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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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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 105ºC 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.

$$\% \text{ Moisture} = \frac{(W1-W2)}{W1} \times 100 \quad (1)$$

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.

$$\%N = \frac{Sample\ Titre - blank\ Titre \times N\ of\ acid \times 1.4}{weight\ of\ sample\ (in\ 10ml)} \quad (2)$$

Crude fat: Round bottomed flask of 250 ml was oven dried at 105ºC. 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.

$$\% \text{ Crude fat} = \frac{W1}{W2} \times 100 \quad (3)$$

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 110ºC and was transferred into a desiccator for cooling before the weight was measured. Equation 4 was used to calculate crude fiber content.

$$\% \text{ Fiber} = \frac{100\ (W1-W2)}{W0} \quad (4)$$

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 muffle furnace pot. The pot with its content was placed in the furnace at 550ºC 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.

$$\% \text{ Ash} = \frac{W2}{W1} \times 100 \quad (5)$$

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

5 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 once 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

Components	Percentage composition (%)
Moisture	11.20
Crude protein	2.60
Crude fat	2.80
Crude fiber	4.10
Ash	0.70
Carbohydrate	78.06
Total	**99.46**

Table 2. Chemical composition of *G. kola* seeds

Components	Percentage composition (%)
Steroids	0.00297
Tannins	0.34200
Flavonoids	0.00764
Saponins	0.00609
Anthraquinones	0.00568
Alkaloids	0.00075
Total	**0.36513**

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*

Retention time (minutes)	Components	Concentration (mg/100 g)	Retention time (minutes)	Components	Concentration (mg/100 g)
	Flavonoids			**Anthraquinones**	
13.739	(+)- Catechin	8.80028e-6	16.465	2,6-dimethoxybenzoquinone	1.76261e-1
15.155	Resveratrol	3.06999e-6	18.006	Hdroxymethoxyquinoline-1-oxide	1.10446e-1
15.495	Genistein	8.37399e-6	18.853	Hydroxymethyl anthraquinone	3.76631
15.620	Daidzein	9.34093e-6	19.638	Soranjidiol	4.12191e-2
16.035	Apigenin	1.38948e-3	20.729	Damnacathal	1.34038e-3
16.779	Biochanin	1.73672e-5	22.699	Damncanthol	5.19679e-4
17.157	Naringenin	1.03758e-3	24.251	Heterrophylline	9.22450e-4
17.767	Luteolin	3.36290e-6		**Sub-total**	**5.68337**
18.049	Kaemferol	11.87276			
18.411	Fisetin	5.22067e-7	**Steroids**		
18.929	Gb1 kolaviron	25.45975	19.548	Cholesterol	7.24640e-4
19.097	Gb2 kolaviron	16.66734	20.764	Cholestanol	1.81416e-3
19.515	Kolaflavanone	19.90848	21.932	Ergosterol	1.59899e-3
19.947	(-)-Epicatechin	4.55074e-4	22.933	Campesterol	8.14445e-1
20.591	(-)-Epigallocatechin	7.86003e-5	23.254	Stigmasterol	4.59230e-1
21.817	Gallocatechin	4.29810e-5	24.910	Savenasterol	6.81305e-2
22.596	Quercetin	2.43998	25.250	Sitosterol	1.62758
22.850	(-)-Epicatechin-3-gallate	2.29837e-4		**Sub-total**	**2.97352**
23.468	(-)-Epigallocatechin-3-gallate	1.98691e-4			
23.963	Astragalin	8.45021e-4	**Tannins**		
24.232	Isorhamnetin	8.08938e-7	19.518	Tannic acid	341.73021
24.536	Robinetin	3.31856e-6		**Sub-total**	**341.73021**
24.997	Myricetin	1.03979e-3			
25.478	Nobiletin	3.15883e-6	**Alkaloids**		
26.012	Isoquercetin	6.54450e-4	6.810	Augustifoline	8.40994e-7
	Sub-total	**76.35435**	7.962	Ellicine	3.35445e-6
			9.013	7-methylxanthine	7.74302e-3
Saponins			9.692	Theophylline	1.85762e-2
17.381	Hispigenin	4.46026e-4	11.369	Theobromine	4.02765e-2
18.046	Taccalin	1.61304e-4	12.251	Xeronine	1.99238e-5

Retention time (minutes)	Components	Concentration (mg/100 g)	Retention time (minutes)	Components	Concentration (mg/100 g)
18.776	Solagenin	5.73264e-3	14.160	Caffeine	6.86236e-1
19.237	Disogenin	8.88613e-5		**Sub-total**	**7.52855e-1**
19.511	Tigogenin	7.27664e-1			
20.357	Neochlorogenin	3.73860			
21.813	Hecogenin	8.31842e-5			
22.589	Sapogenin	8.22321e-1			
23.189	Tribuloin	4.11034e-4			
23.951	Yanogenin	3.98562e-1			
24.776	Conyzorenin	5.74094e-6			
26.472	Saponine	4.00961e-1			
	Sub-total	**6.09503**			

Table 4. Values of serum electrolytes in albino rats treated with ethanolic extract of *G. kola*

Group	Na^+ (mmo/l)	K^+ (mmo/l)	HCO_3^- (mmo/l)	Ur (mmo/l)	Cr (mmo/l)	Cl^- (mmo/l)
1	119.3 ± 4.71^{actg}	3.5±0.30^{actg}	26.5±1.04	7.5±0.52	123±4.5	35±1.78
2	125.5±6.45^{acfg}	3.8±0.15^{acfg}	25.5±1.55	8.25±0.89	122±4.08	38±1.47
3	128±6.38^{acfg}	4.3±0.21^{acfg}	27±1.29	8.9±0.19	125.3±2.14	43.3±0.85
4	149.3±6.07^{*bdeh}	6±0.76^{*bdeh}	26.5±0.96	8.8±0.15	127.3±1.25	47.3±3.27
5	124±5.11^{actg}	3.7±0.44^{actg}	25±0.58	10.2±0.71	148±5.76	39.8±3.27

Values are expressed as mean ± standard error of mean (SEM) for n=4. Values with different superscript letter a,b; c,d; e,f; g,h) in the same columns are significantly different at the 0.05 level (p≤0.05)

**Differ significantly when comparing group 1 with other groups, a,b differ significantly when comparing group 2 with other groups, c,d differ significantly when comparing group 3 with other groups e,f differ significantly when comparing group 4 with other groups, g,h differ significantly when comparing group 5 with other groups. Value with the same superscript letter show no significant difference*

Table 5. Values of serum electrolytes in albino rats treated with n-hexane extract of *G. kola*

Group	Na^+ (mmo/l)	K^+ (mmo/l)	HCO_3^- (mmo/l)	Ur (mmo/l)	Cr (mmo/l)	Cl^- (mmo/l)
1	119.3±4.71^{aceh}	3.5±0.30	26.5±1.04	7.57±0.52^{eg}	123±4.51^{ceh}	35±1.78^{bcfg}
2	132.5±5.5^{aceh}	4.3±0.24	27±1.29	8.90±0.80^{eg}	134.8±6.54^{ceg}	49.50±4.70^{*acfg}
3	133±5.8^{aceh}	4.2±0.22	25.5±1.71	7.32±2.31^{eg}	127.8±16.67^{ceh}	42.25±0.85^{actg}
4	134±2.25^{aceh}	4.3±0.09	28±1.41	6.30±1.82^{eh}	126.3±15.33^{ceh}	59.0±8.55^{*adeh}
5	150±5.61^{*bdfg}	4.7±0.13^{*g}	27±0.58	10.05±1.85^{fg}	155.8±14.22^{*dfg}	41.75±1.03^{acfg}

Values are expressed as mean ± standard error of mean (SEM) for n=4. Values with different superscript letter a,b; c,d; e,f; g,h) in the same columns are significantly different at the 0.05 level (p≤0.05)

**Differ significantly when comparing group 1 with other groups a, b differ significantly when comparing group 2 with other groups, c,d differ significantly when comparing group 3 with other groups, e,f differ significantly when comparing group 4 with other groups, g,h differ significantly when comparing group 5 with other groups. Value with the same superscript letter show no significant difference*

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 effects 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.

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