



## Proximate and Phytochemical Composition of *Cola lateritia* Seeds and its Effect on Lipid Profile Levels in Wistar Albino Rats

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### Abstract

*Cola lateritia* is an indigenous edible tropical fruit species also utilized for health purposes. This study was aimed at determining the proximate, phytochemical composition of *Cola lateritia* seeds and its effect on serum lipid profile levels. Proximate composition and phytochemical analysis were done using standard methods. Crude extraction protocol was done using standard methods. Sixteen albino rats were randomly divided into four groups of four animals each. Group A was the control group fed on food and water only, Group B, C and D received 100 mg/kg, 200 mg/kg and 300 mg/kg respectively per body weight of *Cola lateritia* seeds extract orally for fourteen days. Serum levels of High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglycerides (TG) and cholesterol were determined spectrophotometrically. Results showed that *Cola lateritia* seeds contained 9.44% moisture, 0.64% ash, 0.20% fat, 1.31% fiber, 6.02% protein and 82.39% carbohydrate. Preliminary phytochemical screening revealed the presence of flavonoid (8.35%), saponin (6.59%), terpenoid (5.59%), phenol (2.53%) and tannin (0.098 mg/g). There was a significant ( $p < 0.05$ ) reduction in the serum levels of cholesterol, HDL, LDL and triglycerides in the test groups compared to the control. These findings thereby suggest the hypolipidemic activity of *Cola lateritia* which can be attributed to some of its phytochemical constituents.

**Keywords:** *Cola lateritia*; Lipid profile; Hypolipidemic; Phytochemicals

### Introduction

The use of plants as food and as medicine has been from eon due to the fact that plants contain immense nutrients and phytochemicals adduced to be responsible for some of their observed pharma cotherapeutic effects in man. The occurrence of dyslipidemia, with the commonest form being hyperlipidemia (elevated levels of lipids in the blood) is fast becoming rampant especially in developing countries and is a big threat to health as it also predisposes to other diseases such as

cardiovascular diseases, atherosclerosis etc. Common causes of dyslipidemia are obesity, diabetes, hypothyroidism, metabolic syndrome, genetic factors and some medications [1]. There is therefore a dire need to look for natural agents that can be used in the management of dyslipidemia without presenting with side effects associated with the use of synthetic drugs.

Monkey kola is a common name given to a number of minor relatives of the *Cola spp.* that produce edible tasty fruits which are native to West and central Africa. There are various species of monkey kola based on the colour of the pulp as colas with yellow, white and red pulp are known as *Cola lepidota*, *Cola pachycarpa* and *Cola lateritia* respectively; of which *Cola lateritia* is the least common. Monkey kola fruits are highly nutritious and are rich in minerals, vitamins and fiber. They contain calcium, potassium, phosphorus, iron, zinc and sodium. The fruits are eaten but the leaves are used in traditional medicine to treat eye infections while the bark is used for oral hygiene and to treat tooth ache [2]. The seeds of monkey kola which is the most vital part has been alleged to be a good source of nutrients and a naturally organic health supplement that can be utilised in many therapeutic ways. Lipid profile is a blood test that measures total cholesterol, High Density Lipoprotein (HDL) cholesterol, Low Density Lipoprotein (LDL) cholesterol and Triglyceride (TG) levels as a diagnostic index for determining the risk of cardiovascular diseases. There is paucity of information on *Cola lateritia* among other cola species in the literatures; hence this research was conducted to provide more insight on the potential of *Cola lateritia* as an agent that can be used in the management of hyperlipidemia [3-6]. This study was aimed at determining the chemical composition and the effect of *Cola lateritia* on the lipid profile.

### Materials and Methods

#### Collection, identification and preparation of plant sample

*Cola lateritia* fruits were collected from a forest in Opa, Ile-Ife in Osun State. The sample was identified and authenticated at the Department of Biological Sciences, Obafemi Awolowo University, Ile-Ife, Osun State Nigeria and was assigned the voucher specimen number IFE-17676. The fruits were then cut into pieces and the seeds separated out. The seeds were then gently washed and rinsed in tap water thoroughly and completely air-dried at room temperature for three weeks.

#### Preparation of extract

The dried seeds were pulverized into a coarse powder using a mechanical grinder. 100 g of the powdered dried *Cola lateritia* seeds was cold macerated in 1000 ml of ethanol for 72 hours, after which it was filtered through a filter paper. The residue obtained was further evaporated using a rotary evaporator and the filtrate was stored in an air and water-proof container kept in a refrigerator at 4°C.

#### Proximate analysis

Proximate analysis of the *Cola lateritia* seed powder was carried out to determine the crude protein, crude fibre, total ash, total carbohydrate, crude lipid and moisture content following methods described.

## Mineral content

The materials used were *Escherichia coli* ATCC® 11775 (pathogen) and *Bacillus subtilis* ATCC® 6633 (probiotic) obtained from the Veterinary Center (BVET), and *Lactobacillus acidophilus* (probiotic) obtained from the collection of the UGM Inter-University Center (PAU), Yogyakarta, Indonesia, Broth Heart Infusion (BHI, Merck™), phosphate buffered saline (pH 7.4, Sigma™), *Mueller Hilton agar* (MHA, Merck™), curcuma aquadest extract (concentration: 25%, 12.5%, 6.25%, 3.13%, 1.56%), Lombok honey (100% concentration), distilled water, blank disc (Oxoid™), chloramphenicol antibiotic disc (C 30 µg, Oxoid™). For the determination of the mineral content, the seeds of *Cola lateritia* was weighed into a ready crucible and placed in the muffle furnace chambers at 700°C until the samples turned into ashes within three hours. Afterwards, standard solutions of the minerals (sodium, potassium, calcium, magnesium, phosphorus, iron, zinc and copper) to be determined were prepared using Atomic Absorption Spectrophotometer (AAS). The standard metal solutions were then loaded into the AAS in order to calibrate it and acetylene was used as the carrier gas. An aliquot of the mineral solution obtained from the ash sample of *Cola lateritia* seeds digest was then also loaded into the spectrophotometer and the amount of each metal present was determined from the AAS.

## Determination of Vitamin C content

This was done following the methods described, 9.5 mL of standard ascorbic acid (100 µg/mL) was weighed in a conical flask containing 10 mL 4% oxalic acid and was titrated against the 2, 6-dichlorophenol indophenol dye. The appearance and persistence of pink colour was taken as end point. The amount of dye consumed (V1 mL) is equivalent to the amount of ascorbic acid. 5 mL of sample was taken in a conical flask having 15 mL of 4% oxalic acid and titrated against the dye (V2 mL). The amount of ascorbic acid was calculated using the formula below:-

$$\text{Ascorbic acid (mg/100 g)} = (0.5 \text{ mg/V1 mL}) \times (V2/15 \text{ mL}) \times (100 \text{ mL/Wt. of sample}) \times 100$$

where V1=Amount of dye consumed for standard; V2=Amount of dye consumed for sample

## Phytochemicals analysis

**Determination of total phenolic content:** The total phenol content of the sample was determined by the method of 0.20 ml of the plant extract was mixed with 2.5 ml of 10% Folin ciocalteau's reagent and 2.0 ml of 7.50% sodium carbonate solution. The reaction mixture was subsequently incubated at 450°C for 40 minutes and the absorbance of the coloured mixture was read at 700 nm using UV visible spectrophotometer. Gallic acid was used as standard.

## Determination of total flavonoid content

The flavonoid content in the *Cola lateritia* seed powder extract was determined spectrophotometrically according to the procedure of 0.01 g of the extract was dissolved in 5 mL of methanol and made up to 20 mL to give a final concentration of 0.5 mg/ml. To clean, dry test tubes (in triplicate) were pipetted 0.2 mL of working solution of the sample and diluted with 4.8 mL distilled water. To each test tube was then added 0.3 mL of 5% (w/v) NaNO<sub>2</sub>, 0.3 mL of 10% AlCl<sub>3</sub> and 4 mL of 4% (w/v) NaOH. The reaction mixtures were incubated at room temperature for 15 minutes. The absorbance was read at 500 nm

against reagent blank containing all reagents except the extract or standard catechin in the case of standard curve solutions. The standard calibration curve was prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/mL catechin into clean dry test tubes. The volumes were made up to 5 mL with distilled water. To each of the tubes were added 0.3 mL of 5% (w/v) NaNO<sub>2</sub>, 0.3 mL of 5% (w/v) AlCl<sub>3</sub> and 4 mL of 4% (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 minutes. Absorbance was taken at 500 nm and was plotted against the concentration to give the standard calibration curve. The concentrations of the flavonoids in the extract was extrapolated from standard calibration curve and expressed as milligram Catechin (CAT) equivalent per g of extract (mg CAT/g extract).

The value extrapolated from the standard curve gave the concentration in µg CAT/mL. The concentration in mg GAE/g extract was obtained using the equation below:

$$\text{mg CAT/g extract} = \frac{\mu\text{gCAT} \times 1 \text{ mg} \times \text{mL of solvent used in dissolving the sample} \times \text{dilution factor}}{\text{mL} \times 1000 \mu\text{g} \times \text{mass of the sample used}}$$

## Determination of tannin content

This was done using the method described; 500 mg of the sample was weighed into a plastic bottle. 50 ml of distilled water was added and shaken for one hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and the volume made up to the mark. 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.10 M FeCl<sub>3</sub> in 0.10 N HCl and 0.008 M potassium ferro cyanide. The absorbance was then measured at 120 nm.

## Determination of alkaloid content

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for four hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete [7]. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

## Determination of saponin

The samples were ground and 20 g of each were put into conical flask and 100 cm<sup>3</sup> of 20% aqueous ethanol were added. The samples were heated over a hot water bath for four hours with continuous stirring at about 55°C. The mixtures were filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of N-butanol was added. The combined N-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride [8-12]. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

## Experimental animals

A total of 16 mice of 8-10 weeks old, weighing between 24-27 grams were used in this study. Wood shavings were used as beddings

to keep each compartment dry. Here, normal standard ambient conditions of temperature between 28-31°C, relative humidity between 50%-60% and a photoperiodicity of 12 hours natural light and 12 hours dark were maintained. The animals were maintained on a standard diet and water provided ad libitum.

### Experimental design

At the end of the acclimatization period, the animals were then randomly divided into four groups as follows:

**Group A:** Was the control group fed on food and water only throughout the study.

**Group B:** Received 100 mg/kg per body weight of *Cola lateritia* seed extract orally.

**Group C:** Received 200 mg/kg per body weight of *Cola lateritia* seed extract orally.

**Group D:** Received 300 mg/kg per body weight of *Cola lateritia* seed extract orally.

At the end of two weeks, the animals were anaesthetized using ether and blood samples were collected in clean, dry centrifuge tubes, which were left at room temperature for ten minutes to clot. The tubes were later centrifuged at 503 g for ten minutes. The sera were thereafter aspirated using a Pasteur pipette into clean, dry, sample bottles and were used within 12 hours.

### Assay for cholesterol level

This was done enzymatically using wet reagent diagnostic kits following the method. Three test tubes were set up in a test tube rack and labelled blank, standard, and sample respectively. 10 µl each of distilled water, standard specimen and the serum samples was pipetted into the blank, standard and sample test tubes respectively. To each test tube was then added 1000 µl of reagent. The contents were thoroughly mixed and incubated for ten minutes at room temperature (28 ± 2°C). The absorbance of the blank, standard and samples was then read using a spectrophotometer at 500 nm. The concentration of cholesterol in each sample was then calculated and expressed in mg/dl.

### Assay for triglyceride levels

Total triglyceride in plasma was determined by the enzymatic method, using wet reagent diagnostic kits, a modification of the method. The reagent (1000 µl) was pipetted into three different test tubes labelled A, B and C. Distilled water, a standard solution and plasma (sample) of 10 µl each were then pipetted into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to allow for colour change [13]. The absorbance of the blank, standard and sample were read at 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of the triglyceride in sample was then calculated and expressed in mg/dl.

### Assay for high density lipoprotein

Concentration of High Density Lipoproteins (HDL) was determined according to the method. 0.3 ml of the serum samples were pipetted into a set of clean labelled test tubes. One drop of precipitate solution (dextran sulphate and magnesium acetate) was added to the serum in

each of the test tube, mixed and allowed to stand for 15 minutes at 25°C. It was centrifuged at 3000 rpm for 10 minutes. 1 ml each of cholesterol esterase, cholesterol oxidase, peroxidase, buffer (pH 6.8), phenol 2,3-dichlorophenol, 4-amino antipyrine were added to another set of test tubes labelled standard 2, blank and sample tests. Also, 0.1 ml of the supernatant derived from centrifugation of the precipitate-sample mixture was added to the appropriately labelled test tubes. The entire solution was mixed thoroughly and allowed to stand for 10 minutes at 25°C, after which 0.1 ml of the standard (equivalent to 200 mg/dl HDL cholesterol) was added to each of the test tubes labelled standard 1, standard 2, mixed well and allowed to stand for 10 minutes at 25°C. The absorbance of the sample and the standard against the sample blank were read at a wavelength of 505 nm in a spectrophotometer. The HDL-Cholesterol content of each sample was then calculated using the formula below:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

The result was then expressed in mg/dl

### Assay for low density lipoproteins

0.3 ml of serum samples was pipetted into clean labelled test tubes. Three drops of precipitate solution (polyvinyl sulphate, sodium EDTA, polyethylene glycol mono ethyl ether) were added to the serum in the test tubes, mixed properly and allowed to stand for 10 minutes at 25°C. Thereafter, the mixture was centrifuged at 3000 rpm for 10 minutes. 1 ml each of cholesterol esterase, cholesterol oxidase, peroxidase, buffer (pH 6.8), phenol, 2,3-dichlorophenol, 4-amino antipyrine was added to another set of test tubes labelled standard 1, standard 2, blank and sample tests. Also, 0.1 ml of the supernatant derived from centrifugation of the precipitate- sample mixture was added to the appropriately labelled test tubes. The entire solution was mixed thoroughly and allowed to stand for exactly 10 minutes at 25°C, after which 0.1 ml of the standard (equivalent to 200 mg/dl HDL cholesterol) was added to each test tube labelled standard 1, standard 2, mixed thoroughly and allowed to for 10 minutes at 25°C. The absorbance of the sample and the standards against the sample blank was read at a wavelength of 505 nm. The amount of LDL was then determined using the formular below:

### Calculation

$$\text{LDL-Cholesterol (mg/dl)} = \text{Total cholesterol (mg/dl)} - 1.5 \times \text{Supernatant cholesterol (mg/dl)}$$

## Results

### Proximate composition

The results obtained in this study show the proximate composition, vitamin C and minerals in *Cola lateritia* seed. As shown in Table 1, the seed contains a high amount of carbohydrate as compared to other nutrients and fat was present in the least amount. Table 2 shows the mineral contents of *Cola lateritia* seed. Copper was the most abundant in the seed (12.44 mg/100 g ± 1.18 mg/100 g) while iron was present in the least amount (1.11 mg/100 g ± 0.00 mg/100 g). The result obtained for vitamin C content shows that *Cola lateritia* seed contained 12.83 mg/100 g of vitamin C.

Parameter	<i>Cola lateritia</i> seed
Moisture (%)	9.64 ± 0.09
Ash (%)	0.99 ± 0.02
Fat (%)	0.24 ± 0.01
Fibre (%)	2.02 ± 0.01
Protein (%)	6.07 ± 0.04
Carbohydrate (%)	81.04 ± 0.15

**Table 1:** Proximate composition of *Cola lateritia* seed.

Mineral	<i>Cola lateritia</i> seed
Na (mg/100 g)	2.93 ± 0.00
K (mg/100 g)	2.33 ± 0.01
P (mg/100 g)	3.08 ± 0.02
Mg (mg/100 g)	4.85 ± 0.01
Fe (mg/100 g)	1.11 ± 0.00
Cu (mg/100 g)	12.44 ± 1.18
Zn (mg/100 g)	10.34 ± 0.26

**Table 2:** Mineral composition of *Cola lateritia* seed.

### Phytochemical analysis

Preliminary phytochemical screening of seed revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycosides. Quantitative phytochemical analysis of the seed as shown in Table 3 revealed that flavonoids was present in the highest amount (8.35% ± 2.05%) while tannins was found to be in the least amount (0.098 mg/100 g ± 0.003 mg/100 g).

Phytochemical	<i>Cola lateritia</i> seed
Tannin (mg/g)	0.098 ± 0.003
Phenol (%)	2.53 ± 0.03
Terpenoid (%)	5.59 ± 0.33
Saponin (%)	6.59 ± 0.03
Alkaloid (%)	0.46 ± 0.00
FLavonoid (%)	8.35 ± 2.05

**Table 3:** Quantitative phytochemical analysis of *Cola lateritia* seed.

### Effect of seed on serum lipid profile levels in wistar rats

Table 4 shows the effect of *Cola lateritia* seed on lipid profile levels in wistar rats. As shown in the table, there was a significant ( $p < 0.05$ ) reduction in the cholesterol, LDL and triglycerides levels in the rats that received seed extract of *Cola lateritia* as compared to the control group. However, there was a significant ( $p < 0.05$ ) increase in

the HDL levels of the rats that received *Cola lateritia* seed extract at the different doses administered as compared to the control group.

Group	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
A (control)	22.43 ± 0.33	43.70 ± 0.5	209.20 ± 0.11	143.48 ± 0.05
B (100 mg/kg)	13.53 ± 0.15	66.90 ± 0.79	129.54 ± 0.07	128.43 ± 0.13
C (200 mg/kg)	13.92 ± 0.16	62.65 ± 0.77	121.04 ± 0.07	82.37 ± 0.27
D (300 mg/kg)	6.57 ± 0.04	61.87 ± 0.73	106.34 ± 0.05	81.48 ± 0.07

**Table 4:** Effect of *Cola lateritia* seed extract on the serum cholesterol, HDL, LDL and triglyceride levels in rats.

### Discussion

In this study, the proximate, phytochemical analysis and the effect of *Cola lateritia* seed on the serum lipid profile in mice was evaluated. As seen from the results obtained from the proximate analysis, *Cola lateritia* seed contains a very high amount of carbohydrate. Carbohydrates provide energy, as they are the body's main source of fuel, needed for physical activity [14], brain function and operation of the organs. They are also important for intestinal health and waste elimination. Proximate analysis of the seed flour of the other two varieties of monkey kola (*Cola parchycarpa* and *Cola lepidota*) revealed that they are also very rich in carbohydrates above 70%; though our own results revealed that *Cola lateritia* seeds contain higher carbohydrate content than the other varieties. There were however slight discrepancies in the other proximate analysis parameters in the two other varieties as compared to that of *Cola lateritia*. This might be due to species distribution, soil and climatic factors and geographical location. *Cola lateritia* seeds contain small amounts of proteins and crude protein serves as a source of fuel and it is a nutrient required by the human body for growth and preservation.

The most abundant mineral present in *Cola lateritia* seeds is copper, followed by zinc and it contains trace amounts of magnesium and phosphorus. Copper has been reported to be important for proper insulin function as its deficiency can impair glucose metabolism and increase insulin resistance [15-18]. Zinc and magnesium plays an important role as cofactors in enzyme catalysis in the body. *Cola lateritia* seeds contain relatively significant amounts of vitamin C (12.83 mg/100 g). Vitamin C is required for immune function, collagen and thyroxin synthesis and also boosts the absorption of iron.

Phytochemical analysis of *Cola lateritia* seeds revealed that it contained alkaloids, flavonoids, saponins, tannins, phenol and terpenoid. Alkaloids have demonstrated many pharmacological activities such as antihypertensive effects antiarrhythmic effect, antimalarial activity (quinine), anticancer and analgesic effects. Epidemiology studies have demonstrated that consumption of flavonoids lowers the risk of the incidence of mortality from coronary heart diseases and heart attack [19]. Flavonoids have been stated to demonstrate multiple biological effects such as antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the hallmark of flavonoids is their ability to act as potent antioxidants which can shield the human body from free radicals and reactive oxygen species.

Phenols are plant secondary metabolites, and they possess antioxidant properties which are adduced to their role as protecting agents against free radical-mediated disease processes. Saponins are known to possess antimicrobial property and also ability to significantly affect growth, feed intake and reproduction in animals. Terpenoids have been reported to possess medicinal properties such as anti-carcinogenic, antimalarial, anti-ulcer, antimicrobial or diuretic activity and anticancer property.

The results obtained from this study on the effect of *Cola lateritia* seeds on lipid profile levels as shown in Table 4 revealed that administration of the seeds extract resulted in a significant ( $p < 0.05$ ) reduction in LDL, cholesterol and triglyceride levels and an increase in the HDL levels of the experimental animals as compared to the control group [20-23]. Furthermore, administration of *Cola lateritia* seeds extract to the animals brought about a reduction in the atherogenicity index represented as (TG/HDL) ratio; as increase in HDL is an index of anti-atherogenicity. This illustrates the positive effect of the seed on lipid metabolism. HDL carries cholesterol from peripheral tissues to the liver for use [25]. The mechanism of the anti-atherogenic effect of HDL is by inhibiting the oxidation of LDL [24]. It also prevents oxidation of LDL by transition metal ions and also deters mediated formation of 12-lipoxygenase by lipid hydroperoxide. The reduction in the cholesterol, triglycerides and LDL levels in the experimental animals clearly demonstrates the hypolipidemic activity of *Cola lateritia* seeds. Other plants like *Cola lepidota* and *Saccharum barberi* have also been reported to demonstrate hypolipidemic properties.

## Conclusion

The use of plants could be an efficacious means of preventing and even managing diseases and complications associated with hyperlipidemia. This helps to achieve the control of major risk factors such as levels of blood cholesterol and triacylglycerol that predispose to these disorders. *Cola lateritia* seeds contain different phytochemicals as reported earlier and its hypolipidemic effect might be due to the synergistic action of some of these phytochemicals. Saponins have been reported to inhibit the absorption of lipids and also help to lower blood cholesterol by binding to it. The presence of flavonoids in the seed might also play a role in its hypolipidemic activity as flavonoids have been reported to inhibit hepatic HMG-CoA. However, the general underlying mechanism behind the hypolipidemic activity of *Cola lateritia* might be attributed to inhibition of cholesterol esterase, activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate in the experimental animals.

In conclusion, *Cola lateritia* demonstrated hypolipidemic activity which might be attributed to some of its phytochemical constituents and can therefore be useful in the management of hyperlipidemia and other associated diseases. However, more studies to elucidate the actual mechanism responsible for the hypolipidemic activity of *Cola lateritia* are recommended.

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