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Proximate, Mineral and Anti nutritional Composition of Kola nut (*Cola nitida)*

Benjamin, I.[1]*, Benjamin, A. Anhwange[1], Terhile, M. Iortile[1], Torna, T. Weor[1], Solomon, D. Igbawase[1], Tor, P. Ngunoon[2], Teghtegh F. Samoh[3], John O. Ajegi[4]

[1]Department of Chemistry, Benue State University, Makurdi, Nigeria
[2]Departments of Chemistry, Joseph Saruman Tarka University, Makurdi, Nigeria
[3]Departments of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria
[4]College of Education Oju, Benue State, Nigeria
*Corresponding Author: Benjamin. I. Department of Chemistry, Benue State University, Makurdi, Nigeria
Email: ishwahlizer@gmail.com

Abstract The Nutritional and Phytochemical Screening of *Cola nitida* were investigated. The Nutritional analyses include proximate analyses; Anti-Nutrient composition and Mineral composition. The Phytochemical parameters carried out were Flavonoids, Alkaloids, Saponin and Tannin. The proximate Analyses showed that the sample has a high level of Carbohydrate 68.24%, little amount of Crude Fibre and Ash 3.29% and 2.94% were shown in the analyzed samples. Also a considerable amount of protein and moisture content were shown 14.52% and 10.37 % respectively and negligible amount of Crude fat 0.64%. This composition show that the sample could be a good source of Carbohydrate, dietary fibre and protein. The Anti-Nutrients present are in negligible amount. The parameters are Oxalate (0.31%), Phytate (0.46%). The Mineral composition was determined by Atomic Absorption Spectrometry method. The concentrations of the minerals analyzed were: 0.017, 1.54, 0.007, 0.17 and 0.10 mg/g for Zn, Mg, Mn, Ca and Fe respectively. The concentration of copper (Cu) was not detected in the sample been analyzed. Quantitative Phytochemical analysis was also carried out on the Kola nut sample. The result obtained was 14.96, 13.50, 13.82, 9.40 and 14.13% for Flavonoid, alkaloids, tannins, cardiac glycosides and saponins respectively.

Keywords *Cola nitida*, Anti-nutrients, Mineral, Phytochemicals, Proximate

Introduction

Plants are important in human being everyday existence. They provide our foods, produce the oxygen that we breathe, and use as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our buildings and in the manufacture of dyes, perfumes, pesticides and drugs [1]. Kola (a member of the family *Sterculiaceae*) mostly produced in Africa and is cultivated to a large degree in Nigeria, but also in Ghana, Ivory Coast, Brazil and the West Indian Islands [2]. Annual production from these countries alone is in excess of 250,000 tons, while the world production is about 300,000 tons. Two species *Cola nitida* (ventenat) schott *and* Endlicher and *Cola acuminata* (Beauvoir) Schott and Endlicher are of major economic importance. *Cola nitida*, which is referred to as "the true kola of commerce" has featured in the internal trade of

West Africa for a number of centuries. Kola nuts are a common sight in Nigerian markets, cities and villages. They are often sold by street vendors at bus and train depots. Many Nigerians consume kola nuts regularly, even daily, for the medicinal, stimulating and sustaining properties [3]. Kola is an important economic cash crop to a significant proportion of Nigerian population who are involved in kola farming, trading and industrial utilization. However, Nigeria accounts for about 70% of the total world production of kola nuts. The kola nut is used as a masticatory and stimulant in the tropics and has social and traditional significance as it features in many traditional ceremonies in Nigeria. It also has industrial usage in pharmaceuticals, production of soft drinks, wines and in confectionaries [4]. It is commercially grown in the West where it is known as Obi in Yoruba, consumed by the Northerners where it is known as Goro in Hausa, and revered in the East where it is called Oji in Igbos, Goor in Tiv, Ikpali in Idoma and Egbêên in Igede. It has been reported that the seeds of the plant has the potential to meet year-round carbohydrate and protein requirements of the most vulnerable population if domesticated [5]. The seeds are known to contain high amount of carbohydrate, protein, minerals and fibre content. However, *C nitida* is also known to contain anti-nutritional substances. Anti- nutritional factors are secondary metabolites found in plants and are known to be biologically active substances. These substances are found in fruits, seeds, and other parts. They occur in varying amounts depending on the kind of crop, and their m ode of propagation [6]. Research revealed that anti-nutrients are chemicals produced by plants for their defense and other biological functions. Most of these secondary metabolites have deleterious effect on human beings. However, they could be beneficial to humans and animals if consumed in appropriate amounts. It has been reported that the intake of phytate, tannins and Saponins at low levels reduces blood glucose and increases insulin responses to starchy foods, while phytates, tannins, saponins, protease inhibitors and oxalate reduces cancer risks in both human and animals [7].

Research has proved that oxalate and phytate form chelates with di and trivalent metallic ions such as Iron, Cadmium, Magnesium, to form poorly soluble compounds that are not readily absorbed by the gastrointrotestinal tract, thereby reducing their bioavailability in the body. These anti-nutritional factors can be reduced by some processing methods such as roasting, soaking, boiling, and fermentation, among others. [8].

Objectives of the Research Study

The aims and Objective s of this research work is to qualitatively and quantitatively analyze the Nutritional components of *C. nitida* and to qualitatively and quantitatively carry out the Phytochemical screening of the sample.

Materials and Methods

Study Area

The research was conducted in Makurdi, town the Benue State capital. The town is located at latitude 7^{o} 38'N - 7^{o} 50'N and longitude 8^{o} 24’E - 8^{o} 38'N. It is situated in the Benue valley in the North Central Nigeria.

Sample Collection and Preparation

Fresh samples of the Kola nut seeds were purchased from Adikpo market, Kwande Local Government Area of Benue State and were taken to Department of Biology Science of Benue State University Makurdi for Identification and authentification

Dirt's and other extraneous materials were removed from the nuts with a stainless-steel knife. The seeds were washed, chopped into pieces and allowed to air dried in the Laboratory for 3 weeks and thereafter reduced to powdered form using mortar and pestle.

Sample Digestion

Exactly 2g of the powdered Kola nut seeds was weighed into a crucible and muffled in the furnace. The ash sample was transferred quantitatively into a conical flask and dissolved in 2 mL of concentrated HNO_3 and 1 mL of concentrated HCl and the mixture was heated on a hot plate. The solution was then filtered into a 100 mL volumetric flask and made up to the mark with distilled water. The digested sample was then stored in a 100mL volumetric flask prior to analysis with Atomic Absorption Spectrometer.

Determination of Mineral Contents

Calcium, magnesium, zinc, iron, copper and manganese were analyzed after digestion with HNO_3 and HCl using Atomic Absorption Spectrophotometer (Model Buck 2006, Buck Scientific, USA) with appropriate hollow cathode lamps. Accuracy was assessed by analyzing the samples in triplicate.

Determination of Proximate Composition

Moisture content

The method of Association of Official Analytical Chemists (AOAC) (1995) was employed using hot air-drying oven. Empty clean crucible dish was dried in the oven at a temperature of 105ºC for one hour and cooled in a desiccator. 5g of the sample was sample weighed and put in the dish and heated for 3 hours at a temperature of 105ºC [9]. The dish was then removed from the oven, cooled in a desiccator and weighed. The moisture content was calculated as:

$$\text{Moisture (\%)} = \frac{\text{weight loss}}{\text{ample weight}} \times 100 \quad (1)$$

Ash content

The method of AOAC (1995) was used to determine the percentage of ash content. Exactly 5 g of the sample was weighed in to a pre-heated and cooled crucible and incinerated in a muffle furnace at 200ºC for 4 hours. The ash was then cooled in a desiccator and weighed. The ash content was calculated using;

$$\text{Ash (\%)} = \frac{\text{weight of Ash}}{\text{weight of sample}} \times 100 \quad (2)$$

Crude fibre

The powdered sample (5 g) was weighed and placed in 500mL conical flask containing 200 cm^3 of 1.25% H_2SO_4 and were boiled gently for 30 minutes. The content was filtered and the residue was scrapped back in to the flask with a spatula. 200 cm^3 of 1.25% NaOH was added and were allowed to boil gently for 30 minutes. The content was filtered and washed thoroughly with hot distilled water. The precipitate was rinsed once with 10% HCl and twice with ethanol. The content was allowed to dry and the residue was scraped into a weighed crucible and was dried overnight at 105ºC in hot oven. It was cooled in desiccator. The sample was then heated at 600ºC for 90 minutes in a furnace. It was finally cooled in a desiccator and weighed again [9, 10].

The percentage crude fibre was calculated using the equation below:

$$\text{Crude fibre (\%)} = \frac{\text{weight loss on ignition}}{\text{weight of sample}} \times 100 \quad (3)$$

Crude fat

The dried sample (2 g) was weighed into a porous thimble, and its mouth covered with cotton. The thimble was then placed in an extraction chamber, and then suspended above a receiving flask containing petroleum ether (BP. 40 – 60ºC). The flask was then heated on hot mantle and the oil was extracted. The extraction continued for eight hours after which the thimble were removed from the Soxhlet and heated over water bath, the flask containing the oil was disconnected, cleaned up and placed in an oven at 100ºC for 30 minutes. The flask was then cooled in desiccator and weighed. The percentage crude lipid content was calculated using [10].

$$\text{Crude fat (\%)} = \frac{\text{weight of oil extractd}}{\text{weight of sample}} \times 100 \quad (4)$$

Crude proteins

5g of the sample was weighed in to a Kjeldahl digestion flask and catalyst Na_2SO_4, $CuSO_4$ and selenium Oxide in (10:5:1) were added to the sample which were followed by 10 cm^3 of concentrated H_2SO_4. The content in the flask was then heated in the Kjeldahl digestion flask for one and half hour, ensuring that digestion was completed. The flask was cooled and the content diluted with 10mL distilled water. The diluted content was filtered in to 100mL volumetric flask and was made up to the mark with distilled water. 10 cm^3 of the aliquot was taken into digestion

flask and 20cm^3 of 45% NaOH solution were added to it. The content was diluted to about 200 cm^3 with distilled water and distilled using micro Kjeldahl distilled apparatus. The distillate was received into a flask containing 10 cm^3 boric acid solution indicators after the distillation.

The distillate was then titrated against 0.01 M HCl to the end point [10].

$$\text{Crude protein (\%)} = \frac{\text{TV} \times \text{C} \times \text{F} \times \text{V1}}{\text{W} \times \text{V2}} \times 100 \quad (5)$$

Where: TV = Titre Value of the Acid; C = Concentration of Acid used; V1 = Volume of the distilled water used for diluting the digest; V2 = Volume of aliquot used for titration;

W = weight of Sample used; F = protein Multiplication Factor 6.25

Carbohydrate

The total amount of carbohydrate in the sample was obtained by using the weight difference percentage. This was done by subtracting the percentage sum of the food nutrients (% crude protein, % crude fat, % crude fibre, % moisture content and % ash) from 100% dry weight. Percentage carbohydrate was calculated, using the formula below [10].

Carbohydrate (%) = 100 – (Protein + Fat + Fibre + Ash + Moisture). (6)

Quantification of phytochemicals

The quantity of the bioactive compounds such as tannins, saponins, flavonoids, cardiac glycosides, alkaloids and phenols present were analyzed using standard methods as follows.

Determination of tannins

Exactly 1g of the sample was weighed and transferred into a bottle. 10mL of distilled water was and added and stirred at 5 minute interval for 30 minutes then filtered. A total volume of 2.5mL of the filtrate, (sample) standard tannic acid solution and distilled water was added into test tubes, labelled sample standard and blank respectively. 1.0 of Folin-Denis reagent was added to all the test tubes followed by 2.5mL of saturated sodium bicarbonate solution was then added and allowed to incubate at room temperature for 90 minutes. The absorbance of the sample and the standard was read against the blank at 490nm [11].

The percentage of tannin will be calculated thus:

$$\text{Tannin (\%)} = \frac{\text{AT} \times 100 \times \text{Vf}}{\text{AS} \times \text{W} \times \text{Va}} \times \text{C} \quad (7)$$

Where: AT = Absorbance of the test sample

AS = Absorbance of the standard solution

C = Concentration of standard solution

W = Weight of the sample used

Vf = Total volume of the extract

Va = Volume of the extract analyzed

Determination of saponins

Exactly 2g of powdered sample were put into a conical flask and 100mL of 20% aqueous ethanol added. The sample was heated over a hot water bath for 4 hours with continuous stirring at 55ºC. The solution will be filtered and the residue re-extracted with 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at 90ºC. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Furthermore, 60 mL of n-butanol was added and washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight in a measured crucible. The saponins content was calculated using standard formulae [11].

$$\text{Saponins (\%)} = \frac{(\text{Weight of crucible+saponin}) - (\text{Weight of empty crucible})}{\text{weight of sample used}} \times 100 \quad (8)$$

Determination of alkaloids

About 5 g of the sample was weighed and added into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered with aluminum foil and allowed to stand for 4 hours. After which the solution will be filtered with whatman filter paper. 12 mL of ammonium hydroxide solution will be added to the filtrate and allowed to cool. The precipitate will be dried in the oven at 60ºC and reweighed to determine the weight of the alkaloid.

$$\text{Alkaloid (\%)} = \frac{(\text{Weight of filter paper+alkaloid})-(\text{weight of filter paper})}{\text{Weight of sample used}} \times 100 \quad (9)$$

Determination of cardiac glycosides

The sample (1g) was soaked in 10 mL of 70% alcohol for 2 hours and then filtered. The extract obtained will then be purified using lead acetate and Na_2HPO_4 solution. Freshly prepared Bullet's reagent (containing 95 mL Aqueous picric acid and 5 mL 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gives the absorbance and is proportional to the concentration of the glycosides [11]

Determination of flavonoids

10g sample was weighed and dispersed into 100 mL of 80% aqueous ethanol, mixed properly and allowed to stand for 1 hour after which it will be filtered with Whatman filter paper into a weighed crucible and concentrated to dryness in an oven at 60ºC. The crucible would then be weighed to determine the both weight.

$$\text{Flavonoid (\%)} = \frac{(\text{Weight of crucible+flavonoid})-(\text{weight of empty crucible})}{\text{weight of sample used}} \times 100 \quad (10)$$

Results and Discussion

Table 1: Proximate composition of Kola nut (*Cola nitida*)

Component	% Composition
Moisture	10.37±0.31
Ash	2.94±0.55
Crude Fat	0.64±0.01
Protein	14.52±0.43
Crude Fibre	3.29±0.18
Carbohydrate	68.24±1.04

Values are averages ± mean deviation of triplicate determination.

Table 2: Mineral composition of Kola nut (*Cola nitida*)

Mineral	Amount (mg/g)
Zinc	0.017±0.000
Magnesium	1.541±0.004
Manganese	0.001±0.000
Calcium	1.166±0.001
Iron	0.100±0.001
Copper	Not detected

Values are mean ± standard deviation of triplicate determination.

Table 3: Quantitative Phytochemical screening of Kola nut (*Cola nitida*)

Parameter	Amount (%)
Flavonoid	14.98±0.03
Alkaloids	13.70±0.28
Tannins	13.87±0.07
Cardiac Glycosides	9.15±0.35
Saponins	14.18±0.07

Values are mean± standard deviation of duplicate determination

Table 4: Quantitative anti-nutrient screening

Parameter	Amount (%)
Oxalate	0.521±0.01
Phytate	0.620±0.01
Trypsin inhibitor	0.450±0.05

Values are mean± standard deviation of duplicate determination

Table 5: Qualitative anti-nutrient screening

Parameter	Amount (%)
Oxalate	++
Phytate	++
Trypsin inhibitor	++

(+): Trace Amount Present; (+) (+), Abundant Amount Present; (-): NO Amount Present

Moisture content

The value of the moisture content obtained for this study was 10.37% for *C. nitida*. The value is slightly below for that obtained by some researchers on *C. nitida* with the moisture content of 12.46% [12] and 12.85 [13]. On the other hand, the value obtained for moisture content is above the value reported by [14]. The variation may be the effect of the drying time for the samples before analysis. Some authors reported in their study that the moisture content of a sample varies by drying time of the samples. The high moisture content is an index of spoilage. Too much of moisture in any food sample can make the sample viable for microorganism's growth. This accounts for most of the biochemical and physiological reactions in the plant [14].

Ash content

The result for Ash obtained in this study is shown in Table 1. The value obtained was 2.94%. This value is high when compared to that reported by some authors on the preliminary works on *C. nitida* [15] obtained 2.15% and [16] obtained 1.5%. Similarly, the result obtained for this present study was low to the obtained with *C. nitida* (4.30%) as reported by [17].

Crude fat content

The value of crude fat obtained in this study as shown in Table 1 is 0.64%. This result can be comparable to 0.87% obtained by some authors on the study of *C. nitida* [18]. Contrary to our study, some researchers have showed that the fat content of *C. nitida* seeds (5.71%) was high to those obtained with *C. nitida* (0.64%). The low lipid content of the *C. nitida* is predictable because these seeds are not oleaginous [18]

Protein content

Proteins, another class of food often times referred to as the 'Nitrogen-containing natural product' has been proved to be essential for the survival of human beings and Animals. Proteins have also been reported to be composed of building blocks known as Amino Acids [19]. The sample shows a considerable amount of protein (14.52 %) as shown in Table 1. This value can be comparable with those reported by other researchers on C. *nitida (*10.06%) [20]. On the other hand, the result of this study is lower than those obtained by [21] on C. *nitida* (15.24%). The difference between these values may be due to soil and ecological condition because the water deficit influences the nitrogen supply in the plant. The protein content relatively high could be compliment the body's need of these essential nutrients for growth and development because proteins, another class of food often times referred to as the "Nitrogen containing natural product" has been proved to be essential for the survival of human beings and animals [21, 22].

Crude fibre content

The value for crude fibre obtained in this study was 3.29% as shown in Table 1. This result is comparable with that obtained by other works for preliminary screening of *C. nitida*. The value obtained in this study is slightly above that obtained by other work on *C. nitida* (6.45%) [23]. This implies that *C. nitida* contain little amount of cellulose, hemicelluloses and lignin which aid digestion. Dietary Fibre is reported to lower the risk of coronary heart disease.

Carbohydrate content

Carbohydrate or Saccharrides are the most abundant biological molecules. They play important roles in the body as sources of energy as well as structural material [24]. The result of in this study confirmed that that *C. nitida* has a very high percentage of carbohydrate as also reported by other researchers. The value obtained in this study was 68.24%. Some researchers reported in their study high content of carbohydrate 70.01 and 73.20% respectively [24, 25]. This implies that Cola species is a good source of carbohydrate. The varying composition reported by different authors may imply that the proximate composition of these nuts varies with season, environment and/or condition or time of evaluation.

Mineral content

Minerals are important component of diet because of their physiological and metabolic functions in the body. The concentrations of the six mineral elements in the sample investigated are shown in the Table 2.

Zinc

The value for zinc obtained from this study as shown in Table 2 (0.017mg/g) revealed that the concentration of zinc is low. The value is low compared to the values observed in the research of [26] with 0.69mg/g. Zinc is very essential as co-factor for enzymes in protein and oxidation process. Zn ion (Zn^{2+}) is active in all aspects of intermediary, storage, synthesis and action of peptide hormones and structural maintenance of chromatin and bio membranes.

Magnesium

The result of magnesium in the Table 2 shows that the concentration of magnesium in the current study is 1.54mg/g which shows that magnesium is the most abundant mineral in Kola nut amongst the other minerals analyzed. The value is however low when compared to the values obtained by [26, 27] which are 2.32mg/g and 11.48mg/g. The recommended daily dietary intake of magnesium is between 50-400mg. Magnesium is important, especially within cells, being the second most common intracellular cation after potassium, with both of these elements being vital for numerous physiological functions [27].

Manganese

The Table 2 shows manganese to be present in the research with the concentration of 0.0007mg/g. From the result manganese had the lowest concentration amongst the detected mineral elements in the study. Manganese is considered as part of the enzyme body system

Calcium

From the Table 2, the concentration of Calcium is 1.17mg/g. The concentration of calcium in this study is relatively high compared to the level reported by [28] with concentration of 0.72mg/g. On the other hand, the concentration is relatively low when compared to the 4.33mg/ g reported by [29]. The recommended daily dietary intake is between 360-1200mg. This mineral element supports human biochemical processes by serving structural and function as role as electrolytes [30]

Iron

From the Table 2, the concentration of iron is 0.10mg/g. The concentration of iron in Kola nut in this study is relatively low when compared with the values reported by [31, 32]. Iron plays a vital role for human health. Iron is required by the body for the synthesis of oxygen transport proteins, hemoglobin and myoglobin in particular and also for the formation of enzymes involved in electron transfer.

Copper

From the Table 2, copper was not detected in the analysis of the mineral composition of Kola nut. However, other researchers reported the presence of copper with the concentration of 0.59mg/g [33].

Quantitative Phytochemical Screening

Flavonoids

The result of flavonoid is presented in Table 3. The value obtained for this study was 14.96%. This value is above the values reported by other authors (6.33% and, (5.00%) [34] for white and red Kola nut. This result shows that the total flavonoids represent a large portion in *C. nitida* and can be a good dietary source of flavonoids. Flavonoids which are generally found in a variety of foods such as kola nuts, oranges, tangerines, berries, apples and onions have protective effects including anti-inflammatory, anti-oxidant, antiviral and anti-carcinogenic properties [34].

Alkaloids

The concentration of alkaloid in the *C. nitida* was found to be 13.50%. The result from this study is above the results obtained by other researchers on the Phytochemical screening of kola nut cultivated in the Southern part of Nigeria with values 2.06% and 0.80 % [35], but lower compared to the value obtained by [36] 14.40% and 16.07% for white and red Kola nut respectively. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and anti-bacterial properties. The alkaloid compounds found in kola nuts can therefore be useful in pharmaceutical preparations [36].

Tannins

The quantitative Phytochemical screening of the *Cola nitida* in this study revealed the presence of tannins with a concentration of 13.82%. This result is not consistent with that reported by other researchers on phytochemical evaluation of three species of Kola nuts cultivated in Niger State with values ranging from 13.20, 14.83 and 15.43 mg/g respectively. The presence of tannin in the plants implies they may have astringent properties and in addition, could quicken the healing of wounds and burns. Tannins are water soluble phenolic compounds with reported anti-nutritional effects and may be by their ability to form complex with proteins. Tannins have been reported to impact beneficial effects because they reduce the wasteful protein degradation in the body by the formation of a protein-tannin complex [37].

Cardiac glycosides

The value for glycosides obtained from this study was 9.40% been lower than that of alkaloid, flavonoids, saponins and tannins. This showed that *C. nitida* has a low concentration of glycosides. The value obtained from this study is lower than that obtained by some researchers on the antioxidant and neuroprotective effects of estrogens and phenolic compounds with a value of 10.28mg/g. The beneficial medicinal uses of Kola nut are as treatments for congestive failure, cardiac arrhythmias, cough, asthma, migraine, diarrhea and anti-depressant can be attributed to the presence of Cardiac glycosides present in the seed [38]**.**

Saponins

The value for saponins obtained from this study (14.13%) revealed that *C. nitida* is a good source of saponins. The result obtained in this study is slightly below that reported by other authors with values 19.43mg/g, 18.30mg/g and

15.42mg/g for three different species of Kola nut cultivated in Benin. The high value of saponins could be attributed to the fact that Kola nut seeds are glycosides containing a polycyclic aglycone moiety either C_{27} steroid or C_{30} triterpenoid attached to carbohydrate and characterized by a bitter, antibacterial and foaming properties

The quantitative and qualitative anti-nutrient composition revealed that C. *nitida has* some anti-nutrients. Oxalate was found to be 0.521%, Phytate 0.620% and Trypsin inhibitor 0.450% respectively. Oxalate combines with divalent metallic cations like Iron, Magnesium and Calcium to form crystals of corresponding oxalates which are then excreted in the urine. Phytate on the other provides a medium for phosphorus storage and as a source of cation and myoinositol. Trypsin inhibitor is a protein that reduces the biological activity of Trypsin by controlling the activation and catalytic reactions of proteins [38 39]. Trypsin inhibitor therefore acts as an irreversible and competitive substrate thereby interfering with chymotrypsin function [40].

Conclusion

From the research, it has to be established through Nutritional screening that *Cola nitida* can be used as a good source of Carbohydrate and protein. It also contains moderately amount of minerals, proximate needed for growth and metabolic activities in the body, development of bones, regulation of acid base balance and the transmissions of nerve impulses, despite the trace amount of anti-nutrients. The Phytochemical composition also revealed that *Cola nitida* can be useful in the medicinal and pharmaceutical industries to make vaccines and supplements that can prevent diseases. *Cola nitida* can also be useful in various manufacturing industries as a raw material

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