

Tropical Journal of Phytochemistry & Pharmaceutical SciencesAvailable online at <https://www.tjpps.org>**Original Research Article****Proximate, Phytochemicals, and Mineral Analysis of *Cola acuminata* Aqueous Leaf Extract**

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ABSTRACT

Medicinal plants have been the focus of recent natural product research due to their safety and availability. *Cola acuminata* (CA) has been used in managing anemia, diarrhoea, cough, and dysentery. In this study, phytochemical composition, nutritional content, and *In vitro* antioxidant capacity of aqueous extract of CA leaves were ascertained. Fresh leaves of CA obtained from a farm in Benin City, Edo State, were air-dried and ground into powder. Exactly 500g of the powdered leaves was weighed and macerated in distilled water for 72 hours in an air-tight container with continuous stirring. This was filtered and concentrated with a rotary evaporator at 30°C. Proximate analysis, phytochemical screening, and mineral analysis were carried out in accordance with standard methods. Results revealed nutritional content as crude fibre (31.7%), carbohydrate (31.24%), crude protein (4.7%), crude lipid (5.9%), moisture (16.22%), and ash (10.24%), phytochemical content includes cardiac glycosides, saponins, terpenoids, tannins and reducing sugars. Mineral analysis indicates high concentrations of magnesium ($22.34 \pm 0.34\text{mg}/100\text{g}$) potassium ($33.47 \pm 1.34\text{mg}/100\text{g}$), phosphorous ($60.68 \pm 1.26\text{mg}/100\text{g}$) and Sodium ($26.66 \pm 0.51\text{mg}/100\text{g}$) and low concentrations of calcium ($8.63 \pm 0.11\text{mg}/100\text{g}$), zinc ($1.63 \pm 0.02\text{mg}/100\text{g}$) and iron ($4.50 \pm 0.03\text{mg}/100\text{g}$). The results show that CA leaves are a viable reservoir of natural products that are beneficial for the maintenance of health and drug design.

Keywords: *Cola accuminata*, Drug design, Natural products, Medicinal plant, Minerals, Phytochemicals.

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Copyright: © 2025 Okungbowa *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.**Introduction**

Cola acuminata is an evergreen tree belonging to the Malvaceae family. It is native to the rainforests of tropical West Africa and is commonly referred to as kola nut.¹ The tree grows to about 20–25m, with long, ovoid, leathery-textured leaves pointed at both ends and flowers that are yellow with purple spots. The fruits are star-shaped and borne in pods containing about a dozen seeds per pod. The bark of old trees is rough, corky, and grey in color and often split into squares. The common species of kola nut are *Cola nitida* and *Cola acuminata*.² *Cola acuminata* is a significant plant in various regions of West Africa, as its nuts are believed to bring blessings. Consequently, these nuts are utilized in social and ceremonial activities, particularly during prayers. While the nuts are chewed for pleasure by the elderly, they possess astringent and analgesic effects,³ and contain caffeine, which is an important component of some beverages and pharmaceutical products.^{4,5} They are effective bronchodilators against whooping cough and asthma conditions and exhibit adaptogenic properties.^{6,7,8}

Traditionally, the flowers, leaves, bark, and twigs are prominent constituents of decoctions for coughs, constipation, toothaches, abnormal menstrual cycles, vomiting, depression, diarrhea, sleep apnea, dysentery, chest pains, and ulcers. In addition, traditional medicine practitioners employ extracts of the leaves in treating conditions associated with anaemia and spleen enlargement in toddlers and adults. *Cola acuminata* aqueous leaf extract has been reported to exhibit both antioxidant and antidiabetic properties.⁹ Several researchers have documented the effectiveness of the nuts in treating various diseases; however, there is limited scientific literature supporting the traditional use of the leaves in disease management despite their popularity amongst traditional medicine practitioners. This study, therefore, seeks to provide scientific data for the phytochemical content, nutritional, and mineral composition of *Cola acuminata* aqueous leaf extract.

Materials and Methods*Preparation of Cola acuminata aqueous leaf extract*

The *Cola acuminata* leaves were collected in April 2022, from a farm in Benin City, Edo State, Nigeria. They were identified and authenticated by Prof. H.A. Akinnibosun in the Department of Plant Biology and Biotechnology, University of Benin, and the herbarium specimens were assigned voucher number UBH-C317. They were air-dried and ground into powder. Powdered *Cola acuminata* (500g) sample was macerated in 2.5L of distilled water for 72 hours while stirring at intervals, while stirring at intervals. Thereafter, a muslin cloth was used to filter the mixture and obtain the filtrate, which was concentrated with a rotary evaporator at 30°C and further dried in an oven to obtain the crude extract.

Proximate Analysis of Leaves

The proximate analysis of the plant leaves was carried out in accordance with the Association of Official Analytical Chemists methods.¹⁰

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Moisture content

Sample (10 g) was put in a porcelain dish and allowed to dry in an oven for 24 hours at 105°C. It was then cooled in a desiccator and weighed again to obtain a final weight. % moisture content was estimated as (equation 1):

$$\% M.C = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100$$

% Ash Content

Three grams (3g) of powdered leaf sample was placed in a porcelain dish, then dried for three hours at 100°C and ashed for six hours in a muffle furnace at 500 °C until a greyish-white residue was observed. It was then moistened with some drops of water, dried in an oven at 100 °C for three hours, ashed again at 500 °C in a furnace for an hour, cooled, and weighed. The percentage ash of the sample was evaluated as follows (equation 2):

$$\% A.C = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude lipids

A 250 mL round-bottom flask was dried at 105°C in an oven, cooled, and weighed. About 250 mL of N-hexane was measured into the flask, attached to a Soxhlet extractor, and heated in a heating mantle. Sample weighing 1g was placed in a porous thimble and extracted for 6 hours. Thereafter, N-hexane was evaporated using a rotary evaporator. This was cooled in a desiccator, weighed, and the crude lipid content of the sample was calculated according to the equation below (equation 3):

$$\% C.L = \frac{\text{Weight of flask and extracted fat} - \text{weight of empty flask}}{\text{Weight of sample}} \times 100$$

% Crude fibre content

Five (5 g) sample was added successively to a boiling solution containing 0.26N H₂SO₄ and 0.28N KOH. The residue obtained from filtration was transferred to a crucible and dried for 24 hr in an oven at 65 °C. The weight of sample and crucible was recorded and again placed in a muffle furnace for 24 hr at 500 °C, after which it was cooled in a desiccator, re-weighed, and crude fibre content calculated as follows (equation 4):

$$\% C.F = \frac{(\text{Dry weight of residue before ashing} - \text{Weight of residue after ashing})}{(\text{Weight of sample})} \times 100$$

% Crude protein content

The crude protein content was determined using the method described in AOAC.¹¹ A 100 mL round-bottom flask was attached to a Soxhlet apparatus reflux condenser. About 0.5g dried powdered sample was wrapped in different filter papers and put into a Kjeldahl round-bottom flask. The sample was digested with selenium powder (catalyst) and 10 mL of concentrated sulfuric acid for about 2 hours until a clear solution was obtained. For distillation, 100 mL distilled water and 10 mL 40% sodium hydroxide, along with sodium thiosulfate, were added to prevent ammonia loss. A receiving flask containing 10 mL boric acid and three drops of methyl red was set up. Distillation continued until 100 mL was collected, and titrated with 0.1 M sodium hydroxide to a yellow-red endpoint, and the titre value was recorded and calculated as follows (equation 5):

$$\% C.P = \frac{0.001410 \times 6.25 \times 25 T}{W}$$

Where; W= weight of sample; T = titre value; 6.25 = protein conversion Factor

Carbohydrates

The total carbohydrate content was evaluated by the weight difference percent method, calculated by subtracting the combined percentages of crude fat, crude protein, crude fibre, moisture content, and ash from 100% of the dry weight. The carbohydrate content was then computed using the following formula (equation 6):

$$\text{Carbohydrate (\%)} = 100 - (\% \text{Protein} + \% \text{Fat} + \% \text{Fibre} + \% \text{Ash} + \% \text{Moisture})$$

Qualitative Phytochemical Screening of Leaf Extracts

Phytochemical screening was determined by the method referenced in literature.¹²

Alkaloids: Three drops of Hager's reagent were mixed with 1 mL of the leaf extract. A yellow precipitate formed, indicated the presence of alkaloids.

Saponins: Five millilitres (5 mL) of distilled water were added to 1 mL of the leaf extract in a test tube. This was mixed thoroughly by shaking. The presence of stable foam indicated the presence of saponins.

Cardiac glycosides: One millilitre (1 mL) of the sample was combined with a drop of 15% ferric chloride (FeCl₃) and 2 mL ethanoic acid. Concentrated sulfuric acid (1 mL), when added, resulted in the formation of a separate layer. The appearance of a brown ring in-between the layers confirmed deoxysugars in cardiac glycosides.

Tannins: One millilitre (1 mL) of the extract was subjected to heat for 5 minutes, after which a few drops of 15% ferric chloride (FeCl₃) were added and thoroughly mixed. The development of a dark blue or dirty green color indicated the presence of tannins.

Flavonoids: One millilitre (1 mL) each of dilute ammonia and concentrated sulfuric acid was added to 1 mL of the leaf extract. The development of a yellow coloration indicated the presence of flavonoids.

Determination of Mineral Content

The leaf sample was preserved according to recommended practices as contained in the United States Environmental Protection Agency (USEPA) and American Standard for Testing and Materials (ASTM). Laboratory analysis was conducted using approved standard test method. A 10 mL aliquot of sample in a beaker was digested with 0.04 mL of concentrated nitric acid (HNO₃) and 0.25 mL of concentrated hydrochloric acid (HCl). It was then covered with a ribbed watch glass and heated at 95°C on a steam bath until the volume was reduced to 2 mL. The beaker was removed from the bath and allowed to cool. Thereafter, the filtrate obtained after filtration was made up to 100 mL with distilled water. The resulting solution was used to determine Ca, P, Mg, K, Fe, Se, and Na with an atomic absorption spectrophotometer (Buck Scientific VGP 210) at 660 nm.

Results and Discussion

Medicinal plants are a reservoir of a wide variety of nutrients and phytochemicals, including tannins, phenols, proanthocyanidins, flavonoids, carotenoids, saponins, and alkaloids, which exhibit a diverse spectrum of bioactivities.¹³ *Cola acuminata* aqueous leaf extract in this study has been shown to contain a significant amount of these phytonutrients, as indicated in the results (Table 1-3).

Table 1: Proximate analysis of aqueous extract of *Cola acuminata* leaves

Parameters	Composition (%)
Moisture	16.22 ± 0.96
Ash	10.24 ± 0.05
Crude Lipid	5.9 ± 0.17
Crude Fibre	31.7 ± 1.10
Crude Protein	4.7 ± 0.07
Carbohydrate	31.24 ± 0.02

All values were expressed in mean ± standard deviation, n=3

Proximate Composition of Aqueous Extract of Cola acuminata Leaves

The proximate analysis of plant leaf extracts is a fundamental process in evaluating their chemical composition and potential applications in food and drug manufacture.⁷ It provides valuable insights into the nutritional, medicinal, and industrial significance of the leaves. Moisture content affects the texture, taste, microbial stability, and shelf life of food products. Excess moisture can result in spoilage, whereas insufficient moisture can cause products to become dry or brittle. In both food and pharmaceuticals, managing moisture is essential to prevent the growth of microbes such as bacteria, mold, and yeast. High moisture levels in certain materials can lead to decay during storage. Proximate analysis of *Cola acuminata* leaves (Table 1) shows that crude fibre and carbohydrate content were higher compared to the other nutrients, while crude protein and lipid were the least. Moisture content and ash content were rather low. In this study, the moisture content was higher than some previous reports,¹⁴⁻¹⁶ but lower than the values reported by others in *Cola acuminata* nuts.¹⁷ Different plant parts may vary in their nutrient composition, explaining the disparity in the results obtained. Protein in the diet is a source of amino acids, serving as an essential food constituent responsible for tissue repair, water balancing, nutrient transport, muscle contractions, the formation of enzymes, antibodies, and hormones.¹⁸ Essential amino acids are not synthesized by the body thus must be obtained from the diet. Adequate protein intake supports muscle protein synthesis, metabolic regulation, and overall physiological function.¹⁹ High-quality proteins from both animal and plant sources contribute to optimal health, particularly in vulnerable populations such as the elderly and recovering patients.²⁰ Protein also plays a role in appetite regulation and body composition. The protein content in this work (Table 1) was lower than values reported by Dah-Nouvlessounon *et al.*²¹ but higher than values reported by Adesuyi *et al.*²² A high protein constituent in *Cola acuminata* leaves indicates potential nutritional and therapeutic value. These proteins may help address dietary deficiencies, support immune function, and aid tissue repair. Their use could benefit conditions like malnutrition, infections, and metabolic disorders. However, further research is needed to assess amino acid composition, bioavailability, and safety.

Table 2. Qualitative phytochemical screening of aqueous extract of *Cola acuminata* leave extract

Phytochemicals	Result
Flavonoid	–
Tannins	+
Cardiac glycosides	+
Saponins	+
Steroids	–
Alkaloids	–
Terpenoids	+
Coumarins	–
Reducing Sugar	+

Key: +Present – = Not detected

Fibre is important to improve bowel motility as well as increase intestinal peristalsis by surface extension of the food in the intestinal tract.²³ It plays a crucial role in curing nutritional disorders while lowering the risk of coronary diseases.²³ Although they worked on the seeds, the fibre content from the report of Atanda *et al.*²⁴ was lower than the crude fibre of *Cola acuminata* leaf extract presented in this study (Table 1). Therefore, the leaves of *Cola acuminata* may be a good dietary alternative for fibre. Dietary fibre plays vital roles in maintaining digestive health by promoting bowel regularity and preventing constipation. It also aids in controlling blood glucose levels, lowering cholesterol, and supporting weight management. Fibre-rich diets are directly linked with a reduced risk of type 2 diabetes, cardiovascular disease, and certain cancers, particularly colorectal cancer. The inclusion of *Cola acuminata* leaf fibre in the diet could therefore contribute significantly to ameliorating the deleterious health implications of these conditions. Additionally, fibre may support gut microbiota balance, thus enhancing immune function and reducing inflammation.

Table 3: Mineral composition of *Cola acuminata* aqueous leaf extract

Minerals	Amount (mg/100g)
Phosphorous	60.68 ± 1.26
Potassium	33.47 ± 1.34
Sodium	26.66 ± 0.51
Magnesium	22.34 ± 0.34
Calcium	8.63 ± 0.11
Iron	4.50 ± 0.03
Zinc	1.63 ± 0.02

Values are means ± standard deviations of triplicate determinations.

Carbohydrates are significant for their role in the proper functioning of the brain, heart, neurological system, digestive system, and immune system. It serves as the primary energy source for these organs, especially the brain, which relies heavily on glucose for optimal performance. Complex carbohydrates, rich in fibre, also support digestive health and glycemic control²⁵. Adequate carbohydrate intake is essential for preventing fatigue, cognitive impairment, and metabolic imbalances. Therefore, its deprivation results in the loss of bodily tissue and organ effectiveness. The carbohydrate content in this study was observed to be lower than the report of similar research.²⁶ The low carbohydrate content of *Cola acuminata* leaves suggests potential benefits for individuals managing metabolic disorders such as diabetes and obesity. The extract may be suitable for low-carbohydrate or ketogenic diets, helping to control blood glucose levels and reduce caloric intake. Their minimal impact on postprandial blood sugar makes them useful in glycemic management, while also offering value in calorie-restricted diets.

Fat is a vital macronutrient that serves as a dense source of energy, aids in the absorption of fat-soluble vitamins (A, D, E, and K), and is essential for various physiological functions, including hormone production and cell membrane integrity.²³ However, excessive consumption of dietary fats, particularly trans fats and saturated fats, has been strongly associated with increased adiposity and the development of obesity. Obesity, in turn, is a major risk factor for non-communicable diseases such as cardiovascular diseases, type 2 diabetes, and certain cancers. Current dietary guidelines emphasize the importance of moderating total fat intake and prioritizing unsaturated fats from plant-based sources to support metabolic health and prevent chronic diseases.²⁷ The crude lipid content of *Cola acuminata* leaves

herein recorded (Table 1) was lower than the values obtained by Atanda *et al.*²⁴ but higher than those documented by Durand *et al.*²⁸ The low crude lipid content of the leaves of *Cola acuminata* implies that they have low caloric value, thus are less likely to contribute to lipid-related disorders such as obesity, atherosclerosis, hyperlipidemia, and cardiovascular diseases. The ash content of biological samples measures their mineral constituents, providing valuable insight into the mineral composition and nutritional value of the sample. Ash content is a reflection of the total inorganic matter, including essential minerals such as magnesium, sodium, potassium, calcium, and trace elements like iron and zinc, which are vital for various physiological functions. By determining the ash content, researchers can assess the potential of a biological sample as a source of micronutrients.²⁹ In this study ash content value obtained (Table 1) was less than those of Dewole *et al.*¹⁶ as well as those of Ajai *et al.*³⁰ for *Cola acuminata* seeds. The values obtained in this study were in disparity with those obtained by Owuoye *et al.*¹⁴, which were lower, but Mgbemena *et al.*³¹ reported higher values.

Qualitative phytochemical composition of aqueous extract of Cola acuminata leaves

The phytochemical composition of *Cola acuminata* aqueous leaf extract shows that saponins were strongly present, while tannins, terpenoids, cardiac glycosides, and reducing sugars were moderately present, while flavonoids, steroids, alkaloids, and coumarins were not detected (Table 2).

Phytochemicals are plant chemical compounds that elicit widely varied pharmacological activities and have been isolated for their health benefits.³² Otoide and Olanipekun³³ reported a different result for flavonoids and alkaloids, but similar findings for tannins and saponins. Kanoma *et al.*³⁴ reported similar results for flavonoids but differed in other phytochemicals studied. Omwirhiren *et al.*³⁵ reported the presence of alkaloids, tannins, saponins, flavonoids, steroids, and cardiac glycosides in the aqueous extract of *Cola acuminata* seeds.

Alkaloids are nitrogenous organic compounds that elicit sedative, analgesic, anti-inflammatory, and antimalarial effects. Plant-derived alkaloids have shown potential in managing chronic pain and neurological disorders as they modulate neurotransmission pathways and interact with the central nervous system receptors.^{31,32} Alkaloids in *Cola acuminata* leaves therefore show potential therapeutic roles in managing infections, stress, and symptoms of depression.

Saponins are known to form foam when mixed in aqueous solutions, bind cholesterol, are bitter to taste, and coagulate red blood cells.^{35,36} The presence of saponins in this study shows that *Cola acuminata* leaf extract may act as an anti-inflammatory agent. In recent times, saponins are infused in household products like tooth paste, shampoos, liquid soap, cosmetics and in fire extinguisher foam.

Tannins are a large group of polyphenol having numerous hydroxyl groups and other groups like the carboxyl which form strong interactions with other macromolecules.³⁷ *Cola acuminata* leaves may elicit anti-secretolytic, anti-irritant, anti-phlogistic, anti-parasitic and antimicrobial effects due to the presence of tannins.³⁸ They are mostly used to produce leather in the tanning process and also for the management of burn, inflammation, gonorrhea and piles.³⁹

Cardiac glycosides are bioactive organic compounds which modulate heart function by inhibiting sodium-potassium ATPase pump, thereby increasing the availability of calcium in heart muscles which are needed for heart contraction. Cardiac glycosides in *Cola acuminata* leaves indicates that the extract can be harnessed for the management of heart related conditions like heart failure and some form of arrhythmias.⁴⁰

Minerals composition of Cola acuminata aqueous leaf extract

Minerals are very essential inorganic compounds required by the body for maintaining fluid and electrolyte balance, enzyme activation, nerve impulse transmission, muscle contraction, bone formation immune functions and other physiological roles.⁴¹ Deficiency or imbalances in mineral nutrients may result in adverse health conditions such as anemia, osteoporosis, cardiovascular disease, impaired immune responses, goitre and hypertension. Table 3 reveals the mineral constituent of *Cola acuminata* aqueous leaf extract contains phosphorus, potassium, sodium, magnesium, calcium, iron and zinc.

The amount of phosphorous, was highest, followed by potassium, sodium then magnesium. Calcium, zinc and iron were present in lower amounts. These essential minerals, including calcium, potassium, and magnesium, play a very vital role in maintaining overall well-being and enhancing various biochemical processes that are necessary for optimal cellular function. The result of this study was not in agreement with the reports by some researchers,^{14,42-46} but had some similarities with those obtained by Masittha *et al.*⁴⁷ This extract can thus be exploited as a rich source of novel drugs relevant for the management of anomalies related to mineral deficiencies as well as diseases associated with malnutrition.

Conclusion

Considering the results of the present research, it can be deduced that *Cola acuminata* aqueous leaf extract can be exploited as a valuable natural reserve of phytochemicals, nutrients and minerals that are essential for disease management. Further research is warranted to identify and isolate bioactive compounds present in the leaves. This would not only enhance our understanding of its therapeutic potential but also pave the way for the discovery of new, effective treatments derived from natural sources. Also, the results have corroborated evidence for the use of the plant in phytomedicine as well as contribute to the pool of scientific literature.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the article will be borne by them.

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