

Chemical Composition and Invitro Antioxidant Investigation of Garcinia Kola (Bitter Kola) Pulp

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ABSTRACT

The use of fruits in providing nutritional values for proper growth and total development of humans cannot be over emphasised. One of such is Garcinia kola seed, reported to have tremendous medicinal values, thereby addressing good health and wellbeing (Target 3) of Sustainable Development Goals. However, the pericap and mesocap are discarded as pollutants to the environment. This project was aimed at evaluation of Garcinia kola pulp (biowaste), for proximate, minerals compositions, antioxidant activity and total phenolic content, using standard analytical methods. The results showed percentage proximate composition as: Moisture (6.060.08), crude protein (10.8020.28), fat (1.2910.01), ash(2.53±0.04), crude fibre (5.11±0.01) and carbohydrate (73.85 ±0.25). The calculated fatty acid and metabolizable energy were found to be 1.03%, 1486.70KJ/Mol respectively. Minerals in (ppm) of the Garcinia kola pulp were Ca (416.50), K (118.50), Na (8.20), Mg(12.60), and P(339.00), Fe, Cu, Zn and Mn were found to be significantly low. Na-K ratio was found to be less than 1 suggesting that Garcinia kola pulp can be utilized to reduce the risk of obesity and hypertension while Ca-P ratio greater than 1 meaning, it can be used for food formulation to reduce risk of central obesity. Antioxidant activity for the Garcinia kola pulp was found to be low (0.03%) while the total phenolic content is significantly high (33.1 1mgGAE/g). This indicates that Garcinia kola pulp regarded as agricultural waste can be utilized as a veritable source of energy. It can also serve as source of protein supplement for animal feeds, essential minerals, especially Ca for bone formation in livestock.

Keywords: Garcinia kola, livestock, Biowaste, Nutrients

Introduction

Fruits are referred to as juicy seed-bearing structure of flowering plants that may be eaten as food [1]. The use of fruits in providing nutritional values for proper growth and total development of humans cannot be over emphasised. However, the Pericap and Mesocap are discarded as wastes (biowaste), thereby polluting environment. The wastes from agricultural products and food sources are referred to as biowaste. These are material derived from living organisms and organic compound such as agricultural wastes and foods wastes, these can serve as potential sources of energy example include coconut and palm kernel shells, Garcinia kola (bitter kola) pulp [2]. pollution is harmful, too much of something in the wrong place. In appropriate quantities, some erstwhile pollutants are beneficial. Phosphates and other plant nutrients are essential to aquatic life; too much of these nutrients, however, and eutrophication results. Carbon dioxide in the atmosphere helps keep Earth warm enough to be

habitable, but the buildup of vast quantities of excess carbon dioxide from fossil fuel use and other sources now threatens to alter the planet's climate and releases of free radicals to the atmosphere. Other pollutants, like dioxin and P.C.B.s, are so toxic that even the most minute amounts pose health hazards, such as cancer and reproductive impairment. Releases of pollutants to the environment are most often, the casual by-product of some useful activity, such as generating electricity or raising cows, agricultural deposit and human activities. (sewage). Pollution of this type is a form of waste disposal [3]. The occurrence of diseases in human has been attributed to the production of free radicals generated by environmental pollution and essential metabolic processes in the body. The free radicals can however be mopped up by substance known as antioxidants. Antioxidants are reported to play a very important role in the body defense system against ROS [4]. In response to goal 3 sustainability develop goals (SDGs), Good Health and Well-Being. Discarded waste of Garcinia Kola pulp constitute environmental waste pollution, which is harmful to bio-existence. This creates an

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insight for this investigation in order to make the environment greener and achieve the above target goal of SDGs. The results of this investigation show better way to make use of the *Garcinia kola* (waste) as medicine rather than waste and hence make environment greener.

Literature Review

Garcinia kola (Bitter kola) components are described in the fig below:

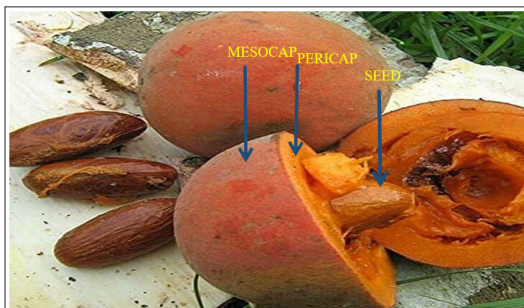


Figure 1: Bitter Kola Fruit Source: (Guedje, 2012)

Bitter kola (*Garcinia kola*) seeds are smooth, ellipsoidally shaped, with yellow pulp and brown seed coat. *Garcinia 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 medicine, many herbal formulations and have potential therapeutic benefits largely to the activity of their flavonoids and other bioactive compounds [Adegoke et al., 2008]. The potential utilization of *Garcinia kola* in brewing operations, a shop substitutes in lager-beer brewing has also been reported [5].

These applications require only the pulp while the hull is discarded. The presence of beneficial bioactive compounds been reported in the seed coats or Almonds, Peanut (*Archishypogea*), Lotus seeds (*Nelumbonucifera*) and African yambean (*Sphenostylisstenocarpa*), thereby opening up the possibility of the presence of potentially beneficial compounds in the seed coat of the plant materials with bioactive components in their pulp [6]. If beneficial compounds are found in the seed coat, it would add value to the hull, either to be regarded as waste, and open up an array of studies into their potential utilization in various food formulations and mammalian systems. Although there has been considerable interest in the. Bioactive compounds of *Garcinia kola* seed mainly from the medicinal perspectives; there are limited reports on the chemical composition of this seed and its hull with a view to investigating its potentials from a nutritional perspective. The objective of this study was therefore to determine the chemical composition of *Garcinia kola* seeds and its hulls and examine their potential for use in food and feed formulations.

Materials And Methods

Collection And Samples Preparation

Twenty-five Bitter kola fruits (*Garcinia kola*) were gotten from Ojumu farmland, Erijiyan Ekiti, Ekiti State. The seeds of *Garcinia kola* were removed in order to retain the pulp. The pulp was dried at room temperature for 30 days. The sample was then grinded into fine powder.

Moisture Content Determination

The moisture content was determined using oven-drying method as described by Association of Official Analytical Chemists

(A.O.A.C, 2005). Clean and dry can was weighed and its weight was recorded (W_1). About 2g of the powdered sample was weighed into the can. The sample and can were weighed and recorded as W_2 . The can containing the sample was transferred into the oven maintained at a temperature of 105°C and dried for three hours. The can was transferred to the desiccator to cool and the weight was noted. The process was continued until a constant weight (W_3) was obtained. The percentage loss in weight during drying was taken to be the percentage moisture content.

$$\text{Calculation: \%Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Ash Content Determination

The ash content was determined by the method described in A.O.A.C. (2005). About 1g of finely ground sample was weighed into clean, dried, pre-weighed crucibles with lid (W_1). The organic matter was burnt off by igniting the sample over a low flame (with lid removed) until the sample became charred. The crucibles were then transferred to the muffle furnace at 550°C (lid removed). The ashing continued until a light grey or white ash was observed. The crucibles were then cooled in a desiccator and weighed (W_2). The percentage ash content was calculated as follows:

$$\% \text{ Ash} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Crude Fat Determination

Semi-continuous solvent extraction method (Soxhlet method) (A.O.A.C, 2005) was used for fat determination. 2g of the sample was weighed into a weighed filter paper

and folded neatly. This was put inside a pre-weighed thimble (W_0). The thimble with the sample was weighed (W_1) and inserted into Soxhlet apparatus and extraction under reflux was carried out with petroleum ether (40–60°C boiling range) for five hours using a pre-weighed extraction flask. At the end of the extraction, the extraction flask was removed from the heating mantle when it was almost free of petroleum ether. The extraction flask with the oil was put in the oven. 50ml of 2% boric acid was pipette into a 100ml conical flask (the receiver flask) and four drops of screened methyl red indicator were added. 50% NaOH was continually added to the digested sample until the solution became turbid, which indicated that the solution had become alkaline. Distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the process of distillation was still going on, the pink colour solution of the receiver flask changed to blue which indicated the presence of ammonia. The distillation was continued until the content of the round bottom flask was about 50ml after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1 M HCl [7].

Carbohydrate Determination

The carbohydrate content was determined by difference. The percentage carbohydrate content is equal to the sum of the percentages of moisture, ash, fat, fibre and protein content subtracted from 100.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fibre} + \% \text{ Fat} + \% \text{ Protein}).$$

Mineral Content Determination

Ash was dissolved in 10% HCl, heated, cooled, filtered and made up to the mark in 100ml standard flask with distilled water. The mineral contents of the samples were analyzed for with the aid of atomic absorption spectrophotometer (AAS) (Buck Scientific Instrument Model-200A/2010, Norwalk, Connecticut, 06855) (Pearson, 1976). Sodium and potassium were analyzed using flame photometer and AAS was used for Mn, Mg, Fe, Zn, Cu, Ca etc.

Antioxidant Capacities Determination Using Dpph

50mg of the ground sample was extracted with 20mL of ethanol (99%) and filtered giving a concentration of 2.5mg/mL of sample. Working solution of DPPH having absorbance of the range 800–1000 at 525nm was prepared fresh. 0.1mL of the extract was pipette into a test tube and 9.9mL of the DPPH solution added and incubated at room temperature for 30mins (enough time to reach a stable value). This was then transferred into a cuvette and the absorbance read at 525nm. Trolox was used as a standard reference to convert the inhibition capacity of each sample to the trolox equivalent antioxidant capacity (TEAC). Standard trolox solution in ethanol were prepared at a concentration of range between 0 and 600ug/ml. 0.1mL of each trolox solution was added to 9.9mL of DPPH radical solution (0-24 um trolox in radical solution). The absorbance was read after 30mins of incubation at room temperature using Jenway 6705 uv/vis spectrophotometer. Standard calibration curves were conducted by plotting percentage against concentration of trolox at 525m.

$$\text{Inhibition(trolox / sample)} = \frac{\text{Abs of control} - \text{Abs of troloxorsample}}{X100}$$

Abs of control

The ratio between % inhibition of the sample and the slope of the trolox calibration curve was defined as the TEAC which was used to indicate the scavenging free radical capability of the sample by the equation below

$$\text{TEAC} = \% \text{Inhibition} / S \times M$$

Where, S= slope of trolox calibration curve, M = mass of sample used

Results and Discussion

Results

Proximate Analysis

The results of the proximate analysis of Garcinia kola (Bitter kola) pulp biowaste are presented in Table 1.

Table 1: % Proximate Analysis of Garcinia kola (Bitter kola) Pulp Biowaste

Parameters	A1	A2	Mean	SD	%CV
Moisture	6.00	6.12	6.06	0.08	1.32
Protein	10.60	11.00	10.80	0.28	2.60
Fat	1.30	1.28	1.29	0.01	0.78
Ash	2.56	2.50	2.53	0.04	1.58
Crude Fiber	5.10	5.11	5.11	0.01	0.11
Carbohydrate	74.02	73.67	73.85	0.25	0.34

Fatty Acid	1.040	1.024	1.03	0.01	1.00
Metabolizable	1486.64	1486.75	1486.70	0.08	0.01
Energy					
(KJ/mol)					

Notes: ^aResults for two replicates

S.D: Standard deviation C.V: Coefficient of variation: ND: Not Detected

¹Carbohydrate (%) was calculated as: 100 – (Moisture + Protein + Fat + Ash + Crude Fiber)

²Calculated Fatty Acids = 0.8 × Crude Fat

³Metabolizable Energy = (Protein × 17) + (Fat × 37) + (Carbohydrate × 17)

Mineral Composition

The mineral composition of Garcinia kola (Bitter kola) pulp biowaste is presented in Table 2.

Table 2: Mineral Composition of Garcinia kola (Bitter kola) Pulp Biowaste (ppm)

Minerals	A1	A2	Mean	SD	%CV
Na	8.20	8.14	8.17	0.04	0.50
K	118.00	119.00	118.50	0.71	0.60
Ca	415.00	418.00	416.50	2.12	0.51
Mg	12.40	12.80	12.60	0.28	2.24
P	380.00	298.00	339.00	57.98	17.10
Fe	8.22	8.25	8.24	0.02	0.26
Cu	5.20	5.40	5.30	0.14	2.67
Zn	1.22	1.00	1.11	0.16	14.01
Mn	0.82	0.85	0.84	0.02	2.54
Na/K	0.069	0.068	0.07	0.00	1.12
K/(Ca+Mg)	0.276	0.276	0.28	0.00	0.04
K/Na	14.39	14.62	14.50	0.16	1.12
Ca/Mg	33.47	32.66	33.06	0.57	1.74
Ca/P	1.09	1.40	1.25	0.22	17.61

^aResults for two replicates

S.D: Standard deviation C.V: Coefficient of variation:

ND: Not Detected

The results of the Total Antioxidants activity of Garcinia Kola (Bitter Kola) Pulp are presented in Table 3

Table 3: % Total Antioxidants Activity of Garcinia kola (Bitter kola) Pulp Biowaste

SAMPLES	A1	A2	MEAN	SD	%CV
TAA	0.03	0.04	0.03	0.0025	7.692308

The results of the Total Phenolic Content of Garcinia Kola (Bitter Kola) pulp foe two replica are represented in table 4

Table 4: Total Phenolic Content (mg/GAE/g) of Garcinia kola (Bitter kola) Pulp Biowaste

SAMPLES	A1	A2	MEAN	SD	%CV
TPC	33.40	32.81	33.11	0.295	0.891104

Discussion

Proximate and Minerals Composition

The investigations done on *Garcinia kola* pulp revealed that the pulp possesses medicinal activities against some disease. The investigation done show high level of phenolic content and low percentage of antioxidant activity. The results showed percentage proximate composition as: Moisture (6.06 ± 0.08), crude protein (10.80 ± 0.28), fat (1.29 ± 0.01), ash (2.53 ± 0.04), crude fibre (5.11 ± 0.01) and carbohydrate (73.85 ± 0.25). The calculated fatty acid and metabolizable energy was found to be 1.03%, 1486.70Kj/mol respectively. Minerals in (ppm) of the *Garcinia kola* pulp were Ca (416.50), K (118.50), Na (8.20), Mg (12.60), P (399.00), Fe, Cu, Zn and Mn were found to be significantly low. Na-K ratio was found to be less than 1 suggesting that *Garcinia kola* pulp can be utilized to reduce the risk of obesity and hypertension while Ca-P ratio greater than 1 meaning it can be used for food formulation to reduce the risk of central obesity. *Garcinia kola* pulp regarded as agricultural waste can be utilized as a veritable source of energy. The most abundant phytochemicals in *G. kola* seeds are flavonoids. Presence of saponins, tannins, phenols, glycosides, and alkaloids has also been confirmed by various authors. Even though anti-nutrients such as oxalate and phytate were detected, the seeds are safe for consumption and there are no reports on harmful overdosing so far [8]. Phytochemicals present in *Garcinia kola* revealed that presence of flavonoids, tannins, cardiac glycoside, steroids and saponins are reducing sugars. These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The medicinal values of medicinal plants lie in these phytochemical compounds and as such produce a definite physiological action on the human body. Flavonoids which are part of the phytochemical constituents of *Garcinia kola* exhibit a wide range of biological activities one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus health promoting in action.

These observations support the usefulness of *G. kola* in folklore remedies for the treatment of various infections. Another important phytochemical compound present in *Garcinia kola* is tannins. Tannins exert antimicrobial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells.

Antioxidant Activity Using 2,2-Diphenyl-1- Picrylhydrazyl (DPPH)

Free radical scavenging activity was evaluated using DPPH, which estimates antioxidant activity as TAA. Trolox standard represent DPPH standard reference solution; therefore, low percentage value (0.03%) obtained indicates that *Garcinia kola* pulp has low antioxidant activity. In contrast to other fruits, *Garcinia kola* pulp, mature mesocarp and pericarp regarded as waste, has significantly low antioxidant activity than other fruits [9]. These results suggest that the composition of mesocarp and pericarp of *Garcinia kola* pulp does not follow other fruits patterns in antioxidant activity. It is interesting to notice that *Garcinia kola* pulp has significantly low antioxidant activity than other fruits [10-15].

Total Phenolic Content (Mg/Gae/G) of *Garcinia Kola* Pulp

Significantly high value of phenolic content (33.11mg/GAE/g) obtained for *Garcinia kola* pulp, symbolize that *Garcinia kola* pulp is a good anti-inflammatory, and antiproliferative agents.

It should be noted that *Garcinia kola* pulp present higher phenolic content than some others fruits [16]. Due to the high level of phenolic content in *Garcinia kola* pulp, considering that high level of phenolic content was contained in *Garcinia kola* pulp, it is proposed that an important component of this activity in accessions could be ascribed to such phytochemicals [17]. Human consumption of *C. chinense* pods may supply a substantial amount of the antioxidants needed to promote a better health and to prevent diseases and ailments. Our results can be the basis for the selection of suitable accessions for antimicrobial and anticarcinogenic trials of habanero extracts [18].

Conclusion

In conclusion, the presence of nutritionally valuable compounds in the *Garcinia kola* pulp suggests that it can be further used in some food industries and some food industries for industrial raw material applications. At the experimented showed, greater consumption of *Garcinia kola* pulp consumption of *Garcinia kola* pulp experiment showed greater materials for many industries and pharmaceutical industries. At the experimented consumption of *Garcinia kola* pulp showed greater presence in *Garcinia kola* pulp especially Ca for bone formation in livestock.

Recommendations

Garcinia kola pulp is very rich in carbohydrate with reasonable level of protein. Carbohydrate that presents in the *Garcinia Kola* pulp digest slowly in the body if ingested and thereby recommended as source of artificial energy for livestock. It's thereby recommended that:

- Medicines or foods formulated or enriched with bitter kola pulp are safe to eat and good for health. Due to the presence of some beneficiary nutrients and some bioactive component revealed by the experiment and the literature review works on *Garcinia kola* pulp waste.
- *Garcinia kola* pulp should not be discarded as waste, but it should be utilized for commercial benefits or further research works.

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