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Quantitative Phytochemical, Proximate Analysis and Hypolipidemic Effect of *Garcinia kola*

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Authors' contributions

This work was carried out in collaboration between all authors. This work was carried out in collaboration between all authors. Author YNO designed the study and wrote the protocol. Author SOO performed the statistical analysis, and wrote the first draft of the manuscript. Author MIE did the literature search and also wrote part of the manuscript. Authors WCU, EO and MR managed the animals, analyses of the study and collected all data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was aimed at evaluating the phytochemical and nutrient composition of *G. kola* seed. Also to examine the effect of *G. kola* seed on the serum lipid profile of fed rats.
Study Design: Quantitative phytochemical, proximate analysis and *in vivo* effect on serum lipid profile.
Place and Duration of Study: Department of Biochemistry, College of Natural sciences, Michael Okpara University of Agriculture, Umudike Abia State, between June 2013 and September 2013.
Methodology: The seeds were cut into small pieces, dried and ground into powder. The quantitative phytochemical and proximate nutrient analyses of the powdered sample were determined using standard methods. The lipid lowering effects of the powdered sample of *G. kola* determined in rats. The rats were fed with feed fortified with graded levels (5, 10, 20 and 50%) of powdered sample of *G. kola* for 21 consecutive days and the effects on total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and high density lipoprotein cholesterol (HDL-C) were compared with a negative control.
Results: The seed sample produced significant ($p<0.05$) dose-dependent decrease in the total serum cholesterol, triglyceride, LDL-C and VLDL-C levels in fed groups of rats when compared to the control group. The seed sample also, caused significant ($p<0.05$) dose-dependent increase in serum HDL-C level in fed groups of rats when compared to the control groups. The phytochemical analysis showed that the sample contained tannins (5.08%), flavonoids (0.93%), saponins (2.54%) and alkaloids (5.13%). The proximate analysis of the nutrient composition of powdered *G. kola* seed showed the presence of moisture, ether extract (EE), crude fibre (CF), crude protein (CP), ash and nitrogen free extracts (NFE) in the following proportion 7.40, 1.48, 2.94, 3.19, 4.39 and 80.58%, respectively.
Conclusion: The sample demonstrated good lipid lowering effects which may suggest that the consumption of *G. kola* seed may help in the reduction of the incidence of cardiovascular diseases in patients.

Keywords: Garcinia kola; hypolipidemia; phytochemicals; proximate analysis; tannins; saponins.

1. INTRODUCTION

In recent time, cardiovascular diseases such as atherosclerosis which are caused by hyperlipidemia, have caused increased mortality rate and reduced life expectancy [1]. Reducing serum hyperlipidemia is very important; a 1% reduction in serum cholesterol concentration results in a 2% reduction in the prevalence of coronary artery diseases [2]. It has been established that long-term consumption of a high fat diet accelerates the development of Coronary Heart Disease (CHD). Dietary cholesterol can increase the level of serum cholesterol to levels which can place an individual at increased risk for the development or exacerbation of atherosclerosis [2,3]. Coronary Heart Disease (CHD) increases dramatically as the plasma concentration of LDL cholesterol increases [3]. The use of orthodox hypolipidemic agents is expensive and associated with some side effects like flatulence, constipation and dyspepsia [4]. The screening of herbs used in the traditional medicine in the management of cardiovascular diseases for lipid lowering effect has gained

wide scientific interest in the last decades. Among the plants used in the traditional medicine for the management of hyperlipidemia related disease is *G. kola*.

Garcinia kola also called aku ilu (Igbo), Orogbo (Yoruba), Namijin goro (Hausa) and Bitter kola (English) belong to the family Guttiferae. It is commonly found in the West African countries. Its natural habitat is subtropical or tropical moist low land forests [1]. The growing tree and roots provide chew-sticks and the seed is eaten [5]. Also, the seed of *G. kola* is traditionally served to visitors for entertainment. The raw seed of *G. kola* is extensively used by herbalist in southern part of Nigeria to treat various disease conditions in humans [5]. The extract of the seed has been proven scientifically to have several pharmacological activities which include anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant and hepatoprotective activities [5-8]. The present work was aimed at evaluating the phytochemical and nutrient composition of *G. kola* seed. Also, to examine the effect of *G. kola* seed on the serum lipid profile of fed rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The freshly harvested fruit of *G. kola* (Bitter kola) were bought from Ndoro market, Oboro in Ikwuano LGA of Abia State in the month of July 2013. It was authenticated by Dr. I. C. Okwulehie of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The voucher specimen catalogued MOUAU/COLNAS/BCM/2013/03 was kept for reference purposes in the departmental herbarium.

2.2 Preparation of Seed Sample

The outer testa of each *G. kola* seed was removed, washed and air dried for about 24h. Each seed was cut into smaller pieces and the resulting pellets were dried in hot air oven (Gallenkamp) for 12h at 40ºC. The dry seed pellets were ground to fine powder. The resulting powder was stored in an air tight container throughout the duration of the experiment.

2.3 Experimental Animals

Mature Wistar albino rats bred in the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used for the experiments. They were housed in an environment of normal ambient temperature and relative humidity of 40–60%, and the lighting period was about 12h daily. The weight of the rats varied between 107 and 185g. The rats were kept in stainless steel cages, supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Broiler finisher) (Vital feed®, Nigeria). Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea [9], and the experimental protocol was approved by Michael Okpara University of Agriculture Umudike ethical committee.

2.4 Effect of Seed Sample of *Garcinia kola* on the Serum Lipid Profile of Rats

Thirty male albino Wistar rats were randomly divided into five groups of six animals each. Group 1 served as the control and received plain feed without seed sample. Group 2-5

received feed fortified with graded levels (5, 10, 20 and 50%, respectively) of *G. kola* seed sample. The animals were fed 10gram of feed per 100gram body weight daily for 21 days. Twenty-four hours after the last feed administration, the animals were anaesthetized with chloroform vapour. Overnight fasting blood sample for sera preparation were collected by direct cardiac puncture into sterile plain tubes. The serum samples were separated from the clot by centrifugation at 3000g for 5min using bench top centrifuge (MSE minor, England). Serum samples were separated into sterile plain tubes and stored in refrigerator for serum lipid profile analysis.

2.5 Biochemical Analysis

The serum lipid profiles of the rats were evaluated using a commercially available assay kit (Randox, UK). The serum level of total cholesterol (TC) was measured by enzymatic hydrolysis and oxidation method as described by Stein [10]. The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by Tietz [11]. The serum level of HDL-C was measured by the method of Wacnic and Albers [12]. The serum VLDLC was calculated as 1/5 of the serum triglyceride [13], while the serum LDLC was calculated using the Friedewald formula [14].

2.6. Quantitative Analysis on Phytochemical Constituents of *Garcinia kola*

Total alkaloids, flavonoids, tannins and saponins were determined using the method described by Krishnaiah et al. [15] and Edoga et al. [16] in triplicates.

2.6.1 Determination of alkaloids

Five grams of the plant sample was placed in a 250ml beaker and 200ml of 10% ethanoic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated ammonium hydroxide was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute ammonium hydroxide. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference. This was done in triplicate.

2.6.2 Determination of flavonoids

Ten grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference. This was done in triplicate.

2.6.3 Determination of saponins

Twenty grams of seed sample was weighed into a 250ml conical flask. One hundred mililitre of 20% ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55ºC. It was then filtered with a Whatman No.1 paper. The residue was re-extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over a water bath at about 90ºC. The concentrated extract was then transferred into a 250ml separator funnel and 20ml of diethyl ether was added to the extract

and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded. This purification process was repeated. Sixty (60)ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% sodium chloride. The remaining solution was then heated on a water-bath in a pre-weighed 250ml beaker. After evaporation the residue was dried in a Gallenkamp moisture extraction oven (Size 1) to a constant weight. The % saponin was calculated by difference. This was done in triplicate.

2.6.4 Tannin determination

The seed sample, 500mg, was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filterate was pipetted out into a test tube and mixed with 2ml of 0.1M ferric chloride in 0.IN hydrochloric acid and 0.008M potassium ferrocyanide. The absorbance was measured at 700nm within 10min. This was done in triplicate.

2.7 Proximate Nutritional Analysis

Standard methods of the Association of Official Analytical Chemists [17] were used to determine the moisture, crude protein (CP), ether extract (EE), ash, nitrogen free extract (NFE) and crude fiber (CF) contents of each sample. Moisture content was determined by heating 2.0g of each fresh sample to a constant weight in a crucible placed in an oven maintained at 105ºC. The dry matter was used in the determination of the other parameters. Crude protein (% total nitrogenx6.25) was determined by the Kjeldahl method, using 2.0g samples. Ether extract was obtained by exhaustively extracting 5.0g of each sample in a Soxhlet apparatus using petroleum ether (boiling point range 40-60ºC) as the extractant. Ash was determined by the incineration of 2.0g sample placed in a muffle furnace maintained at 550ºC for 5h. Crude fiber was obtained by digesting 2.0g of sample with 1.25% w/v sulphoric acid and 1.25% w/v sodium hydroxide, the fiber was filtered, dried at 120ºC, weighed and incinerating the residue in a muffle furnace maintained at 550ºC for 5h. Each analysis was carried out in triplicates.

Nitrogen free extracts (NFE) which represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed were determined by mathematical calculation. It was obtained by subtracting the sum of the percentages of all the nutrients already determined from 100.

$$\%NFE=100-(\%moisture+\%CF+\%CP+\%EE+\%Ash)$$

2.8 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) and the variant means were separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The feeding of the albino Wistar rats with feed fortified with powdered *G. kola* seed produced significant ($p<0.05$) dose-dependent decrease in the total serum cholesterol, triglyceride, LDL-C and VLDL-C levels and a significant ($p<0.05$) dose-dependent increase in the serum HDL-C level in fed group rats when compared to the control groups (Table 1).

The result of the phytochemical analysis showed that the seed of *G. kola* contained tannins (5.08%), flavonoids (0.93%), saponins (2.54%) and alkaloids (5.13%) (Table 2).

The result of the proximate analysis of the nutrient composition of powdered *G. kola* seed showed the presence of moisture, lipid, crude fibre, crude protein, ash and nitrogen free extracts in the following proportion 7.40, 1.48, 2.94, 3.19, 4.39 and 80.58% respectively (Table 3).

Table 1. Effect of seed sample of *G. kola* on the serum lipid profile (mean±SEM) of fed rats

Treatment group	Cholesterol (mg/dl)	HDL-C (mg/dl)	Triglyceride (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Control (plain feed)	97.77±2.05	36.63±0.24	116.13±3.72	23.23±0.74	37.91±2.69
5% feed	70.51±1.57*	31.94±0.28*	47.31±2.15*	9.46±0.43*	29.11±1.32*
10% feed	68.74±0.59*	34.34±0.24*	43.01±2.13*	8.60±0.34*	25.79±0.39*
20% feed	68.14±0.59*	42.96±0.37*	38.70±3.72*	7.74±0.47*	17.44±0.64*
50% feed	66.96±1.19*	48.33±0.24*	21.51±2.02*	4.30±0.43*	14.33±1.21*

**P<0.05 when compared to the negative control*

Table 2. Phytochemical content of *G. kola* seed

Composition	Mean values±SEM(%)
Tannin	5.08±0.02
Flavonoid	0.93±0.03
Alkaloid	5.13±0.67
Saponin	2.54±0.01

Table 3. Proximate composition of *G. kola* seed

Composition	Mean values±SEM (%)
Moisture content	7.40±0.79
Lipids	1.48±0.01
Crude fibre	2.94±0.01
Protein	3.19±0.01
Ash	4.39±0.01
Nitrogen free extracts	80.58±0.82

3.2 Discussion

Garcinia kola (Heckel), an angiospermae, belonging to the family Guttiferae, is commonly known as bitter cola. On chewing, *G. kola* seed has a bitter astringent and resinous taste, somewhat resembling that of raw coffee, followed by a slight sweetness. The bitter astringent properties may be due to the presence of tannin and bitter principles [18,19]. *Garcinia kola* has been shown to posses several pharmacological activities; anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant and hepatoprotective activities, which have been attributed to some of its phytochemical compositions [20,21].

In the present study the feeding of the albino Wistar rats with feed fortified with powdered *G. kola* seed produced a significant ($p<0.05$) dose-dependent serum-lipid lowering effects which may be mediated by some of its phytochemical and nutritional constituents. Though the mechanism of lipid lowering effect is not known, it could be either through reduction in absorption of cholesterol from the gut or by reduction in the biosynthesis of cholesterol [22]. The possible mechanism of reducing dietary cholesterol absorption from the gut is by binding of the phytochemical constituent such as phytosterols to the cholesterol receptor site in the gut mucosa [19].

Another possible mechanism through which the powdered seed sample of *G. kola* may have caused the lipid lowering effect may be by binding to bile acid in intestine, which will impede their reabsorption. This will subsequently deplete the bile acid pool leading to up regulation of cholesterol 7-α-hydroxylase and increased conversion of cholesterol to bile acids. This causes an increased demand for cholesterol in the liver cells, resulting in the dual effect of increasing transcription and activity of the cholesterol biosynthetic enzyme, HMG-CoA reductase and increase in the number of hepatic LDL receptors. These compensatory effects result in increased clearance of LDL-C from the blood, resulting in decreased serum LDL-C levels. Serum TG levels may increase or remain unchanged [23].

The result of the phytochemical and nutrient analysis obtained for the seed sample of *G. kola* in this work, differs from reports of previous workers. Adesuyi et al. [18] reported that *G. kola* seed contained 0.342, 2.471, 0.645 and 2.041% of tannins, saponins, alkaloids and flavonoids respectively. Eleyinmi et al. [8] reported that *G. kola* seed contained 97.31, 39.52, 43.25, 11.40, 114.02 and 694.48 g/kg dwt of moisture, CP, EE, ash, CF and NFE respectively. Also Odebunmi et al. [24] reported that *G. kola* seed contained 60.48, 2.48, 4.51, 0.79, 5.23 and 35.64% of moisture, CP, EE, ash, CF and NFE respectively.

The differences may be due to difference in the source of the materials used. According to Evans [25] plants may grow well in different situations but fails to produce the same constituents. Plant growth and development, and often the nature and quantity of secondary metabolites are affected by temperature, rainfall, length of day (quantity of light) and altitude. Light determine the amount of glycosides or alkaloids present in a plant. Also, continuous rain can lead to a loss of water-soluble substances from leaves and roots by leaching; this is known to apply to some plants producing alkaloids, glycosides and even volatile oils [25].

4. CONCLUSION

In conclusion, the lipid lowering effects as demonstrated by this study suggest that the consumption of *G. Kola* seed may help in the reduction of the incidence of cardiovascular

diseases in patients. This study has provided information on the benefits of *G. kola* consumption.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declarations of Helsinki and Michael Okpara University of Agriculture, Umudike, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alli SYR, Adanlawo IG. Tissue lipid profile of rats administered saponin extract from the root of bitter kola. Advances in Biochemistry. 2013;1(1):1-4.
2. Onyeneke EC, Adebisi KE, Eriyamremu GE, Ojeaburu SI, Asagba SO, Oluba OM. Effect of lipid-based diet on some lipid-metabolizing enzymes. J. Med. Sci. 2007;7(8):1283-1289.
3. Oluba OM, Adeyemi O, Ojieh GC, Adebisi KE, Isiosio IO, Aboluwoye CO. Effect of dietary cholesterol on some serum enzymes. J. Med. Sci. 2008;8(4):390-394.
4. *Knapp* HH*,* Schrott H, Ma P, Knopp R, Chin B, Gaziano JM, et al. Efficacy and safety of combination simvastatin and colesevelam in patients with primary hypercholesterolemia. The American Journal of Medicine. 2001;110:352-360.
5. Madubunyi II. Antihepatotoxic principles of *Garcinia kola* seeds. Comp. Clin. Pathol. 2010;21:481-485.
6. Farombi EO, Akanni OO, Emerole GO. Antioxidant and scavenging activities of flavonoid extract (kolaviron) of *Garcinia kola* seeds in vitro. Pharm. Biol. 2002;91:129–134.
7. Okunji CO, Ware TA, Hicks RP, Iwu MM, Skanchy DJ. Capillary electrophoresis determination of biflavanones from *Garcinia kola* in three traditional African medicinal formulations. Planta Med. 2002;68:440–444.
8. Eleyinmi AF, Bressler DC, Amoo IA, Sporns P, Oshodi AA. Chemical composition of bitter cola (*Garcinia kola*) seed and hulls. Pol. J. Food Nutr. Sci. 2006;15(4):395–400.
9. Ward JW, Elsea JR. Animal case and use in drug fate and metabolism. In: Edward RG, Jean LH (eds) Methods and techniques, 1st edn., New York: Markel Dekker; 1997.
10. Stein EA. Lipids, lipoproteins and apolipoproteins. In: Tietz, N. W. (Ed). Fundamentals of Clinical Chemistry. 3rd Edn., Philadelphia: W . B Saunders; 1987.

11. Tietz NW. Clinical Guide to Laboratory Test, Second Edition, Philadelphia: W.B. Saunders Company; 1990.
12. Wacnic RG, Alber JJ. A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholestsrol. J. Lipid Res. 1978;19:65-76.
13. Rifai N, Warmick GR, Remaley AT. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. Pages 402 – 430. In: Burtis CA, Ashwood ER, Bruns DE. (Eds.), Tietz Fundamentals of Clinical Chemistry, 6th ed. Missouri: Saunders Elsevier; 2008
14. Friedewald VT, Levy RI, Fredrickson DS. Estimation of low density lipoprotein cholesterol in plasma, without use of preparative centrifuge. Clin. Chem. 1972;18:499-502.
15. Krishnaiah D, Devi T, Bano A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants. Res. 2009;3(2):67-72.
16. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 2005;4(7):685-688.
17. Association of Official Analytical Chemist. Official Methods of Analysis, 15th Ed., Arlington, VA; 1990.
18. Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGM. Nutritional and Phytochemical Screening of *Garcinia kola*. Advance Journal of Food Science and Technology. 2012;4(1):9-14.
19. Bruneton J. Pharmacognosy, phytochemistry, medicinal plants (2nd edition) translated by Hatton CK. Paris: lavoisier publishing; 1995.
20. Iwu MM, Igboko AO, Okunji CO, Tempesta MS. Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. J. Pharm. Pharmacol. 1990;42:290-292.
21. Braide VP. Antihepatotoxic Biochemical effects of kola viron, a Biflavonoid of *Garcinia kola* seeds. Phytother. Res. 1991;5:35-37.
22. Rang HP, Dale MM, Ritter JM, Moore RK. Pharmacology 6th edition, Edinburgh: Churchill livingstone; 2007.
23. Fonseca VA, Rosenstock J, Wang AC, Truitt KE Jones MR. Colesevelam HCl improves glycemic control and reduces LDL cholesterol in patients with inadequately controlled Type 2 diabetes on sulfonylurea-based therapy. Diab. Care. 2008;31(8):1479–1484.
24. Odebunmi EO, Oluwaniyi OO, Awolola GV, Adediji OD. Proximate and nutritional composition of kola nut (*Cola nitida*), bitter cola (*Garcinia kola*) and alligator pepper (*Afromomum melegueta*). Afr. J. Biotechnol. 2009;8(2):308-310.
25. Evans WC. Trease and Evans Pharmacognosy. 16th ed. Edinburgh: Saunders Elsevier. 2009;16:87-89.

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