Chemical Composition of the Seeds and the Defatted Meal of *Garcinia kola* Heckel (Guttiferae) from Benin

P. Yété¹, V. Ndayishimiye¹, P.C. Agbangnan², S.T. Djènontin², V.D. Wotto¹ and D.C.K. Sohounhloué²*

¹Lab. of Phy. Chem., Faculty of Science and Tech., Uni. of Abomey-Calavi (LCP/FAST/UAC), 01 BP 2009 Cotonou, Benin
²Lab. of Study and Research in App. Chemistry, University of Abomey Calavi (LERCA/EPAC/UAC)

*E-Mail: ksohoun@bj.refer.org, cssohoun@gmail.com

Abstract

*Garcinia kola* is a medium sized tree found in the moist forests and widely distributed throughout West and Central Africa. The edible nut is highly valued in these countries. Seeds, commonly known as the bitter kola are appreciated. The seeds are used in traditional medicine and in many herbal preparations for the treatment of diseases such as laryngitis, liver disorders and bronchitis. The objective of this work is to determine the chemical composition of the seeds and defatted meal of *Garcinia kola* from Benin. The results of proximate analysis have showed that the seeds and defatted meal of *Garcinia kola* Heckel contained several families of chemical compounds: flavonoids, tannins, alkaloids, anthraquinons, saponosids, leucoanthocyanins, anthocyanins and heterosids. The contents of total phenols (1.65±0.08 mg/g), flavonoids (0.35±0.30 mg/g), anthocyanins (0.80±0.15 mg/g) in the seeds are present in significant amounts. *Garcinia kola* can be used as anti-inflammatory and as active components of drugs derived from plants. The average percentages in starch, total sugars, and crude protein are: 62.9±0.4; 12.0±0.2; 3.69±0.50 % respectively. The mineral elements: N (0.59±0.10%), P (0.09±0.00%), K (0.34±0.00%), Mg (0.07±00%), Ca (0.10±0.00%) show that *Garcinia kola* may be a good source of minerals. These results suggest that *Garcinia kola* possesses nutritional and healthy benefits.

Keywords: Traditional Medicine, Chemical Compounds, *Garcinia kola* Heckel, Nutrients

1. Introduction

Medicinal plants have always and will continue to have an important place in the therapeutic arsenal of humanity. According to the World Health Organization (WHO), about 65 to 80% of the world populations in developing countries rely on medicinal plants for their primary health care (WHO, 2002). Benin (West Africa) is rich in diverse flora whose mysteries of the nature are not yet perceived by humans, hence the need of finding a new source of natural products for solving health problems which are actual and acute in humans (de Souza, 2001). The preventive and curative capacities of foods depend on their organoleptic quality which is related to the chemical composition, in particular in mineral elements. Thus, chemical families and the elements found in fruits play an important role in maintaining the organism and this improve its response capabilities against diseases (Ogbe & George, 2012).

*Garcinia* is a tropical plant genus including several species in Africa, America and Asia. These species are commonly useful for many purposes (Rai, 2003). *Garcinia kola* Heckel is a highly valued and multi-purpose tree for its fruits, seeds, stems, roots and barks that are used in Western and Central African regions. *Garcinia kola*’s interest is proved as one of the many non-timber forest products that detain high socio-economic importance (Adebisi, 2004). It occupies the third rank of medicinal plants in Benin in terms of number of recipes in which the species belongs (de Souza, 2001) and investigations proved that *Garcinia kola* seeds contain compounds useful in curing several diseases e.g., Cancer, diabetes, upset stomach (Esimone et al, 2002 and Farombi et al, 2005). Because of its high interest resulting in its over exploitation, *Garcinia kola* is extinction-threatened in several West and Central African
countries such as Ivory Coast (FAO, 1996), Togo, Congo and Cameroon (Tchata, 1999). Considering its importance and to prevent genetic erosion, appropriate strategies should be developed to promote its sustainable use. It is therefore useful to undertake on farm conservation by small farmers through agro-forestry systems. This will decrease the pressure over wild individuals. In Benin, non-timber forest products such as *Irvingia gabonensis* (Ahoussi et al, 2012) and four oleaginous cokes (Djenontin et al, 2012) were studied and their richness in natural substances useful in traditional medicine in Africa was highlighted. Therefore, no investigation in traditional pharmacopoeia and human consumption was still described on the *Garcinia kola* from Benin, in spite of its intense consumption. *Garcinia kola* presents opportunities for food and medicinal use. It is in this context the present work may contribute to the study of chemical composition of *Garcinia kola* seeds used by traditional healers in Benin for their therapeutic properties in the treatment of certain diseases such as liver disorder, hepatitis, diarrhoea, diabetes and cancer (Adaramoye & Adeyemi, 2006).

2. Materials and Methods

2.1. Vegetable Material

The matter constituting this study was composed of *Garcinia kola* seeds collected in Oueme, Benin Republic department. The wholesome seeds were peeled manually to remove the brown seed coat. The peeled seeds were adequately dried in an oven at 50 °C and milled. The milled samples were packed in a sterile screw capped sample bottles and stored at ambient temperature for analysis.

2.2. Chemical Screening and Dosage

The chemical groups determination (Sterols and terpenes, Polyphenols, flavonoids, tannins, coumarins, saponaoids, alkaloids, reduced compounds, anthocyanins and quinones) has been made through reactions of colouration and precipitation (N'guessan et al, 2009).

2.2.1. Dosage of the Flavonic Aglycones and Anthocyanidins

For the research of the flavonic aglycones and anthocyanidins, a hydrolysis was done on 2 g plant material in presence of 160 ml of 2 N HCl. The solutions were then placed in the double boiler at 100 °C during 40 min. After cooling, the flavonic aglycones and the anthocyanidins were extracted according to the following analyzes

2.2.2. Flavonic Aglycones Proportion

The differential proportion of flavonic aglycones was carried out based on their chelating properties with AlCl₃ 1% in ethanol (95%). The absorbance was measured by using an UV–Vis spectrophotometer (JENWAY50/60 Hz) at the wavelength of 380 nm to 460 nm. The differential height of the peaks against a sample consisting of an extract solution with ethanol 95% without AlCl₃ is proportional to concentration of flavonic aglycones in the sample. The content in aglycones, expressed in equivalent of quercetol, was calculated according to the formula:

\[ T_{ag} = \frac{A \times M \times d}{\varepsilon \times m} \]  

(1)  

Where:

- \( T_{ag} \): Content in flavonic aglycones expressed in mg/g of quercetol equivalent
- \( A \): Absorbance of differential peaks
- \( M \): Molar mass of quercetol (302 g/mol)
- \( V \): Volume of ethanolic solution (ml)
- \( d \): Dilution factor
- \( m \): Mass of dry vegetable material (g)

2.2.3. Anthocyanidins

The aqueous phase was extracted three times with the n-butanol that extracted the anthocyanidins of red colour. The absorbance of the butanolic phase has been measured by the spectrophotometer (JENWAY 50/60 Hz) between 480 nm and 600 nm and the most elevated absorbance was used against a white constituted of the butanol to quantify the anthocyanidins according to the following formula:

\[ T_{ant} = \frac{\alpha \times M \times d}{\varepsilon \times m} \]  

(2)  

Where:

- \( T_{ant} \): Content in anthocyanidins expressed in mg/g (Cyanidols equivalent)
- \( \alpha \): Factor of correction (α=6) of proanthocyanidins transformation output (17%)
- \( A \): Absorbance at the maximum wavelength
- \( \varepsilon \): Molar absorption coefficient of the cyanidol (34700 L/mol.cm)
- \( M \): Molar mass of leucocyanidol (306 g/mol)
- \( V \): Volume of butanolic solution (ml)
- \( d \): Dilution factor
- \( m \): Mass of dry vegetable material (g)

2.2.4. Quantitative Evaluation of the Polyphenolic Compounds

The total polyphenolic content (TPC) was determined by a Folin-ciocalteu assay using Gallic acid as the standard (Singleton & Lamuela-Raventos, 1999). The last one was constituted by a mixture of phosphotungstic acid and phosphomolybdic acid that were reduced, at the time of the oxidation to the phenols in mixture of blue oxides of tungsten and molybdenum. The produced blue colouration possessed a maximal absorption around 765 nm absorbance, by reference to a range stallion gotten with phenolic...
acids (gallic acid), permitted to determine the quantity of total polyphenols present in an extract. It was expressed in mg of equivalent gallic acid per g of dry matter (mgGAE/gDM with DM=dry matter). The method of the aluminium trichloride (AlCl\sub{3}) was used to quantify the total flavonoids (Agbangnan et al, 2012). That technique was based in the formation of the complex flavonoids-aluminium that possessed a maximal absorbance at 500 nm.

The dosage of the tannins condensed was achieved by the method of the sulphuric vanillin. The principle of this dosage was based on the fixing of the group aldehydic of the vanillin on the carbon in position 6 of the cycle to the catechin to form a complex red colour chromophore that absorbed at 510 nm and the absorbance was measured to the spectrophotometer (JENWAY 50/60 Hz) after 10 min rest of the mixture in the darkness.

The quantitative analysis of anthocyanins was carried out using the transformation into colourless derivatives under the action of certain reagents like bisulphite ions. Thus, variation of the absorbance at 520 nm after addition of excess bisulphite ions was proportional to the anthocyanins (Ribereau & Stonestreet, 1965 and Agbangnan et al, 2012). The total content of anthocyanins expressed as mg/g of standard of extract was given according to the formula:

\[ T_{\text{anthocyanin}} = (\text{OD}_A - \text{OD}_B) \cdot P/C \]

Where:
- \( \text{OD}_A \): Absorbance of witnesses solution (without bisulfite)
- \( \text{OD}_B \): Absorbance of the mixture
- \( P \): Line slope obtained starting from the standard, (\( P = 875 \) for malvidine- 3-glucoside)

2.3. Nutrients

Ascorbic acid was determined by titration (iodometric) method reported in the literature by Morabandza et al (2013). The nitrogen content was determined by the Kjeldahl method (AOAC, 2003) from the micro-distillation of mineral deposit in the sample digested with sulphuric acid in the presence of a catalyst based on selenium. The nitrogen content thus measured was multiplied by the factor 6.25 to obtain the protein content of the sample. The Proximate analyses were performed in a conventional system of analysis that gave the quantitative as well as qualitative idea of nutrients present in a particular food sample. Individual nutrients such as amino acids, fatty acids, monosaccharids were not considered. The gross components considered were Moisture content, Ash content, Crude Protein, Crude Fat, Crude Fibre and Carbohydrate.

2.4. Mineral Elements

Mineral elements (P, Ca, Mg, Na) have been dosed after ash mineralization according to the dry way procedure, by ICP (Inductive Couplage Plasma) with a Varia-Vista apparatus at a wavelength \( \lambda = 214.914 \) nm for phosphorus and at \( \lambda = 589.592 \) nm for sodium. The spectrophotometer Varia-Vista was equipped with a detector CCD (Coupled Charge Device). The measurement has been done realizing stallionage in respect to the analyzed medium conditions (matrix acidity). The calculations have been done by interpolation in relation with stallionage range. Analytical results validation has been carried out by internal reference samples analysis, named proves, in which the mineral elements content was known.

2.5. Cellulose and Lignocellulososes Measurement

The crude cellulose measurement and parietal fibres sequential determination have been carried out according to AFNOR Standard, NF V 03-040 and Van Soest protocols, standardized AFNOR, NF V 18-122 (1990). The interest of these methods was to isolate different parietal fractions permitting to predict foods energetic value. This method is a general application for simple and composed foods. The successive treatments with neutral and acidic detergents, then with sulphuric acid at 72% lead to three residues named: Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL). NDF, ADF and ADL represented respectively Total Insoluble Fibres estimation, lignocellulosic and lignin complex.

3. Results and Discussion

3.1. Screening and Dosage

The chemical screening results were showed in Table 1. The analysis of this table displayed a marked presence of polyphenolic compounds (catechic tannins, flavonoids, anthocyanins, leuco-anthocyanins), alkaloids, anthraquinons, the C-glycosides, O-glycosides and saponins. Flavonoids, tannins, alkaloids present in our samples were also reported in the literature Aderibigbe (2012) and Adesuyi et al (2012) for the species naturalized in Nigeria. These compounds were absent in samples from Cameroon studied by Niemenak et al (2008). The compounds such as saponosis, anthraquinons, O-glycosids aglycons present and abundant in the sample studied were absent in the Nigerian one studied by Adesuyi et al (2012).

Flavonoids are recognized for possessing anti-inflammatory, antioxidant, anticancer, antiviral and anti-proliferative protective effects. They are present in a variety of foods, such as oranges, tangerines, berries, apples and onions (Middleton et al, 2000). Mazi et al (2013) reported that flavonoids got potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, got anti-cancer activity and protect against all stages of carcinogenesis. Garcinia kola may be a good
dietary source of flavonoids.

Table 1. Chemical Screening

<table>
<thead>
<tr>
<th>Chemical Family</th>
<th>Garcinia kola Seeds</th>
<th>Garcinia kola Defatted Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponosids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catechic Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gallic Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leuco-anthocyanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinons</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O-heterosids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O-heterosids of gennins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C-heterosids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+=Very abundant, - = absent

Alkaloids are specific compounds of the leaves, bark roots or seeds. They stimulate the nervous system, act as analgesics, tranquilizers, “but can also cause paralysis and blood pressure (Michael, 2008)”. Some extraction and use of alkaloids from Garcinia kola may be useful in medicine in the manufacturing of vaccines to prevent some diseases.

Relatively to phenols, they are widely used in the manufacturing of resins, plastics, insecticides, explosives, dyes, detergents and raw materials for the production of medicinal drugs such as aspirin (Michael, 2008). Garcinia kola can then play an economic role in this field.

The overall results of the chemical screening explain the popularity of traditional healers in general using the plant Garcinia kola in traditional medicine. Many examples in scientific works from the literature emphasize our hypothesis. Indeed a number of compounds have been isolated from the seeds of Garcinia kola, the most important consisted of a mixture of bioflavonoids called kolaviron (Iwu, 1985).

Phenolic compounds were composed of three main categories: phenolic acids, flavonoids and tannins. The contents of total and individual phenols measured using the Folin-Ciocalteu varied from 1.64 to 1.65 mg of gallic acid equivalent (GAE) (Table 2), showing a low difference between the levels of these compounds in the seed and defatted meals of the Garcinia kola (Figure 1). These values were in the same order of magnitude like that of Nigeria (1.47 mg/g) reported by Adesuyi et al (2012). Our results were also comparable to those of Niemenak et al (2008) with the sample from Cameroon. The contents of flavonoids were 0.35 and 0.19 mg/g respectively in almonds and in defatted meal. Contents in anthocyanidins, anthocyanins and in tannins were respectively 0.56 and 0.27 mg/g; 0.80 and 0.43 mg/g; 3.87 mg/g and 1.29 mg/g. The latter amount was comparable to that reported by Adesuyi et al (2012). Seeds richness in tannins (Figure 2) was a source of antioxidants, substances of human health interest. These interactions affect the digestibility of proteins and carbohydrates. Our results were in agreement with other studies which highlighted the presence of flavonoids and tannins in the barks and the roots of the same species. Other authors have revealed the presence of the saponins, tannins and antraquinons in the seeds (Adejumo et al, 2011) in one part, and alkaloids, tannins and reducing carbohydrates in the barks of stem in other part (Yemoa et al, 2008).

Table 2. Chemical Compounds Content

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Garcinia kola Seeds</th>
<th>Defatted Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenols</td>
<td>1.65±0.08</td>
<td>1.64±0.00</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.35±0.30</td>
<td>0.19±0.11</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>0.80±0.15</td>
<td>0.43±0.00</td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>0.56±0.00</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td>Tannins</td>
<td>3.87±0.01</td>
<td>1.25±0.01</td>
</tr>
<tr>
<td>Aglycons flavonic</td>
<td>1.44±0.05</td>
<td>1.12±0.00</td>
</tr>
</tbody>
</table>

Figure 1. Content of Total Polyphenolic Compounds

3.2. Defatted Meals Mineral Elements

The minerals of the defatted meal of Garcinia kola were presented in Table 3. Values were in Triplicate Mean ± Standard Deviation. The study of this table showed that defatted meal of Garcinia kola was low in total minerals percentage (1.30%) that was, however higher than the one obtained from the sample of Nigeria which was only 0.47% (Essien et al, 1995). The composition of individual
minerals was marked by the predominance of nitrogen (0.59%) and potassium (0.34%). It was also noted that the sample of Nigeria got higher percentage than the one studied meal which was only 95 ppm in sodium (852 ppm). Globally, phosphorus, nitrogen and calcium were the main minerals of these defatted meals. However, their proportions were still weak comparatively to those of oleaginous in general (soya, rape seed, cotton, groundnut, palm oil nut); according to these results, the contents in N, P, K apart the one in phosphorus, were weak comparing to these reported in the literature by Adesuyi et al (2012). Those data were sufficiently weak to permit the defatted meals use in order to replace the chemical fertilizers in agriculture (Leclerc, 2012). But their mineral elements composition and in organic matters allow their use in animals feeding.

![Figure 2. Content of Individual Polyphenolic Compounds](image)

Table 3. Mineral Composition of Defatted Meal

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Garcinia kola*</th>
<th>Garcinia kola Nigeria*</th>
<th>Garcinia mangostana Nigeria*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Mineral</td>
<td>1.30±0.10</td>
<td>0.47±0.09</td>
<td>1.99±0.30</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.59±0.10</td>
<td>0.09±0.00</td>
<td>-</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.09±0.00</td>
<td>0.002±0.001</td>
<td>-</td>
</tr>
<tr>
<td>K (%)</td>
<td>0.34±0.00</td>
<td>0.10±0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.07±0.00</td>
<td>0.02±0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.10±0.00</td>
<td>0.22±0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Na (ppm)</td>
<td>95.0±2.00</td>
<td>852±0.71</td>
<td>26</td>
</tr>
</tbody>
</table>

Aedesuyi et al, 2012; Ajayi et al, 2007; *This study

3.3. Defatted Meals Organic Mineral Composition

Table 4 assembled the data on organic matters of defatted meals extracted from Garcinia kola studied and those from Nigeria. These results expressed their potential use for digestion and in constituents for motor fuel of second generation. The sample showed a considerable content of proteins in defatted meal (3.69%) even though it was low compared to the protein level in some commonly consumed oil seed in Benin like rapeseed (25%) and Sunflower (28.7%) as reported by Silou et al (2004). Garcinia kola can still be used as a source of Protein. This value was greater than that reported by Adesuyi et al (2012) for a naturalized species in Nigeria is 1.86%. Protein and starch were in the normal range compared to those published by FAO in 1995 (protein: 2-15%, starch: 60-75%).

Table 4. Proximate Composition of Defatted Meal

<table>
<thead>
<tr>
<th>Parameters (g/100g-Dry Matter)</th>
<th>Garcinia kola*</th>
<th>Garcinia kola Nigeria*</th>
<th>Garcinia mongostana Nigeria*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter</td>
<td>98.70±0.00</td>
<td>90.16±0.23</td>
<td>50.07</td>
</tr>
<tr>
<td>Proteins</td>
<td>3.69±0.50</td>
<td>1.86±0.15</td>
<td>6.57</td>
</tr>
<tr>
<td>Crude fibres</td>
<td>4.50±0.10</td>
<td>1.23±0.15</td>
<td>13.7</td>
</tr>
<tr>
<td>Lignins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lignocellulose</td>
<td>5.00±0.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>19.80±0.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>62.90±0.40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Sugar</td>
<td>12.00±0.20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Aedesuyi et al, 2012; Ajayi et al, 2007; *This study

The other gross components were present in minute quantities. Crude fibre content (4.5%) showed that the sample contained little defatted meal of Cellulose, Hemi-cellulose and Lignin. However, the high proportion of starch and total sugars suggested strong operating value of these seeds in agro-business opportunities. These defatted meals rich in nutritive elements could, however, constitute a lingo cellulosic raw material for motor fuel of second generation (Couleau, 2012). The high content in starch and total sugars was indicative for digestive character of the Garcinia kola defatted meals studied and tubers utilization for milk extraction (Chopra et al, 1986).

The result of this research has confirmed that Garcinia kola got a higher percentage of carbohydrate (Table 4) than Sorghum bicolore. L stem flour content (44.52%) as reported by Adetuyi & Akpambang (2005) and could be used as a source of energy. They also provide readily accessible fuel for physical performance and regulate nerve tissue.

4. Conclusion

This study initiated to characterize the physical chemical of the seeds and defatted meal of Garcinia kola showed that it contained several chemical compounds as antioxidiant and the mineral elements in appreciable quantities. The
composition of the defatted meals showed that it was a good food providing a nutritional of biogenic salts and can be destined to livestock feeding or to an intermediate product of biscuit factory. In addition, the minerals in significant quantities showed that *Garcinia kola* could also play a role in the prevention and treatment of certain diseases. From the research, it has been established through the nutritional screening that *Garcinia kola* could be used as a good source of carbohydrate and Protein, also a good source of minerals necessary for metabolic activities in the body despite the trace seeds of micronutrients. The physical chemical composition also showed that *Garcinia kola* could be useful in the pharmaceutical and medical science to promote those industries as raw material.

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