ash content. The ash may not usually be the same as the inorganic matter present in the original food since there may be losses due to volatilization or chemical interaction between the constituents. The value is useful in assessing the quality or grading of certain edible materials. The importance of the ash lies in the fact that it gives an idea of the number of mineral elements present in the sample, while the organic matter gives an estimate of proteins, lipids (fats), carbohydrate, and nucleic acid content in the sample (FAO, 1983).

0.5 g of *Garcinia kola* seed sample was transferred to the muffle furnace set at 550°C. (lid removed). Ashing was continued until a light grey or white ash was obtained. The crucibles were

then cooled in desiccators and weighed. The ash content was calculated using the equation below:

$$Ash\ (\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad (5)$$

where W_1 is the weight of crucibles, W_2 is the weight of crucibles + sample, and W_3 is the weight of crucible + ash.

2.3.1.5. Fat contents determination

Clean and dried thimble was weighed (W_1) and about 0.5 g oven dried sample was added and re-weighed (W_2). The round bottom flask was filled with petroleum ether (40-60℃) up to 3/4 of the flask. The Soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boils gently. The sample(s) were placed inside the thimble and inserted into the Soxhlet apparatus for 6 h of reflux extraction with petroleum ether (40-60℃). After the barrel of the extractor was empty, the condenser and thimble were removed and taken into the oven at 100℃ for one hour, and later cooled in the desiccators and weighed again (W_3) (A.O.A.C, 1990). The samples were oven dried again and weighed. Percentage fat content was then calculated using the equation:

$$Fat\ content\ (\%) = \frac{Weight\ loss\ of\ sample\ (extracted\ fat)}{Original\ weight\ of\ sample} \times 100 \quad (6)$$

$$Fat\ content\ (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (7)$$

2.3.1.6. Carbohydrate content determination

The carbohydrate content of the fruit pulp and seed was determined by subtracting the sum of percentage ash, crude fiber, protein, and moisture content from 100, which is the whole percentage on which the analysis was based.

2.3.2. Anti-Nutrient analysis of Garcinia kola

2.3.2.1. Determination of phytate contents

Wheeler and Ferrel (1971) method was employed for phytin determination. Four grams (4 g) of finely ground samples were soaked in 100ml of 2% HCl for 3 hours and then filtered. 25 ml of the filtrate was placed in a 100ml conical flask and 5ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added as an indicator. 53.5 ml of distilled water was then added to give it the proper acidity. It was then titrated with Ferric Chloride solution, which contained about 0.195mg of Fe per cm of FeCl used, until a brownish yellow color persisted for 5 min. The phytate content in mg/100g was calculated using the equation below:

$$Phytate\ acid\ (mg/g) = Titre\ value \times 1.19 \times 1.95 \times 3.55 \quad (8)$$

2.3.2.2. Determination of saponification values

Saponification is a rough index of the molecular weight of the fat and oil. The smaller the saponification value, the higher the molecular weight. It also indicates the quantity of alkali required for the conversion of a definite amount of fat or oil into soap. It is used to check the adulteration of fat and oil. One gram of the powdered sample was weighed into a conical flask and to this was added 25 ml of alcoholic potassium hydroxide solution. The flask with its content was heated in a boiling water bath for 30 mins with occasional shaking. 1 ml of phenolphthalein indicator was added to the solution and titrated while hot with 0.5 M hydrochloric acid (a, ml). A blank titrate was carried out which contained the entire reagent without a sample (b, ml). The saponification value was computed using the formula below:

$$Saponification\ value = (b - a) \times M \times 56 \quad (9)$$

where a is the titre value, b is the blank titre value and M is the molarity of acid used.

2.3.2.3. Determination of tannin contents

About 200 mg of finely ground sample was weighed into a 50ml plastic bottle. 10 ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker and shaken for 2 h at 30℃. Each solution was then centrifuged, and the supernatant was stored on ice. 0.2 ml of each solution was pipetted into test tubes and 0.8 ml of distilled water was added. A standard tannic acid solution was prepared from a 0.5 mg/ml stock and the solution was made up to 1ml with distilled water. 0.5 mg/ml folin-ciocalteau was added to both the sample and the standard, followed by 2.5 ml of 20% Na_2CO_3. The solution was then vortexed and allowed to incubate for 40 min at room temperature, after which absorbance was read at 725 nm against a reagent blank concentration of the samples from a standard tannin acid curve (Makkar & Goodchild, 1996).

2.3.2.4. Determination of oxalate

One gram of the sample was weighed into a 100 ml conical flask. Then, 75 ml of 1.5 NH_2S0_4 was added, and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No. 1 filter paper. 25 ml of sample filtrate (extract) was collected and titrated hot (80-90℃) against 0.1 $KMN0_4$ solution to the point when a faint pink color appeared that persisted for at least 30 seconds (Day & Underwood, 1986). The following equation was used to calculate the oxalate content:

$$oxalate\ value\ (mg/g) = VT \times 0.9004 \quad (10)$$

where VT represents the titre value.

2.3.2.5. Flavonoid test

5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each sample extract followed by the addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicates the presence of flavonoids. The yellow colorations disappeared on standing. A few drops of aluminum solution were added to a portion of each filtrate. A yellow coloration was observed, indicating the presence of flavonoids. A portion of the powdered sample was, in each case, heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution.

A yellow coloration was observed, indicating a positive test for flavonoids.

2.3.2.6. Determination of alkaloid

Using Harborne (1973) method, 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 4 h. This was filtered and the extract was concentrated in water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract, until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3. Results and Discussion

3.1. Comparison of nutritional composition

Table 1 shows the nutritional compositions of the seed kernel, pulp, and pod of *Garcinia kola*. The pod with the measured composition of 0.81% had the highest ash content, which is significantly higher than the ash contents of the fruit pulp with the value of 0.26% and seed kernel of 0.33%. The total ash content as reported by Dah-Nouvlessounon et al. (2015), was 2% and it was a little bit higher in term of percentage composition in comparison with this study in which the total ash percentage composition is 1.4%. The mean moisture content, which ranged from 71.99 to 92.62%, was significantly different in all the fruit parts, which was similar to the result published by Abai et al. (2020). The fruit pulp had the significantly highest moisture content, followed by the fruit pod, which is significantly higher than the moisture content of the seed kernel, while this might be significantly different from the result reported by Dah-Nouvlessounon et al. (2015) with a low moisture content of 8.46 reported for *Garcinia kola*.

The protein contents of the seed kernel and fruit pod were statistically similar and were significantly higher than those of the fruit pulp. Based on the percentage protein content reported by Dah-Nouvlessounon et al. (2015), there was 4.26% of protein recorded for *Garcinia kola*, which was closer to the percent measured. The seed kernel protein was 1.74%, fruit pod was 1.68%, and pulp was 1%; Fat content followed a similar trend as protein content. The fat content was reported as 2.5% in the work of Dah-Nouvlessounon et al. (2015). The seed kernel contains 0.95% of fat, the fruit pulp was 0.38%, and fruit pod was 0.83% as depicted in the Table 1.

Fiber content was significantly different in all the fruit parts, with the seed kernel having the highest, of the 3.2% composition, followed by the fruit pod which was 2.23%, and lastly the fruit pulp with the value of 0.53%. Dah-Nouvlessounon et al. (2015) reported the crude fiber composition of 1.35% which was lower than the total percentage obtained in this study. Carbohydrate concentration in the seed kernel was 21.79%, significantly higher than in the fruit pulp which was 5.8% and fruit pod with the compositional value of 7.6% which were statistically similar.

3.2. Anti-nutritional composition

Fig. 2 depicts the anti-nutritional composition of *G. kola*. Phytate was 2.47 mg/g in the seed which was greater than was what measured in fruit pulp at a proximate composition of 1.5 mg/g. The phytate composition in the fruit pod was 1.5 mg/g which was similar to the measure proximate analysis in the fruit pulp. We can predict that the phytate composition can vary considerably depending on the source.

Phytin phosphorus composition followed the same trend as phytate composition, being higher in the seed kernel with the value of 1.65 mg/g than in the fruit pulp of the proximate composition of about 1.4 mg/g and fruit pod, which is nearly equivalent with fruit pulp composition. Oxalate composition was highest in the seed kernel, followed by the fruit pulp, and lastly by the fruit pod. According to the results, the fruit pod contained the most saponin and alkaloid, while the fruit pulp contained the most tannin. Dah-Nouvlessounon et al. (2015) argued that there was a significant saponin in the *Garcinia kola* which was also responsible for the foaming and bitter taste properties, mainly located in the fruit pod as discovered from the result of this study.

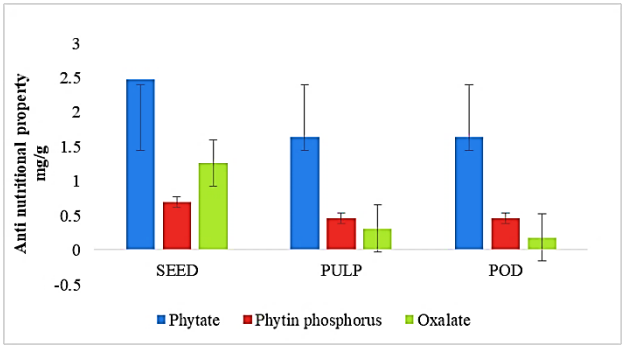

Fig. 2. Anti-nutritional compounds (phyatate, phytin phosphorus and oxalate) of *Garcinia kola*.

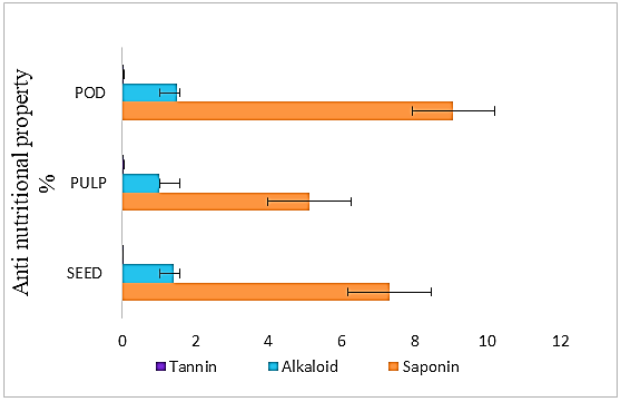

Fig. 3. Anti-nutritional compound (tanin, alkaloid, and saponin) percentage of *Garcinia kola*.

3.3. Percentage antinutritional values

The results of the nutritional composition of *Garcinia kola* revealed that moisture content was highest in the fruit pulp (92.62%), which was closely followed by the fruit pod and the seed. As indicated in Fig. 3, carbohydrate (21.79%), protein (1.74%), fat (0.95%) and fibre (3.22%) were higher in the seed than

in the fruit pulp and pod. Seed is the edible part of the fruit. The result indicated that the seed was a good source of nutrients. However, the values reported for most of the nutrients in this study were lower than those reported by Dosunmu et al. (1995).

The seed of *Garcinia kola* contained more anti-nutrients than the fruit pulp and pod, except for alkaloid, saponin and tannin. The tannin composition reported for *Garcinia kola* by Dosunmu et al. (1995) was much higher than the value obtained in this study. On the other hand, *Garcinia kola* had a higher phytate composition than what was reported by Dosunmu et al. (1995).

4. Conclusion

From the results of this study, the following conclusions can be drawn.

- The seed has higher concentration of most nutrients such as fat, fiber, protein, and carbohydrate than the fruit pulp and fruit pod. The seeds of *Garcinia kola* are the edible parts of the species. The results indicate that they are good sources of nutrients and can be used as nutritional supplements.
- The anti-nutritional composition of the fruit pulp and the seeds of *Garcinia kola* is not high; thus, the consumption of the fruits and seeds of this specie is not detrimental to human health.

Acknowledgment

The author acknowledges everyone that contributed to the success of this work, especially Oyebanji Uthman for providing a conducive environment during the period when the authors are preparing the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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