Comparative Study of the Effects of Ethanolic and n-Hexane Extracts of *Garcinia kola* Seeds on the Serum Electrolyte of Albino Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

*Garcinia kola* (bitter kola) plays an important role in Africa ethnomedicine and traditional hospitality. Proximate and phytochemical composition of *G. kola* seeds as well as the effects of ethanolic and n-hexane extracts on the serum electrolytes of albino rats were studied using standard methods. Thirty-six albino rats of both sexes were used for the experiment. The animals were divided into nine groups of four rats per group. The groups were designated 1-9. Group 1 served as the control which was treated with normal saline. Groups 2-5 served as the groups treated with ethanolic extract of *G. kola* seeds and received 50, 125, 250 and 500 mg/kg body weight, while groups 6-9 served as the groups treated with n-hexane extract. After three weeks of treatment, the animals were sacrificed, and blood samples analyzed. Result of the proximate analysis showed that carbohydrate content was the highest (78.06%) while ash was the lowest (0.70%). Phytochemical result of *G. kola* seeds showed that tannins (0.342%) was the highest in terms of percent composition, followed by flavonoids (0.00764%); while alkaloids (0.00075%) was the lowest. Also, biochemical analysis revealed that the n-hexane extract of *G. kola* seeds was found to have slightly increased the activities of the serum electrolytes than the ethanolic extract. Conclusively, the results of this study showed that both extracts had effect on serum electrolytes of the albino rats, but the n-hexane extract had more toxic effect.

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Keywords: Garcinia kola; phytochemicals; proximate; serum electrolytes; tannins.

1. INTRODUCTION

Medicinal plants for long have been used as remedies for human diseases because of their therapeutic values [1]. The medicinal values of plants lie in the chemical substances present in parts of the plant such as the seed, leaf, bark, stem and root. These substances produce definite physiological action in the health of humans [2]. Over the years, medicinal plants have played important role in the preservation of health and management of diseases. This is mainly due to the harmful effects produced by the use of synthetic drugs which are comparatively minimal in drugs of plant origin [3].

Garcinia kola is a medicinal plant grown in tropical rainforest in West Africa. Extracts obtained from the bark of this plant are used for the treatment of liver cirrhosis and hepatitis in traditional medicine [4]. Its seeds have been shown to possess various bioactivities in experimental models such as neuroprotective and bronchodilatory effects [5].

Some chemical compounds obtained from plants are known as phytochemicals which are easily seen in fruits, vegetables, grains, nuts and legumes [6]. They are non-nutritive components that exert proactive or disease preventing effects as well as other health benefits. Studies have shown that the level of phytochemicals in some plants can be manipulated by breeding and the content of a single phytochemical may be adjusted [7]. Phytochemicals include terpenoids, alkaloids, saponins, steroids and phenolic compounds [8]. Phytochemical studies have shown that Garcinia kola seed contains varieties of phytochemicals including cardiac glycoside, flavonoids, tannins and saponins [9]. The main bio-active component which is responsible for numerous physiological and pharmacological effects of G. kola seed is flavonoid. Flavonoid from G. kola reduced lipid levels in normal and hypercholesterolemic rats [10].

The kidney is a bean-shaped organ which functions in numerous crucial regulatory roles in animals. It eliminates excess organic substances from the blood and waste products of metabolism. The main functioning part of the kidney is the nephron. Kidney is made up of more than two million nephrons, and each is capable of producing urine. Kidneys are vital to the urinary structure and also perform homeostatic functions such as maintenance of acid-base balance, the regulation of electrolytes, and regulation of blood pressure [11,12]. They act as natural filters of the blood and eliminate water-soluble impurities which are moved to the bladder. Kidneys excrete wastes such as urea; and are also accountable for the reabsorption of glucose, amino acids and water. The kidneys also produce hormones including erythropoietin and calcitriol. An essential enzyme renin is also formed in the kidney which acts in negative feedback [13].

This study was undertaken to investigate the effects of ethanolic and n-hexane extracts of G. kola seeds on the serum electrolytes of albino rats.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh seeds of G. kola were obtained from Sangana street market in Port Harcourt, Nigeria. The seeds were washed, peeled, weighed, cut into bits and allowed to air dry. The dried seeds were weighed and ground into coarse powder using a manual grinder. The ground sample was stored in a cool and dry place.

2.2 Extraction

Ground G. kola seeds was subjected to extraction at room temperature using maceration method [14]. Briefly, 2 kg of ground G. kola seeds was soaked in 4 L of n-hexane inside an aspirator bottle and stirred properly to ensure proper mixing of plant sample and solvent. The mixture was filtered after 24 hours into a conical flask. This process was repeated five times using fresh n-hexane each time. The filtrates were combined and concentrated to dryness using a rotary evaporator. The concentrate was labelled n-hexane extract. Extraction process was repeated on the same sample using 4 L of ethanol. The concentrate for this batch was labelled ethanolic extract. The process yielded 108.15 g and 149.5 g of n-hexane and ethanolic extracts respectively with percentage yields of 5.4% and 7.5%.

2.3 Experimental Animals

Thirty-six albino rats comprising of both sexes were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Nigeria and transferred to the animal.
house of the Department of Chemistry, Rivers State University, Nigeria. They were weighed and randomly assigned into metallic cages and were allowed access to growers' mesh feed (Top feeds, Nigeria Ltd) and water throughout the period of experiment. They were allowed to acclimatize for one week before the commencement of administration.

2.4 Proximate Analysis

Proximate analysis of G. kola seeds was carried out using A.O.A.C. method [15].

Moisture content: Dried sample of G. kola seeds (1.0 g) was weighed into an evaporating dish. The evaporating dish containing the sample was placed in the oven for 24 hours at 105°C and was cooled in a desiccator at room temperature. The evaporating dish containing the sample was oven dried again for 2 hours to ensure complete dryness. The process of drying and cooling was repeated until a constant weight was obtained. Moisture content was calculated using equation 1.

\[ \% \text{ Moisture} = \frac{(W_1 - W_2)}{W_1} \times 100 \]  

(1)

where, \( W_1 \) = weight of sample before drying, \( W_2 \) = weight of sample after drying

Crude protein: Total protein was determined by Kjeldahl method. Equation 2 was used to calculate crude protein.

\[ \% \text{N} = \frac{\text{Sample Titre} - \text{blank Titre} \times N \text{ of acid} \times 1.4}{\text{weight of sample (in 10ml)}} \]  

(2)

Crude fat: Round bottomed flask of 250 ml was oven dried at 105°C. Weighed sample of G. kola seeds (0.25 g) was poured into a thimble, 200 ml of petroleum ether was measured and then added to the dried extracting flask. The thimble having the sample was placed in a Soxhlet extractor compartment where the sample was extracted for 5 hours. Crude fat content was calculated using equation 3.

\[ \% \text{Crude Fat} = \frac{W_1}{W_2} \times 100 \]  

(3)

where, \( W_1 \) = weight of ground G. kola seed used, \( W_2 \) = weight of extracted fat

Crude fiber: Dried sample of G. kola seeds (2.0 g) was weighed into 1 L conical flask, 200 ml sulphuric acid was added and allowed to boil gently for 30 minutes; it was filtered and rinsed properly with hot deionized water. The sample was scrapped with spatula into the flask, 200 ml of boiling sodium hydroxide was added and allowed to gently boil for 30 minutes; it was filtered, the residue was later washed with hot deionized water and rinsed with 10% hydrochloric acid. The residue was then oven dried overnight at 110°C and was transferred into a desiccator for cooling before the weight was measured. Equation 4 was used to calculate crude fiber content.

\[ \% \text{Fiber} = \frac{100 (W_1 - W_2)}{W_0} \]  

(4)

where, \( w_0 \) = weight of sample, \( w_1 \) = weight of crucible with sample before ashing, \( w_2 \) = weight of crucible with sample after ashing

Ash content: Dried sample of G. kola seeds (10.0 g) was weighed and placed in a Petri dish. Sample in the Petri dish was emptied into a muffle furnace pot. The pot with its content was placed in the furnace at 550°C for 4 hours and was allowed to cool in a desiccator. This process was repeated until a constant weight was obtained. Ash content was calculated using equation 5.

\[ \% \text{Ash} = \frac{W_2}{W_1} \times 100 \]  

(5)

where, \( w_1 \) = weight of sample, \( w_2 \) = weight of ash

Carbohydrate content: 100% minus the sum of percentages of crude protein, crude fat, crude fiber and moisture content of G. kola seed sample gave the carbohydrate content [16].

2.5 Phytochemical Quantification

The methods of Wenkam [17] and Wills [18] were adopted for the preparation and extraction of G. kola seeds for gas chromatography-mass spectroscopy (GC-MS) analysis.

2.6 Experimental Design

Oro-gastric intubation method was adopted. Animals in each group were administered a volume of extract in accordance with their body weight for twenty-one days [19]. Thirty-six albino rats of both sexes were divided into nine groups of four rats per group. Group 1 served as control which received normal saline water for 21 days. Group 2 received orally 50 mg/100 g body weight of G. kola seeds ethanolic extract once a day for 21 days. Group 3 received orally 125 mg/100 g body weight of G. kola seeds ethanolic extract once a day for 21 days. Group 4 received orally 250 mg/100 g body weight of G. kola seeds ethanolic extract once a day for 21 days. Group
received orally 500 mg/100 g body weight of G. 
kola seeds ethanolic extract once a day for 21 
days. Groups 6-9 received 50 mg/100 g, 125 
mg/100 g, 250 mg/100 g and 500 mg/100 g of n-
hexane extract of G. kola seeds respectively 
one a day for 21 days.

The animals were given access to water and rat 
diet throughout the duration of administration. 
After the administration, the animals were made 
to fast before they were sacrificed. Blood 
samples were collected and transferred into 
heparin bottles while the liver organs were 
transferred into sample bottles containing 
formalin for preservation. These samples were 
then used for analysis.

2.7 Biochemical Analysis

Standard methods were adopted [12,20-25].

2.8 Statistical Analysis

Statistical analysis was done with the aid of 
Statistical Package for Social Sciences (SPSS) 
for windows (SPSS Inc., Chicago, Standard 
version 21.0) to determine difference between 
means using ANOVA. Data was reported as 
mean ± standard error of mean (SEM) and the 
level of significance was set at P< 0.05.

3. RESULTS AND DISCUSSION

The importance of plants in human diet cannot 
be over emphasized; it supplies the human body 
with mineral salts, certain hormone precursors 
and vitamins. Garcinia kola has been reported to 
contain good quantities of phytochemicals, some 
of which maybe toxic to vital human organs and 
tissues [26]. Phytochemical analysis is important 
in the evaluation of bioactive components of 
seeds and other plant parts.

The result in Table 1 shows that G. kola seed 
was composed mainly of carbohydrate (78.06%) 
followed by moisture (11.2%), while ash content 
was the least (0.7%). The proximate composition 
result of this study agrees with the report that 
Garcinia kola contains minimal protein, fat and 
fiber [27,28].

Phytochemical analysis of Garcinia kola revealed 
that the seed contains more of tannins (0.342%), 
followed by flavonoids (0.00764%) and saponins 
(0.00609%), while alkaloids (0.00075%) was the 
least in terms of percentage composition [Table 
2]. These phytochemicals are usually the 
bioactive compounds found in plants and are 
responsible for plant medicinal values. 
Flavonoids are water soluble antioxidant and free 
radical scavengers which prevent cells of living 
things from oxidative damage. Previous 
researchers have reported that G. kola contains 
tannins, saponins, steroids, flavonoids and 
alkaloids [29]. They noted that these components 
were present in significant amount, which 
corroborates the report of this study. Enemchukwu, 
et al. [30] reported that Garcinia 
kola contains flavonoids, tannins, saponins and 
cardiac glycosides; noting that tannins content 
was the highest followed by flavonoids. They 
also reported that extract of Garcinia kola has 
useful pharmacological bioactive compounds 
that could be used for ethnomedicine [30]. Their 
report which agrees with the findings of this study 
also noted that alkaloids was the least in terms of 
percentage composition [30]. The revealed 
phytochemical components from this work 
indicates that Garcinia kola could be of medicinal 
value.

Table 1. Proximate analysis of G. kola seeds

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage composition (%)</th>
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</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.20</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.60</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.80</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>4.10</td>
</tr>
<tr>
<td>Ash</td>
<td>0.70</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>78.06</td>
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<td>Total</td>
<td>99.46</td>
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Table 2. Chemical composition of G. kola seeds

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage composition (%)</th>
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</thead>
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<tr>
<td>Steroids</td>
<td>0.00297</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.34200</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.00764</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.00609</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>0.00568</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>0.00075</td>
</tr>
<tr>
<td>Total</td>
<td>0.36513</td>
</tr>
</tbody>
</table>

Table 3 shows the compounds of each 
phytochemical group present in G. kola seed. 
The highest number of compounds (twenty-five) 
was recorded for flavonoids; similar observation 
was reported by Enemchukwu, et al. [30]. 
Flavonoids are strong antioxidants and free 
radical scavengers which prevent oxidative cell 
damage; they also have protective effects such 
as anti-carcinogenesis, anti-inflammatory and 
platelet aggregation [29]. Tannic acid which may
## Table 3. Phytochemical components of *G. kola*

<table>
<thead>
<tr>
<th>Retention time (minutes)</th>
<th>Components</th>
<th>Concentration (mg/100 g)</th>
<th>Retention time (minutes)</th>
<th>Components</th>
<th>Concentration (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td><strong>Anthraquinones</strong></td>
<td></td>
</tr>
<tr>
<td>13.739</td>
<td>(+)-Catechin</td>
<td>8.80028e-6</td>
<td>16.465</td>
<td>2,6-dimethoxybenzoquinone</td>
<td>1.76261e-1</td>
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<td>15.155</td>
<td>Resveratrol</td>
<td>3.06999e-6</td>
<td>18.006</td>
<td>Hydroxymethoxyquinoline-1-oxide</td>
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<tr>
<td>15.495</td>
<td>Genistein</td>
<td>8.37399e-6</td>
<td>18.853</td>
<td>Hydroxymethyl anthraquinone</td>
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<td>16.035</td>
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<td>Damncathal</td>
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<td>16.779</td>
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<td>Damncanthol</td>
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<td>17.157</td>
<td>Naringenin</td>
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<td>Heterrophylline</td>
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<td>(-)-Epicatechin</td>
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</tbody>
</table>
be responsible for antioxidant, anti-inflammatory, anti-enzymatic and analgesic properties of G. kola [29] was the only tannins compound present in G. kola seed. Tables 4 and 5 show the effect of ethanolic and \( n \)-hexane extracts of G. kola seeds on serum electrolyte composition of albino rats respectively. The values of \( Na^+ \) and \( K^+ \) were not significantly different when compared with the control group, this result is in agreement with the report of Mazi, et al. [29]. It is important to note that \( Na^+ \) and \( K^+ \) help to maintain the osmotic balance of the body fluid and retention of protein during growth. \( Cr \) and \( Ur \) values in the experimental groups were significantly not different when compared with group 1 (control). This is in contrast with the report of Okoko and Awhin, [31], which showed significant increase in \( Cr \) and \( Ur \) values. Comparatively, the results showed that both extracts had effect on serum electrolytes of albino rats, but the \( n \)-hexane extract had more toxic effect than the ethanolic extract; the observed toxic effect could be caused by non-polar components of G. kola seeds.

4. CONCLUSION

Garcinia kola seed contains useful bioactive compounds which may be responsible for its pharmacological effects. This study has shown that \( n \)-hexane extract of Garcinia kola seed has more toxic effect than the ethanolic extract on serum electrolytes of albino rats.

ETHICAL APPROVAL

The procedures followed in this study met the standards of the Local Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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