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Comparative Study on the Effects of Consumption of *Garcinia kola* and *Cola acuminata* on Some Biochemical Parameters of Wistar Albino Rats

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Abstract

This study investigated the comparative effects of consumption of *Garcinia kola* and *Cola acuminata* on some biochemical parameters of Wistar albino rats. Twenty albino rats weighing between 120-180g were divided into four groups. Group A rats fed with normal feed (control), Group B rats fed with 30% of *C. acuminata* diet, group C rats fed with 30% *G. kola* diet, group D rats fed with mixture of 15% *C. acuminata* and 15% *G. kola* diet. The diet was formulated by mixing homogenously 30% weight by volume (W/V) of *G. kola* powder and *C. acuminata* powder with 70% of normal rat feed. The proximate and phytochemical compositions of *G. kola* and *C. acuminata* used were evaluated using standard methods. The liver and kidney functions, haematological and lipid profiles were determined using standard biochemical methods. The proximate analysis shows that *G. kola* has a higher percentage of fat, protein and carbohydrate contents while *C. acuminata* has a higher percentage of moisture, ash and fibre contents. The result obtained for the phytochemical analysis revealed that *G. kola* contained a higher percentage of flavonoid, steroid, saponin, anthocyanine, phytate and tannin while *C. acuminata* has a higher percentage of alkaloids and cardiac glycosides. The results on the kidney function test suggests that *G. kola* and *C. acuminata* have no adverse effect on kidney. The results on the Lipid profile showed that daily consumption of *G. kola* and *C. acuminata* reduced the serum total cholesterol, the results on the liver function test showed that there was a significant ($P<0.05$) increase in the AST and ALT levels of group B and D when compared to control. While *G. kola* has no adverse effect on the liver. The result on haematological analysis showed that group B and D exhibited lower heamatological indices. While group C had no adverse effect on the heamatology. The results shows that, *G. kola* has more health and medicinal benefits compared to *Cola acuminata*.

Keywords: *Garcinia kola, Cola acuminata, phytochemical analysis, proximate analysis, biochemical parameters*

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1. Introduction

Garcinia kola is a largely cultivated forest tree indigenous to sub-Saharan Africa. In Africa, *G. kola* seed is commonly recommended in folklore medicine for the treatment of diabetes and its associated complications (Adedara and Farombi, 2012). Its seed administration significantly ameliorate hyperglycemia mediated damage by decreasing the blood glucose level, enhancement of the antioxidant system, inhibition of lipid peroxidation (Adedara and Farombi, 2012). In addition, *G. kola* seed intervention has been found to restore the kidney and liver function biomarkers, the sperm characteristics as well as the plasma levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), testosterone, Triiodothyronine (T_3), and Thyroxine (T_4) to normal in STZ-induced diabetic rats (Adedara and Farombi, 2013).

Bitter Kola (*G. Kola*) is popular in Southern Nigeria. The plant is extensively used in herbal medicine. It prevails as a multipurpose tree crop in the home gardens of Southern Nigeria Nzegbule and Mbakwe (2001). It could serve as raw material for pharmaceutical industries (Iwu *et al.*, 1989). The raw stem bark of *G. Kola* is purgative. The powdered bark is applied to malignant tumors. The sap is used for curing parasitic skin diseases. The latex or gum is used internally against gonorrhea, and applied externally on fresh wounds. The seeds prevent, relieve or cure headaches, chest colds, and suppressed cough and is often used in the treatment of Cirrhosis and hepatitis (inflammation of the liver) (Ogu and

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Agu, 1995). It contains flavonoid and tannins which are antioxidant properties (Braide, 1993). The seeds are highly valued as oral masticatory agent with bitter astringent taste and stimulant effect. They (as well as other plant parts) are used to treat wide range of diseases, including: gastric and liver disorders, diarrhoea, bronchial diseases, throat infections, colds, fever, malaria, and as an aphrodisiac (Erukainure *et al*. 2021)

Also, extracts of the plant have been traditionally used for treatment of ailments such as laryngitis, and cough (Ayensu, 1978). The seed also has anti-inflammatory, antimicrobial, antibacterial, antidiabetic and antiviral (Iwu *et al*., 1990). Various extracts of *G. kola* have been found to elicit a number of biochemical properties including hepatoprotection, antidiabetic properties and antigenotoxic potentials. The proximate composition of *G. kola* includes the following parameters (in g/100g); moisture content, crude protein, crude fibre, ash, crude fat, and carbohydrate. Its mineral compositions (ppm) include but unlimited to magnesium, zinc, iron, manganese, copper, lead, phosphorus, sodium, potassium and calcium (Adesuyi, 2012). Thus the ability of *G. Kola* to act as antioxidant in the cell underscores its role as an antioxidant and its potential role in the chemoprevention of chemically-induced genotoxicity (Drury *et al*., 1976). Every part of *G. kola* (bitter kola) is an important component in traditional herbal medicine worldwide (Dalziel, 1937).

G. kola is one of such plants that have been frequently used for its nutritional values. All parts of this plant including nut, leaf, stem bark and root have been mentioned in many ethnobotanical and pharmacological studies, although the nut remains the most used part.

G. kola is a species of flowering plant which belongs to a family of tropical plants known as Clusiaceae or Guttiferae (Plowden, 1972). It is a cultivated large forest tree, valued in most parts of West and Central Africa for its edible nuts (Hutchinson and Dalziel, 1956). The plant grows as a medium size tree, up to 12-14m high and produces reddish yellowish or orange coloured fruit (Okwu *et al.,* 2005; Adesanya *et al*., 2007). Each fruit contains 2-4 yellow nuts and a sour tasting pulp. *G. kola* has economic and cultural values across West and Central African countries where the nuts are commonly chewed and used for traditional ceremonies (Eleyinmi, 2006). The seeds contain kolaviron which is an active ingredient for many herbal formulations and have potential therapeutic benefits due to the activity of their flavonoids and other bioactive compounds (Akintonwa and Essien, 1990; Okunji, 2002). In fact, these nuts are sold in the local markets and also at the international level, and constitute an enormous source of incomes for many traders. They are also shared during dowry and wedding ceremonies.

Cola acuminata is a species in the genus Cola, of the family Malvaceae, native to tropical Africa. It is generally known for its fruit, the kola nut. *Ab initio* (from the beginning) it was used in the production of soft drinks like coca-cola and other beverages (Veronique *et al.,* 2016). Its characteristic height is about 13–20 metres (43–66 ft). *C. acuminata* has a bitter taste and high caffeine content (Benjamin *et al*., 1991). It is used traditionally as a caffeine stimulant and it is chewed in many West African cultures individually or in a group setting and is often used ceremonially (Sonibare *et al.,* 2009). Chewing *C. acuminata* can ease hunger cramps, stimulates digestion and also for euphoric purpose. Its effects are comparable to other xanthine containing herbs like cocoa and tea (Endrini *et al.,* 2011). It is thought to enhance alertness and physical energy, elevate mood, increase tactile sensitivity, and suppress appetite. It may also lower body temperature, blood pressure, and respiratory rate. The plant produces fruit pods containing seeds that are used to treat poisoning, digestive disorders (Warner *et al.,* 2007) and asthma (Odebunmi *et al*., 2009). Small doses are used to treat migraine, motion and morning sickness. In addition, it has been used to relieve inflammation disorders such as rheumatism and gout and has been administered to treat pneumonia and typhoid fever when great nervous irritability was present. *C. acuminata* is also used to treat diarrhea and has been used as a diuretic. Research shows that methanolic and aqueous extracts of *C. acuminata* seeds displayed antimicrobial activity. Investigation was done and it was found that mice whose mothers were exposed to the kola nut extract showed a decline in the rate of post natal body weight gain. But they experienced eye opening and hair gain relatively faster than their respective controls (Ajarem and Ahmad, 1994) implying that *C. acuminata* affects the utero development and the effects seem permanent. *C. acuminata* was also shown to exhibit a depressive effect on biphasic locomotive activity in male mice models at high concentrations (10 mg/kg). Results obtained from limited human trials infer that kola nut may have some weight loss properties, positive chronotropic and diuretic properties. Animal studies show analeptic and lipolytic (fat-burning) properties. More recent research shows that it stains the cytoplasm of various rat tissues which shows that it can be a suitable alternative for histological staining and so be more environmentally friendly and cost effective than synthetic dyes (Shehu *et al.,* 2012). The plant contains: moisture, ash, fat, protein, crude fiber and carbohydrate. Kola nuts are rich in xanthine alkaloids such as theobromine (up to 0.1%), caffeine (0.6% - 3.0%) and kolatin (Atawodi *et al.,* 2007). The plant is a source of other xanthine alkaloids, tannins, proanthocyanidins and anthocyanins such as “kola red”. The phenolics and anthocyanins are likely to provide antioxidant activity (Atawodi *et al.,* 2007) and the some of the active ingredients have been identified as non-steroidal compounds that are bioactive against prostate and breast cancer cells and so these compounds could be responsible for the observed bioactivity against breast and prostate cancer cell lines. Considering the enormous relevance of *G. kola* and *C. acuminata* in traditional medicine, this report is focused in order to evaluate the comparative study on the effects of consumption of *G. kola* and *C. acuminata* on some biochemical parameter of albino rats.

Materials and Methods

Materials

Plant materials

Bitter Kola and Oji Igbo used in this study was purchased from Osse market Onitsha, and was identified by a botanist.

Animals
Twenty (20) albino rats weighing between 120-180g were used for the study. The rats were obtained from the Department of Zoology, NnamdiAzikiwe University, Awka.

Normal rat feed
The normal rat feed used in this study is growers mash of Top feed, produced by Premier mill company Calabar, Nigeria

Equipment and Instrument used:
The following equipment were used:
8 Bucket Centrifuge (made by Lab Tech Medical Equipment, India with Model no. AD131), Micro Haematocrit centrifuge (made by Medifield Equiment and Scientific Ltd, England with Model no. YL8025HSL), Microscope (made by Wetzlar Microscope, Germany with Model no. V250), Neubaeur counting chamber, Spectrophotometer (made by Zenith Medical Equipment, England with Model no. S23A), Thermometer, Water bath (made by Medifield Equiment and Scientific Ltd, England with Model no. DK420), Weighing balance (Ohaus), kjehdah flask, oven (precision electro-thermal model BNP 9052 England), Whatman filter paper (NO.42), funnel, water bath (memmert).

Chemicals and reagents
The chemicals and reagents used in this study are of analytical grade. They are the product of Sigma Aldrich (Merck) Germany.

Methodology
Bitter kola powder preparation
Bitter kola seed was washed with water, sliced into small pieces and sun dried. It was then ground into powdered form using electric blender

Oji Igbo powder preparation
Oji Igbo seed was washed with water, sliced into small pieces and sun dried. It was then grinded into powdered form using electric blender

Bitter kola diet formulation
This was formulated by mixing homogenously 30% weight by volume (W/V) of bitter kola powder with 70% of normal rat feed.

Oji Igbo diet formulation
This was formulated by mixing homogenously 30% weight by volume (W/V) of ojiigbo powder with 70% of normal rat feed.

Experimental design
Twenty (20) albino rats weighing between 120-180g were used for the study. The rat was obtained from the department of Zoology, Nnamdi Azikiwe University, Awka. They were then divided into 4 groups:

Group	Diet
A	Rats fed with normal feed (control)
B	Rats fed with 30% of orji igbo diet
C	Rats fed with 30% bitter kola diet
D	Rats fed with mixture 15% orji Igbo and 15% bitter kola diet

All the animals had access to water (*ad libitum*) for 30 days. All the protocols as approved by Institutional Animal Ethics Committee (IAEC) were observed in this study. At the end of the 30 days feeding experiment, the rats were bled from the retro-bulbar plexus canthus of the eye. The blood collected was centrifuged and the serum separated from the cells. Plasma blood sample was collected into EDTA bottle.

Methodology on Proximate Analysis
Moisture content
Procedure: A petri-dish was washed and dried in the oven. Exactly 2g of the sample was weighed into petri dish. The weight of the petri dish and sample was noted before drying. The petridish and sample were put in the oven and heated at 100^0c for 1hr the result noted and heated another 1hr until a steady result is obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained.

$$\%\,Moisture\ content = \frac{W_1 - W_2}{Weight\ of\ sample} x100$$

Where W_1 = weight of petridish and sample before drying
W_2 weigh of petridish and sample after drying.

Carbohydrate determination
(Differential method)
100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

Ash content (AOAC, 1984)
Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.
Procedures: Empty platinum crucible was washed, dried and the weight was noted. Exactly 2g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 500^0c for 3 hours. The sample was cooled in a desiccator after burning and weighed.
Calculations

$$\%\,Ash\ content = \frac{W_3 - W_1}{W_2 - W_1} x\ 100$$

Where, W_1 = weight of empty platinum crucible; W_2 = weight of platinum crucible and sample before burning; W_3 = weight of platinum and ash.

Crude fibre
Procedure: About 2g of material was defatted with petroleum ether (if the fat content if more than 10%). It was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The solution was filtered through linen or several layers of cheese cloth on a fluted funnel. It was then washed with boiling water until the washings are no longer acid. The residue was transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible and then dried in an electric oven and weighed, it was then Incinerated, cooled and weighed.
The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\%Crude\ fibre = \frac{Weight\ of\ fibre}{Weight\ of\ sample} x100$$

Crude fat

Soxhlet Fat Extraction Method

This method is carried out by continuously extracting a food with non- polar organic solvent such as petroleum ether for about 1 hour or more.

Procedure: Two hundred and fifty millilitre (250ml) of clean boiling flasks was dried in oven at 105 - 110^{0}c for about 30 minutes. It was then transferred into a desiccator and allowed to cool. It was weighed correspondingly labeled, cooled boiling flasks. The boiling flasks were filled with about 300ml of petroleum ether (boiling point 40^{0}c - 60^{0}c). The extraction thimble was plugged with cotton wool. The Soxhlet apparatus was assembled and allowed to reflux for about 6 hours. Thimble was removed with care and petroleum ether was collected in the top container of the setup and drained into a container for reuse. When flask was almost free of petroleum ether, it was removed and dried at 105ºC - 110ºC for 1hour. It was then transferred from the oven into a desiccator and allowed to cool, then weighed.

Crude proteins (AOAC, 1984)

Principle: The method is the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline, and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

Procedures: Exactly 0.5g of sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling about 100ml of distilled water was added to avoid caking and then 50ml was transferred to the kjedahl distillation apparatus. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. The 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

% Nitrogen =Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6.25

Methodology on Phytochemical Analysis

Oxalate determination by titration method

This determination involves three major steps digestion, oxalate precipitation and permanganate titration

Digestion

Two grams (2g) of sample was suspended in 190ml of distilled water in a 250ml volumetric flask. Ten microlitre (10ml) of 6m HCl is added and the suspension digested at 100ºC for 1 hour, Cooled, and then made up to 250ml mark before filtration.

Oxalate precipitation

Duplicate portions of 125ml of the filtrate were measured into beakers and four drop of methyl red indicator added. This was followed by the addition of NH_4OH solution (drop-wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion was then heated to 90^{0}c, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90ºC and 10ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25ºC. The solution was then centrifuged at 2500rpm for 5minutes. The supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2S0_4 solution.

Permanganate titration

At this point, the total filtration resulting from digestion of 2g of the sample was made up to 300ml. Aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMNO_4$ solution to a faint pink colour which persists for 30s. The calcium oxalate content was calculated using the formula

$$\%Oxalate = \frac{Tx(Vme)(Df)x10^5(mg/100g)}{(ME)x(Mf)}$$

Where T is the titre of $KMn0_4$(ml), Vme is the volume-mass equivalent (i.e. 1ml of 0.05m $KMn0_4$ solution is equivalent to 0.00225g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300ml) and A is the aliquot used (125ml), ME is the molar equivalent of $KMn0_4$ in oxalate ($KMn0_4$ redox reaction) and Mf is the mass of sample used (Harborne, 1993).

Alkaloids determination

Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25ºC. This was filtered with filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven (precision electrothermal model BNP 9052 England) at 80ºC. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed (Harborne, 1993; Obadoni and Ochuka, 2001).

Calculation:

$$\%\ alkaloid = \frac{Weight\ of filter\ paper\ with\ residue - Weight\ of\ filter\ paper}{Weight\ of\ sample\ analyzed} x100$$

Flavonoids determination

Ten grams (10g) of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a waterbath and weighed to a constant weight (Boham and Kocipai, 1994).

Calculation:

$$\%flavonoids = \frac{Weight\ of\ crucible + residue - Weight\ of\ crucible}{Weight\ of\ sample\ analyzed} x100$$

Determination of saponin

Exactly 5g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a waterbath at 50ºC for 24hours. This was filtered and the extract was concentrated using a waterbath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage (Obadoni and Ochuko, 2001).

Calculation:

$$\%\ saponin\ content = \frac{(Weight\ of filter\ paper + residue) - (Weight\ of\ filter\ paper)}{Weight\ of\ sample\ analyzed} x100$$

Cardiac glycosides determination

Wang and Filled method was used. To 1ml of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50ºC till dryness and weight of the filter paper with residue was noted.

The cardiac glycoside was calculated in %.

Calculation:

$$\%\ cardiac\ glycoside = \frac{(Weight\ of filter\ paper + residue) - (Weight\ of\ filter\ paper)}{Weight\ of\ sample\ analyzed} x100$$

Determination of anthocynanin in the water of life using the gravimetric method of Harborne, 1973

Principle: Acid hydrolysed sample when filtered reacts with ethylacetate to enable extraction of anthocyanin. Upon addition of amyl alcohol, anthocyanin was extracted and after drying, the percent composition was determined in relation to weight of original sample gravimetrically.

Procedure: Five grams (5.0g) of the sample was boiled in 100ml of 2MHCl for 30minutes. The hydrolysate was filtered using Whatman filter paper. The filterate was transferred into separation funnel and equal volume of ethylacetate added, mixed and allowed to separate into two layers. The ethylacetate layer was recovered while the aqueous layer was discarded. The extract was dried over a steam bath. The dry extract was then treated with 50ml of conc. Amylalcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin was determined and expressed as percentage of original sample.

$$Calculation\ (g(\%)) = \frac{Weight\ of\ Anthocyanin}{Weight\ of\ original\ sample} x100$$

Determination of steroid content

One gram (1.0g) of the sample was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. A 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. Then 3ml of ice cold acetic anhydride was added to the mixture in the flask. A 2 drops of (200mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standard and test was measured, zeroing the spectrophotometer with blank at 420nm.

$$Calculation\ (mg/100ml) = \frac{Absorbance\ of\ test}{Absorbance\ of standard} x\ Conc.of\ standard$$

Phenol determination

The quantity of phenols is determined using the spectrophotometer method. The sample is boiled with 50ml of $(CH_3CH_2)_20$ for 15min. 5ml of the boiled sample is then pipette into 50ml flask, and 10ml of distilled water is added. After the addition of distilled water, 2ml of NH_4OH solution and 5ml of concentrated $CH_3(CH_2)_3CH_2O$ His added to the mixture. The samples is made up to the mark and left for 30min to react for colour development and measured at 505nm wavelength using spectrophotometer.

Tannin determination by follins dennis titration

The follins dennis titrating method as described by Pearson (1974) was used. To 20g of the crushed sample in a conical flask was added 100mls of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filter ate collected. 25ml of NH_4OH were added to the filter ate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M NaOH using phenolphthalyne as indicator until a pink end point is reached. Tannin content was then calculated in % ($C_1V_1 = C_2v_2$) molarity.

Calculation:

C_1 = conc. of Tannic Acid

C_2 = conc. Of Base

V_1 = Volume of Tannic acid

V_2= Volume of Base

Therefore

$$C_1 = \frac{C_2 V_2}{V_1}$$

$$\%\ \text{of tannic acid content} = \frac{C_1 x100}{Weight\ of\ sample\ analyzed}$$

Phytate determination

Phytate contents were determined using the method of Young and Greaves (1940) as adopted by Lucas Markakes (1975). About 0.2g of each of the sample was weighed into different 250ml conical flasks. Each sample was soaked in 100ml of 2% concentrated HCl for 3hr, the sample was then filtered. 50ml of each filtrate was placed in 250ml beaker and 100ml distilled water added to each sample. Ten milliliter (10ml) of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (111) chloride solution which contained 0.00195g iron per 1ml.

$$\text{Phytic acid} = \frac{\text{Titre value x 0.00195 x 1.19 x 100}}{Weight\ of\ sample}$$

Methodology on Kidney Profile Test

Determination of urea

Method: Berthelot method.

Principle: Urea in serum is hydrolysed to ammonia in the presence of the urease. The ammonia reacts with phenol and hypochlorite in alkaline medium to form indophenol. The intensity of the colour indicates the concentration of urea in the sample. Nitroprusside is used to catalyse the reaction. This indophenol is then measured photometrically.

Procedure: About 0.01ml of serum was added to test, 0.01ml of urea standard was added to standard and 0.01ml of distilled water, added to blank. Following these additions, 0.1ml of urea reagent 1 was added to all the tubes, mixed thoroughly and incubated at 37ºC for 10 minutes thereafter, 2.5mls of urea reagent 2 and 3 were each added to all the tubes, mixed thoroughly and incubated at 37ºC for 15 mins and read at 540nmλ after blanking the spectrophotometer with the reagent blank.

Determination of creatinine

Method: Jaffe colorimetric method.

Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Procedure

A working reagent composed of equal volumes of Reagent A and B (Alkaline solution and Picric acid solution) was prepared. For each determination, 1ml of Reagent A was mixed with 1ml of Reagent B to give 2mls of working reagent for control and test. 0.1ml of standard serum was added to the tube marked standard, 0.1ml of the test serum was added to the tube marked test. 1ml of the working reagent was added to the tubes. The tubes were mixed and the absorbance of the tubes were read and noted within 30seconds. The absorbance of the tubes were read for the second time at exactly 2minutes. The tubes were read at a wavelength of 500nm.

Determination of bicarbonate

Method: Enzymatic method for the determination of CO_2 in serum.

Principle: Phosphoenol Pyruvate + HCO_3^- PEPC → Oxalate + H_2PO_4

Oxalate + NADH MDH → Malate + NAD

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to CO_2 concentration in the sample.

Procedure: One millilitre (1.0 ml) of CO_2 reagent was delivered into test tubes marked blank, standard and sample. The tubes were incubated for 3minutes at 37^{0}C. The spectrophotometer wavelength was set at 340nm. 5µl of water, standard and serum sample was added to the tubes marked blank, standard and sample respectively. The tubes were mixed gently by inversion and incubated for 5minutes at 37^{0}C. The absorbance of the tubes were read and recorded at 340nm.

Determination of chloride

Method: Colorimetric method.

Principle: $Hg(SCN)_2 + 2\,Cl^- \rightarrow HgCl_2 + 2SCN^-$

$3SCN + Fe^{3+} \rightarrow 4Fe(SCN)_3$ red complex

Chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colored complex that absorbs light at 480nm. The intensity of the color produced is directly proportional to the chloride concentration.

Procedure: One and half milliliter (1.5ml) of chloride reagent was delivered into test tubes marked blank, standard and sample. 10µl of water, standard and sample was added to their respective test tubes and mixed. The tubes were incubated at room temperature for five (5) minutes. The spectrophotometer was set at 480nm and zeroed with the reagent blank. The absorbance of the tubes were read and recorded.

Determination of sodium

Method: Colorimetric method.

Principle: The method is based on the reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly as the concentration of sodium in the test specimen.

Procedure: One thousand microlitres (1000µl) of sodium reagent was delivered into the test tube marked blank, standard and test. Ten microlitres (10µl) of distilled water was added to the tube marked blank, 10µl of standard serum was added to the test tube marked standard and 10µl of the sample was added to the test tube marked sample. The tubes were mixed and incubated at 37ºC for 10 minutes. The absorbance of the blank, standard and sample were read at 630nm.

Determination of potassium

Method: Colorimetric method.

Principle: The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is directly proportional to potassium concentration in the range of 2 – 7 mEq/ L.

Procedure: One thousand microlitres (1000µl) of Potassium reagent was delivered into test tubes marked blank, standard and test. Ten microlitres (10µl) of water was added to the blank test tube, 10µl of standard was added to the standard test tube and 10µl of test sample was added to the sample test tube. The tubes were mixed and allowed to sit at room temperature for 3minutes. The absorbance of all the tubes were read at 500nm.

Liver Function Test Manual Method

Determination of alanine aminotransferase (ALT)

The ALT was assayed using the method of Reitman and Frankel (1957) as outlined in Randox Kit.

Principle

α-oxoglutarate + L-Alanine GPT → L-Glutmate + Pyruvate

Alanine Aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine

Procedure: About half milliliter (0.5ml) of ALT substrate was added to Test, Test Blank, Standard, and Standard Blank. This was incubated for 5mins at 37ºC. Thereafter, 0.1ml of serum was added to Test, 0.1ml of pyruvate standard added to

Standard and 0.1ml of Distilled water added to test blank and standard blank. They were mixed and incubated at 37ºC for 30minutes. After the incubation, 5.0mls of 2,4dinitrophenylhydrazine was added to all the tubes, mixed and incubated for 20min at room temperature (25ºC). This was followed by the addition of 5.0mls of 0.4N NaOH to all the tubes and read at 505nmλ after zeroing the spectrophotometer with the blank. The results were then read off using the calibration curve provided.

Determination of aspartate aminotransferase (AST)

The AST was determined using the method of (Reitman and Frankel, 1957) as outlined in Randox Kit.

Principle

α-oxoglutarate +L aspertate GOT ⟶ L-glutamate + oxaloacetate

AST was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Procedure: About half milliliter (0.5ml) of AST substrate was added to Test, Test Blank, Standard, and Standard Blank. This was incubated for 5mins at 37ºC. Thereafter, 0.1ml of serum was added to Test, 0.1ml of pyruvate standard added to Standard and 0.1ml of Distilled water added to test blank and standard blank. They were mixed and incubated at 37ºC for 30minutes. After the incubation, 5.0mls of 2,4dinitrophenylhydrazine was added to all the tubes, mixed and incubated for 20min at room temperature (25ºC). This was followed by the addition of 5.0mls of 0.4N NaOH to all the tubes and read at 505nmλ after zeroing the spectrophotometer with the blank. The results were then read off using the calibration curve provided.

Determination of alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) was determined using Randox kit as recommended by Deutsche Gesellschaft fur KlinischeChemmie (GSCC)

Principle

P-nitrophenylphosphate + H_2O ALP ⟶ Phosphate + P-nitrophenol (a coloured chromogen)

Procedure: To Test, blank and Standard, was added 1.0ml of Alkaline buffer and phenyl phosphate substrate. They were incubated for 3min at 37ºC. Thereafter 0.1ml of serum was added to test, 0.1ml of phenol standard was added to standard and 0.1ml of distilled water was added to blank they were equally incubated for another 15mins at 37ºC. following the incubation, 1.0ml of 0.5N NaOH, 1.0ml of 0.5N $NaHCO_3$, 0.1ml of 4-amino antipyrine, 0.1ml of potassium ferricyanide were each added to all the tubes, mixed and read immediately after zeroing the spectrophotometer with blank at 510nmλ wavelength.

Determination of bilirubin

The concentrations of conjugated and unconjugated bilirubin were determined using the method of (Jendrassik and Grof, 1938) as outlined in Randox Kit.

Principle

Colorimetric method: Direct (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Procedure for total bilirubin (T BIL)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of Sodium nitrite was added into the sample tube. Then 1000 µl of caffeine was added to both sample blank and sample tube. Also 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 20 – 25ºC. Then 1000 µl of tartrate was added to both sample blank and sample tubes. The tubes were mixed and were incubated for 5- 30 minutes at 25ºC and then, the absorbance of the sample blank was read at wavelength of 578nm.

Procedure for direct bilirubin (D BIL)

The sample blank and sample tubes were set for the analysis. About 200 µl of sulphanilic acid was put into the sample blank and sample tubes. Then 50 µl of Sodium nitrite was added into the sample tube. Then 2000 µl of sodium chloride was added to both sample blank and sample tube. Also 200 µl of the sample was added to both sample blank and sample tubes. The tubes were mixed and allowed to stand for 10 minutes at 20 – 25ºC.

The absorbance of the sample was read at wavelength of 546nm against the sample blank

Lipid Profile Manual Method

Determination of serum total cholesterol

Method: CHOD PAP method using Randox kit.

Principle: Free and esterified cholesterol in the sample originates by means of the coupled reactions described below. A coloured complex that can be measured spectrophotometrically is formed. The intensity of the colour indicates the concentration of cholesterol in the sample.

Cholesterol ester + H_2O Chol. esterase ⟶ Cholesterol + Fatty acid

Cholesterol + ½O_2 + H_20 Chol. Oxidase ⟶ Cholestone + H_20_2

2H_2O_2 + 4-Amino antipyrine + Phenol peroxidase ⟶ Quinoneimine + 4H_20.

Procedure: About 0.01ml of serum was added to test, 0.01ml of Cholesterol standard was added to standard and 0.01ml of distilled water, added to blank. Following these additions, 1.0ml of cholesterol reagent was added to all the tubes, mixed thoroughly and incubated at 37ºC for 10 minutes and read at 500nmλ after blanking the spectrophotometer with the reagent blank.

Calculation:

Conc. of Cholesterol (mmol/L) = Abs. Test/ Abs. Standard x Conc. of std

Determination of triglyceride

Method: Enzymatic method using Randox kit.

Principle: Triglyceride are determined after enzymatic hydrolysis with lipases. The indicator is a quinonemine formed from hydrogen peroxidase, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The intensity of the colour indicates the concentration of the triglyceride in the sample and can be measured spectrophotometrically.

Triglycerides + H_2O Chol. Lipases ⟶ Glycerol + Fatty acids.
Glycerol+ ATP Glucose Kinase ⟶ Glycerol-3-phosphate + ADP
Glycerol-3-phosphate+ O_2 GlucosePeroxidase ⟶ Dihydroxyacetone + phosphate +H_2O_2
$2H_2O_2$ + 4-aminophenazone + 4-chlorophenol

Procedure: About 0.01ml of serum was added to test, 0.01ml of triglyceride standard was added to standard and 0.01ml of distilled water, added to blank. Following these additions, 1.0ml of triglyceride reagent was added to all the tubes, mixed thoroughly and incubated at 37ºC for 10 mins and read at 500nmλ after blanking the spectrophotometer with the reagent blank.

Determination of HDL cholesterol
Method: Enzymatic method using Randox kit.
Principle: Very low density (VLDL) and low density lipoprotein (LDL) in the sample precipitate with phospho-tung state and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL Cholesterol reacts by means of the coupled reactions described below. The intensity of the colour indicates the concentration of the cholesterol in the sample which can be measured spectrophotometrically.
Cholesterol ester + H_2O Chol. esterase ⟶ Cholesterol + Fatty acid
Cholesterol + ½O_2 + H20 Chol. Oxidase⟶ Cholestenone + H_20_2
$2H_2O_2$ + 4-Aminoantipyrine + DCFS Peroxidase Quinoneimine + $4H_2o$.
Procedure:
Stage 1: About 0.2ml of sample was pipetted into labelled centrifuge tubes. To it was added 0.5ml of HDL precipitant reagent they were mixed thoroughly and stood for 10minutes at room temperature (25ºC) thereafter, centrifuged at 4000rpm for 10 minutes.
Stage 2: About 0.05ml of supernatant from stage one was added to test, 0.05ml of HDL standard was added to standard and 0.05ml of distilled water, added to blank. Following these additions, 1.0ml of HDL cholesterol reagent was added to all the tubes, mixed thoroughly and incubated at 37ºC for 10 mins and read at 500nmλ after blanking the spectrophotometer with the reagent blank.

Determination of LDL cholesterol
Method: Enzymatic method using Biosystem kit.
Principle: Low density lipoprotein (LDL) in the sample is precipitated by heparin at their isoelectric point (PH 5.04). After centrifugation the high density lipoproteins (HDL) and the Very low density lipoproteins (VLDL) remain in the supernatant. These can be determined by enzymatic method. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation.
Procedure
Stage 1: About 0.4ml of sample was pipetted into labeled centrifuge tubes. To it was added 0.2ml of LDL precipitant reagent they were mixed thoroughly and stood for 15minutes at room temperature (25ºC) thereafter, centrifuged at a minimum of 4000rpm for 10 minutes.
Stage 2: About 0.02ml of supernatant from stage one was added to test, 0.05ml of LDL standard was added to standard and 0.02ml of distilled water, added to blank. Following these additions, 1.0ml of cholesterol reagent was added to all the tubes, mixed thoroughly and incubated at 37ºC for 10 mins and read at 500nmλ after blanking the spectrophotometer with the reagent blank.

Methodology on Full Blood Count (Manual Method)
Determination of packed cell volume
Principle: When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.
Procedure: Using micro haematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until appropriately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with Bunsen flame and placed in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the open end towards the center. The lid was replaced and centrifuged for five minutes at 11,000rpm. The tubes were removed as soon as the centrifuge had stopped spinning. And the value of the packed cells was read off using the microhaematocrit reader.

Determination of haemoglobin concentration
Principle: When whole blood is added to Drabkin's reagent: a solution containing KCN (Potassium cyanide) and (Potassium ferricyanide) $K_3Fe(CN)_6$, KCN converts Hb-Fe^{2+} (ferrous) to Hb-fe^{3+} (ferric) state to form methaemoglobin which then reacts with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540nm (or using a yellow-green filter). The optical density (OD) of the solution is proportional to the haemoglobin concentration.
Procedure: Using Cyanmethaemoglobin method, exactly 5.0ml of Drabkin's reagent was pipetted into two test tubes 1 and 2. A well-mixed sample of EDTA blood (0.02ml) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions were well mixed and allowed to stand at 25ºC for 10 minutes in order to allow the formation of Cyan – met-haemoglobin. The mixtures were transferred into cuvettes and read in a spectrophotometer at a wavelength of 540nm. The Drabkin's reagent in tube 1 was used as the blank (setting the percentage transmittance at 100%). The readings from each tube were recorded and the actual Hb values in g/dl were determined from a pre- calibrated chart.

Determination of White Blood Cells (WBCS)
Principle: When whole blood is mixed with weak acid solution such as glacial acetic acid solution, it dilutes the blood and haemolyses the RBCs, enabling the WBCs to be counted.
Procedure: The blood specimen was mixed approximately for one minute, using the white blood cell pipette, blood will be drawn to the 0.5mark in the pipette. Blood was removed from the outside of the pipette with clean gauze. The tip of the pipette was placed into the counting diluting fluid to draw it slowly until it reached the 11 mark. The counting chamber and

the cover glass were cleaned with a cloth. The counting chamber was filled with diluted blood. The four corners of the chamber was visualised under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Determination of Red Blood Cells (RBCs)

Principle: To facilitate counting, whole blood is diluted with Gower's solution which hemolyze white blood cell and prevent red blood cell lysis.
Procedure: 1:200 dilution was made by diluting 20µl of EDTA anticoagulated blood in 3.98ml of Gower's solution and mixed for 3 minutes. The counting chamber and cover glass was cleaned appropriately. Ten microlitre of the diluted fluid was used to both chambers of the haemocytometer avoiding air bubble, it was allowed to stand for 3minutes prior to counting. The haemocytometer was carefully placed on the microscope stage, the condenser on the microscope was lowered and the chamber was scanned using 10X objective lens. The cells were counted using the 40X objective lens.
NOTE: Gower's solution contains sodium sulphate 12.5g, glacial acetic acid 33.3ml and distilled water 100ml.

Determination of differential cells

Principle: A drop of blood is smeared on a slide, stained and examined under the microscope, to establish the morphology of red blood cells, leucocytes and platelets and the relative frequency of different leucocytes. The slide is stained with one of the Romanowsky stains (Leishman stain).
Procedure: A drop of well mixed anticoagulated blood was placed on a clean, grease free slide, using a spreader the blood was smeared on the slide and allowed to air dry. The slide was flooded with Leishman stain and allowed to stand for 2minutes. The stain was diluted with twice its volume of buffered distilled water. It was mixed by blowing air gently on the stain to ensure uniform mixing. The stain was allowed to stand for 8minutes. Excess stain were rinsed off with buffered distilled water, the back of the slide was wiped to remove all traces of the stain. The slide was drained and stood upright in a draining rack to dry. The slide was examined microscopically with 100X oil immersion objective lens

Results

Proximate Analysis

Results shows that *Garcinia kola* has higher levels of fat content, protein content and carbohydrate content while *Cola acuminata* has higher levels of moisture content, ash content, and fibre content (fig. 1)

Phytochemical Analysis

Results showed that *G. kola* has higher content of phenol, flavonoid, Steroid, Saponin, oxalate, anthocyanine, phytate and tanin when compared to *C. acuminata*. While *C. acuminata* has higher content of alkaloid and cardiac glycosides when compared to *G. kola* (fig. 2)

Kidney Function Test

Kidney function tests of albino rats administered with *G. kola* and *C. acuminate* were shown in Table 2. The results revealed that the urea level of the albino rats showed a significant ($P<0.05$) decrease in all instances of the treatment group. The creatinine level Group D albino rats was significantly ($P<0.05$) higher than Groups A. The chloride level of the albino rats showed a significant ($P<0.05$) decrease in Group C and Group D when compared to control. The sodium level showed no significant ($P<0.05$) increase in all instances of treatment when compared to control. There was no significant ($P<0.05$) increase in the potassium level of all instances of treatment group.

Liver Function Test

Liver function Test of albino rats administered with *G. kola* and *C. acuminata* were shown in Table 1. The results revealed that the direct bilirubin levels of albino rats fed with group B was higher than rats fed with Groups A. There was a significant ($P<0.05$) increase in the total bilirubin levels of group B when compared to control. The alkaline phosphatase level of Group B and Group C were significantly higher than rats fed with Groups A.

Lipid Profile Test

The results revealed that the cholesterol levels of albino rats fed with Group B, C and D were significantly ($P<0.05$) lower than Group A. There was no significant ($P<0.05$) difference in Triglyceride levels of albino rats in all instances of the treatment groups. There was no significant ($p<0.05$) difference in the HDL level of Group B, Group C and Group D when compared to control. There was a significant ($p<0.05$) decrease in the LDL levels of Group B and Group C when compared to control (Fig. 3).

Haematological Analysis

The result showed that group B and group D exhibited lower PCV (%), Hb (g/dL), RBC (mm^3) and exhibited higher WBC (mm^3). The Hb level was higher in group C (13.3±0.00 g/dL) when compared to control. There was no significant ($P>0.05$) increase in the Hb, PCV and WBC levels in all instances of treatment group. There was a significant ($P<0.05$) increase in the RBC level of Group C compared to controls.

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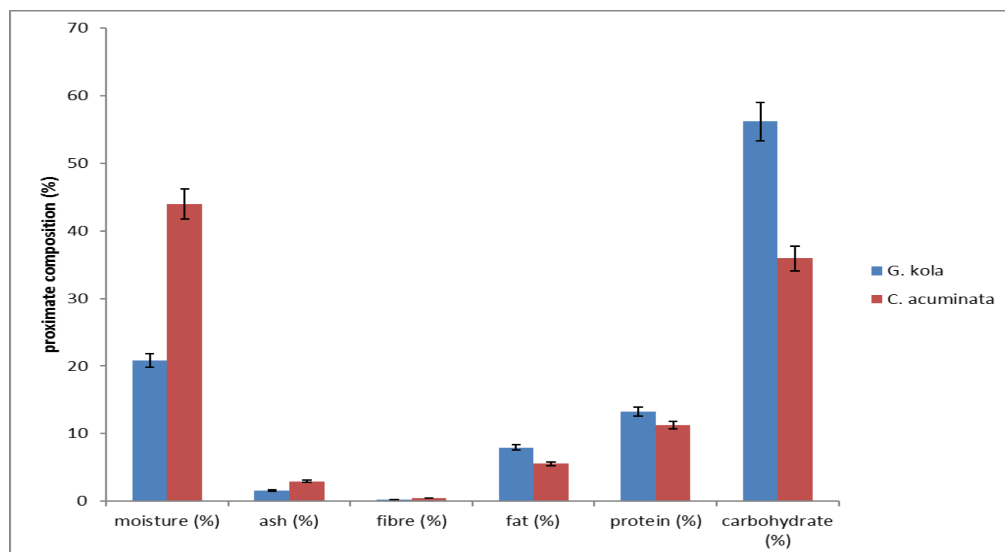

Figure 1: Proximate composition of *G. kola* and *C. acuminate*

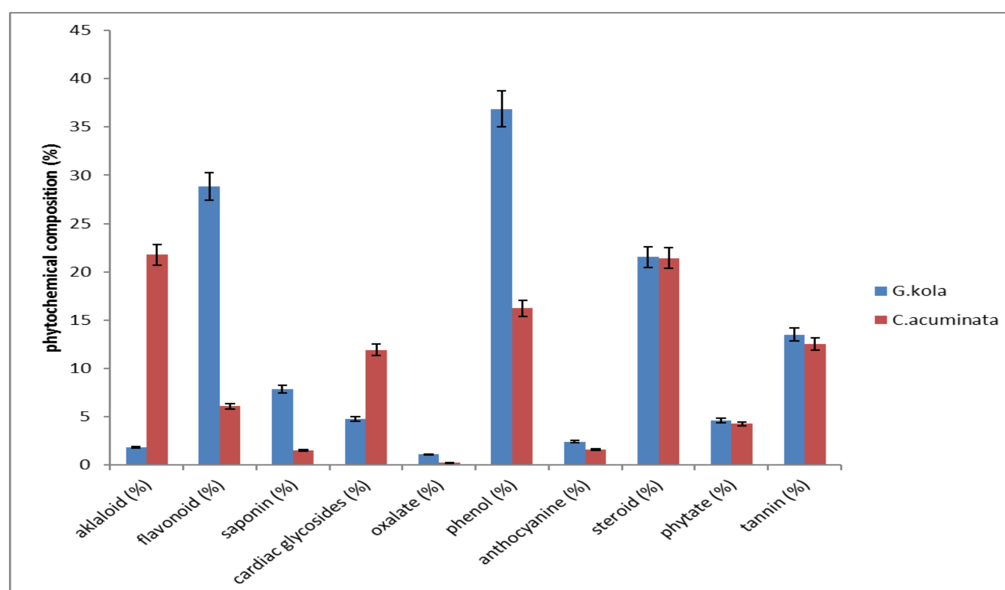

Figure 2: Phytochemical composition of *G. kola* and *C. acuminate*

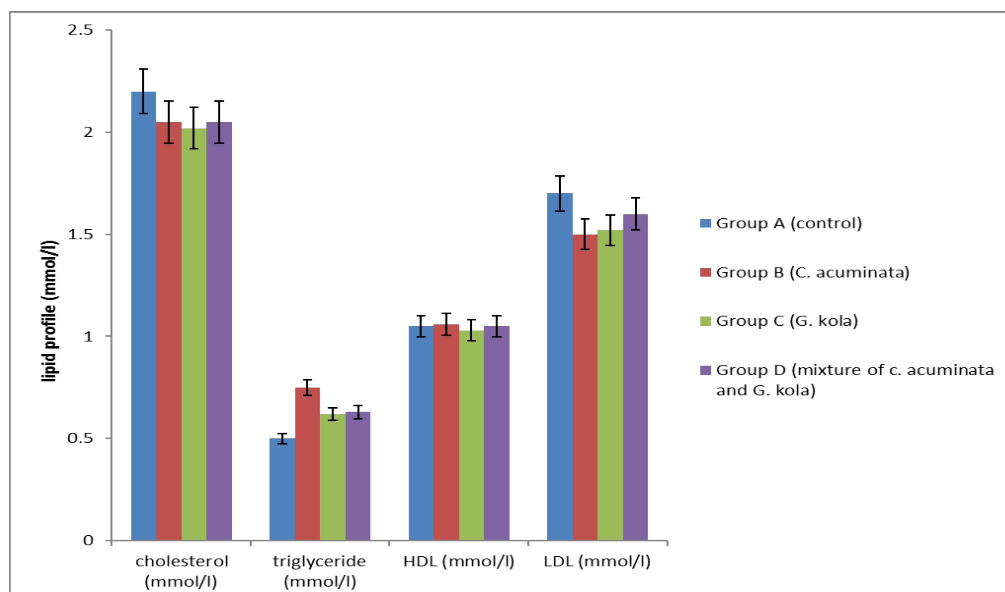

Figure 3: Lipid profile Test of albino rats administered with *G. kola* and *C. acuminate*

Table 1: Liver Function Test of albino rats administered with *G. kola* and *C. acuminate.*

Groups	Direct bilirubin (µmol/l)	Total bilirubin (µmol/l)	Alkaline phosphatase (IU/L)	Aspartate aminotransferase (IU/L)	Alanine transaminase (IU/L)
A	2.10±0.00	17±.00	292±0.00	3±0.00	2±0.00
B	2.20±0.01	20±0.05*	409±0.10*	5±2.00*	4±0.01*
C	2.10±0.00	17±0.00	347±0.00*	4±0.00	3±0.10
D	2.10±0.00	17±0.00	284±0.00	5±0.50*	4±0.50*

Values represent mean ± SD of the liver function levels determined

Table 2: Kidney function tests of albino rats administered with *G. kola* and *C. acuminata*

Groups	Urea (mmol/l)	Creatinine (µmol/l)	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Bicarbonat e (mEq/l)
A	9.3±0.00	34±0.00	93±0.00	4.2±0.01	100±4.15	23±0.00
B	7.1±0.00	34±0.00	95±0.00	4.4±0.03	90±0.00	21±1.00
C	4.8±0.00*	34±0.00	98±0.00	4.3±0.05	80±1.15*	23±0.23
D	7.9±0.00	103±4.45*	98±0.00	3.8±0.00	77±0.00*	21±0.00

Values represent mean ± SD of the kidney function levels determined

Table 3: Haematological analysis of albino rats administered with *G. kola* and *C. acuminata*

Group	Hb (g/dl)	PCV (%)	WBC (mm^3) ($\times 10^3$)	RBC (mm^3) ($\times 10^{12}$)
A	13.0±0.05	39±0.50	4.2 ±0.00	5.1 ±0.02
B	12.6±0.00	38±0.00	4.3 ±0.00	4.6 ±0.00
C	13.3±0.00	40±0.00	4.1±0.00	5.3 ±0.00*
D	12.6±0.20	38±0.00	4.3 ±0.00	4.6 ±0.13

Values represent mean ± SD of the haematological indices determined

Discussion

This research work was carried out to evaluate the comparative effect of the daily consumption of *Garcinia cola* and *Cola acuminata* on some biochemical parameters of albino rats.

Proximate composition

The results obtained for the proximate analysis of *G. Kola* and *C. acuminata* were presented in Fig.1. The result revealed that the presence of constituent of *G. Kola* are moisture content (20.83%), ash content (1.57%), fibre content (0.19%), fat content (7.94%), protein content (13.30%), carbohydrate content (56.17%). The percentage composition of the constituent of *C. acuminata* are moisture content (43.99%), ash content (2.93%), fibre content (0.45%), fat content (5.52%), protein content (11.20%), carbohydrate content (35.92%). A comparison between *G. Kola* and *C. acuminata* showed significance difference in moisture content and carbohydrate content. Results shows that *G. kola* has higher levels of fat content, protein content and carbohydrate content while *C. acuminata* has higher levels of moisture content, ash content and fibre content. This suggests that *G. Kola* could serve as a better source of energy generation than *C. acuminata* because of its higher composition of fats and carbohydrate. Since fats and carbohydrates are noted as good sources of energy when metabolized (Olusanya, 2008). Also results in Figure 1 revealed that *G. kola* used in this study has lower moisture content than *C. acuminata*. This suggests that *G.kola* used could be more durable than *C. acuminata* (Olusanya, 2008). The result in Figure 1 also revealed that *C.acuminata* has higher composition of fibre than *G. kola*. This suggests that *C. acuminata* diet could be more useful in weight control than *G. kola*, since according to Olusanya (2008) a diet high in fibre aids weight control and reduces the risk of developing obesity.

Phytochemical analysis

The result obtained for the phytochemical analysis of *C. acuminata* and *G. kola* were presented in Figure 2. Results shown revealed that *G. kola* contained a higher level of flavonoid (28.84%), compared to *C. acuminata* (6.07%), steroid were found higher in *G. kola* (21.54%), when compared to *C. acuminata* (21.42%), saponin were found higher in *G. kola* (7.88%) compared to *C. acuminata* (0.20%), anthocyanine was higher in *G. kola* (2.38%) when compared to *C. acuminata* (1.55%), phytate were found higher in *G. kola* (4.59%) compared to *C. acuminata* (4.25%), tannin were found higher in *G. kola* (13.50%) when compared to *C. acuminata* (12.5%). Alkaloids were found higher in *C. acuminata* (21.76%) compared to *G. kola* (1.82%), cardiac glycosides were found higher in *C. acuminata* (11.92%) compared to *G. kola* (4.79%). The results are almost similar to the findings of (Ukaoma *et al.,* 2013) who analysed quantitatively the bioactive components of *G. kola* seed extract. Results in Figure 2 revealed that *G. kola* has significantly higher ($P<0.05$) composition of phytate, anthocyanine, tannin, flavonoid and saponin. This suggests that *G. kola* could be more useful in mopping up free radicals and lowering the cholesterol level/high blood pressure than *C. acuminata*, since according to Egbuna *et al* (2019) tannin, flavonoid and saponin are known to possess good antioxidant and cholesterol lowering potentials.

Kidney function test

Kidney function tests of albino rats administered with *G. Kola* and *C. acuminata* were shown in Table 2. The results revealed that the urea level of the albino rats showed a significant ($P<0.05$) decrease in all instances of the treatment group. The urea level of group C albino rats showed a significant ($P<0.05$) decrease when compared to control. The creatinine level of Group D (103±4.45 mmol/L) albino rats was significantly ($P<0.05$) higher than Groups A (34±0.00 mmol/L). The accepted normal urea level range is between 2.1-8.5 mmol/L. The urea level decreased in Group B, C and D when compared to control. This indicates a positive result for rats administered with *G. kola*, *C. acuminata* and mixture of *G. kola* and *C. acuminata*. The accepted normal creatinine level range is between 61.9-114.9 mmol/L. The urea-creatinine ratio has been found to be an estimation factor of other metabolic disorders, besides those intrinsic to the kidney. A urea level raised out of proportion to creatinine level may indicate a metabolic disorder. The accepted normal chloride level is 96-106 mEq/L. The chloride level of the albino rats showed a significant ($P<0.05$) decrease in Group C and Group D when compared to control. There was a reduction of chloride level in group B (90±0.00 mEq/L), C (80±1.15 mEq/L) and D (77±0.00 mEq/L) when compared to control. The sodium level showed no significant ($P<0.05$) increase in all instances of treatment when compared to control. There was no significant ($P<0.05$) increase in the potassium level of all instances of treatment group. However, the results of kidney function parameters analysed revealed that all the parameters were still within normal acceptable range. This suggests that *G. kola* and *C. acuminata* diets used in this study were not toxic to the kidney at dose used.

Liver function test

Liver function Test of albino rats administered with *G. Kola* and *C. acuminata* were shown in Table 1. The results revealed that the direct bilirubin levels of albino rats fed with group B (2.20±0.005 µmol/l) was higher than rats fed with Groups A (2.10±0.00 µmol/l), although there was no significant ($P>0.05$) increase in all instances of the treatment group. The alkaline phosphatase level of Group B (409±0.10 Iu/l) and Group C (347±0.00 Iu/l) were significantly higher than rats fed with Groups A (292±0.00 Iu/l). The aspartate aminotransferase of Group B (5±2.00 Iu/l) and Group D (5±0.50 Iu/l) was significantly ($P<0.05$) higher than Groups A (3±0.00 Iu/l). It is shown in Table 4 that the experimental rats showed no significance increase ($p>0.05$) in direct bilirubin of the entire treatment group. There was a significant ($P<0.05$) increase in the total bilirubin levels of group B when compared to control, although it was still within normal range. There was a significant ($P<0.05$) increase in the aspartate aminotransferase and alanine transferase levels of group B and group D compared to control. Bilirubin is the major breakdown that results from the destruction of old red blood cells, is removed from the blood by the liver, hence it is a good indication of the function of the liver. The accepted total bilirubin level is between 5.1-17 µmol/L. Group B rats administered with *C. acuminata* showed an increase in total bilirubin level which exceeds the accepted level. Total bilirubin level above 20µmol/L suggests liver disease. This observation suggests that too much consumption of *C. acuminata* might have an adverse effect on liver. After the experiment there was no significant change ($p>0.05$) in the AST and ALT of albino rats fed with *G. kola*. This is in agreement with Osifo *et al.,* (2012) which revealed no observational histopathological effect of *G. kola* on liver. The result of this study suggests that too much consumption of *C. acuminata* may increase bilirubin and ALP level which could lead to liver diseases, while *G. kola* has no adverse effect on the liver.

Lipid function test

Lipid profile test of albino rats administered with *G. kola* and *C. acuminata* were shown in Fig 3. The results revealed that the cholesterol levels of albino rats fed with Group B (2.05±0.01 mmol/L), Group C (2.02±0.00 mmol/L) and Group D (2.05±0.00 mmol/L) were significantly ($P<0.05$) lower than Group A (2.20±0.00 mmol/L). There was no significant ($P<0.05$) difference in Triglyceride levels of albino rats in all instances of the treatment groups. The significant ($p>0.05$) decrease in the serum total cholesterol level of group B, C and D when compared to control could be as a result of high fibre content of *G. kola* and *C. acuminata* as revealed from the result of their proximate analysis (Fig.1), since according to Olusanya (2008) soluble fibres could bring about reduction in cholesterol level. Furthermore, reduction in total cholesterol level observed in this study could also be as a result of high content of flavonoid and saponin observed in the phytochemical analysis in this study (Fig.2), since according to Egbuna *et al* (2019) flavonoid and saponin have the potentials to reduce cholesterol level in living system. There was no significant ($p<0.05$) difference in the HDL level of Group B (1.06±0.03 mmol/L), Group C (1.03±0.01 mmol/L) and Group D (1.05±0.05 mmol/L) when compared to control (1.05±0.01 mmol/L). Cholesterol is an important constituent of cellular membranes and is a precursor of steroid hormones and bile acids. However high cholesterol levels in the blood is the primary cause of cardiovascular disease and can result in atherosclerosis, myocardial infarction and coronary heart disease. There was a significant ($p<0.05$) decrease in the LDL levels of Group B (1.50±0.05 mmol/L) and Group C (1.52±0.01 mmol/L) when compared to control (1.70±0.04 mmol/L). The results are similar to the findings of Omeh *et al.,* (2014) who had published a trial on albino rats fed with *G. kola* seed results in the lowering of total cholesterol, LDL while increasing HDL. The results showed that *G. kola* and *C. acuminata* caused a decrease in the serum total cholestrol, which suggests that they could be used in management of hypercholesterolaemia, artherosclerosis and their associated diseases.

Haematological analysis

The results obtained for the Haematological analysis of albino rats administered with *G. kola* and *C. acuminata* were shown in Table 3. The result shows that group B and group D exhibited lower PCV (%), Hb (g/dL), RBC (mm^3) and exhibited higher WBC (mm^3) . The PCV level was higher in group D (38±0.00%) when compared to control. The Hb level was higher in group C (13.3±0.00 g/dL) when compared to control. The lowest Hb level was found in group B (12.6±0.00 g/dL), followed by group D (12.6±0.20 g/dL). Total RBC was highest in group C (5.3±0.00 mm^3) ($\times 10^{12}$) when compared to group A (5.1±0.02 mm^3) ($\times 10^{12}$). This shows that daily

consumption of *C. acuminata* could lead to anaemia when consumed for a long period of time, while *G. kola* has no adverse effect on PCV, RBC and Hb. The recommended haemoglobin level is between 13.5-17.5 g/dL. Group B (12.6±0.00 g/dL) and group D (12.6±0.20 g/dL) showed a reduction in Hb levels when compared to control (13.0±0.046 g/dL). This indicates a negative results for groups administered *C. acuminata* and mixture of 15% *G. kola* and 15% *C. acuminata*. There was an increase in Hb level of group C (13.3±0.00 g/dL) when compared to control (13.0±0.046 g/dL), this indicates a positive result for groups administered *Garcinia kola*. There was no significant ($P>0.05$) increase in the Hb, PCV and WBC levels in all instances of treatment group. There was a significant ($P<0.05$) increase in the RBC level of Group C compared to control. The accepted normal PCV level is 38.3-48.6%. Group C administered with *G. kola* showed improved PCV level whereas group B and group D administered with *C. acuminata* and mixture of 15% *G. kola* and 15% *C. acuminata* respectively showed decreased PCV level. The accepted normal WBC level is 4,500-11,000mm^3. Group B (4.3±0.00 mm^3) ($\times 10^3$) and group D (4.3±0.00 mm^3)($\times 10^3$) showed an increase in WBC level when compared to control (4.2±0.00 mm^3) ($\times 10^3$), this indicates a positive result for rats administred *C. acuminata* and mixture of 15% *G. kola* and 15% *C. acuminata*. This shows that *C. acuminata* has no adverse effect on the immune system, therefore it can boost immunity. The accepted normal RBC level is 4.7-6.1 $mm^3 \times 10^{12}$. Group B and group D, showed a decrease in RBC level when compared to control and group C, this indicates a negative result for rats administered *C. acuminata* and mixture of 15% *G. kola* and 15% *C. acuminata*. The result of this study is in agreement with the report of Esomonu *et al*., (2005) in which the mean Hb, PCV and RBC counts of rats fed with *G. kola* were found to be non-significantly different ($P>0.05$) from control. The result of this study suggests that consuming *C. acuminata* for a long period of time may reduce the haemoglobin level causing anaemia.

4. Conclusion

Garcinia kola and *Cola acuminata* is no doubt a potential medicinal plant. Phytochemical composition showed that *G. kola* and *C. acuminata* could be useful in the development of numerous drugs in the health care industry. *G. kola* increased the packed cell volume (PCV) and red blood cell (RBC) levels, whereas *C. acuminata* increased the white blood cell count (WBC), boosting immunity. *G. kola* had no adverse effect on liver, whereas *C. acuminata* increased the alkaline phosphatase level and bilirubin level. *C. acuminata* and *G. kola* reduced the urea level therefore they have no adverse effect on kidney. *G. kola* and *C. acuminata* caused a decrease in the serum total cholesterol, which suggests that they could be useful in the management of hypercholesterolaemia and its associated diseases. Measures should be taken when consuming *C. acuminata*, as consuming it for a long period of time may reduce the haemoglobin level causing anaemia and also increase bilirubin and ALP levels which are indications of severe liver damages. The results of this study therefore suggests that *G. kola* has more health benefits and should be used in place of *C. acuminata* as a medicinal plant.

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