Niger. J. Physiol. Sci. 40 (June 2025): 197 – 201
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full-length Research Article

Methanolic Extract of Kola Nut (*Cola acuminata*) Decreased Body Weight and Elevated Total Plasma Cholesterol Level in Rats (Rattus norvegicus)

***Asogwa C.N.[1] and Osibe D.[2]**

[1]*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.*
[2]*Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria.*

Summary: Kola and kola-containing beverages are among the most consumed products globally for their stimulatory and energy-boosting potentials. The precise impact of kola nut consumption on various biological indices of consumers remains debatable, necessitated by the fact that the phytochemical compositions of kola nuts are dependent on a range of factors. This study investigated the impacts of a 28-day administration of a methanolic extract of *C. acuminata* on the body weight and serum cholesterol indices of adult male albino rats (Wistar strain). The quantitative photochemical compositions and lethal dose (LD50) of the extract were determined using standard bioassay procedures. Rats were randomized into four experimental groups that received various concentrations of the extract: 0, 100, 150, and 200 mg/kg. Results show an LD_{50} of 3101.37 mg/kg for the extract, whereas flavonoids and tannins were the most abundant phytochemicals. In addition, extract administration caused a dose-dependent reduction in body weight, while significant elevations in total serum cholesterol levels were recorded in rats that received 100 and 150 mg/kg of the extract. These suggest that kola nut consumption may negatively impact body weight and cholesterol metabolism.

Keywords*: Kola nut, Cola acuminata, body weight, cholesterol, lethal dose*

**Author for correspondence:* Email: chinweike.asogwa@unn.edu.ng, *Tel: +234-9037981062*

Manuscript received: December 2024; Accepted: April 2025

DOI: https://doi.org/10.54548/njps.v40i1.23

INTRODUCTION

Kola and kola-containing beverages are one of the heavily consumed nervous system stimulants and energy boosters globally. Common sources of kola include various species of kola nuts, *C. nitida* and *C. acuminata*, and many coca-cola beverages (Adesida *et al*., 2021). Nigeria accounts for about 70 % of world kola nut production (Quarco, 1969; Jacob, 2023), 90% of which are consumed locally (Quarco, 1973). Quantitatively, kola nuts are rich in water, carbohydrates, ash, alkaloids, flavonoids, tannins, and cellulose (Purseglove, 1968, Igbinovia *et al*., 2009). The stimulatory and energy-boosting potentials of kola nuts are due to its caffeine and other methylxanthine alkaloid contents (Jacob *et al*., 2023). Although there is no extant data on the precise rate or dynamics of consumption. Kola nut, especially *C. nitida* and *C. acuminata* are greatly employed as a symbol of hospitality (Purseglove, 1977), and during cultural, religious, and social gatherings (Jacob *et al*., 2023). Kola nuts are also used in the production of various beverages and wines (Beattie, 1970; Ogutuga, 1975; Ajiboye & Afolayan, 2009).

As stimulants and energy boosters, there are undocumented claims of local farmers, especially in Africa, subsisting on water and kola for hours without food. Again, it is common in Nigeria, including among students, to chew kola nut seeds to stay awake for various reasons. For instance, Erinfolami *et al*. (2011) found 11.2%, 29.1%, and 74.8% for 30-day, one-year and lifetime prevalence rate of kola nut consumption among secondary school students most of who started chewing kola nut from the age of 14 in Osogbo, Osun State, Nigeria. That study also found that several factors, including poor school attendance, polygamy, low maternal and high paternal educational attainment, and over-permissiveness on the part of the mother. Consequently, the rate of kola nut consumption appears to be on the increase even in these modern times, strengthening the growing concern about potential negative impacts of kola consumption on crucial biological indices of consumers of different ages, socio-economic status, and health. However, most extant studies on the impact of kola nut consumption focused on *C. nitida* (Ikegwuonu *et al*., 1981; Obidike *et al*., 2011; Nku *et al*., 2014; Ewenighi *et al*., 2016), making us wonder if *C. acuminata* consumption would elicit harmful or beneficial consequences. For instance, Asogwa *et al*. (2014) reported dose and time-dependent changes in the stress response and inflammatory biomarkers of male Albino rats associated with an in vivo exposure to methanolic extract of *C. acuminata*. These changes were typified by elevated serum cortisol levels and leukocytosis. We reasoned that *C. acuminata*, which is most

consumed of all kolas in Eastern Nigeria, may have a wide-ranging effect on different biological systems apart from the stress hormonal and leukopoietic impacts earlier reported (Asogwa *et al*., 2014). So, we extended our investigation to evaluate the potential effects of *C. acuminata* extract on the body weight and total plasma cholesterol levels of adult male Albino rats (Rattus norvegicus) for 28 days. We first determined the quantitative phytochemical composition and lethal dose of *C. acuminata* used in this study. Our findings are consistent with the hypothesis that *C. acuminata*¸ like C. nitida, has weight-reducing potentials and an obvious tendency to spike total plasma cholesterol levels. The extent to which *C. acuminata* impacts on the body weight and lipid biochemical indices of human consumers remain to be ascertained.

MATERIALS AND METHODS

Procurement of kola nut seeds: Seeds of *C. acuminata* were purchased from Ogige Market, Nsukka, Enugu State, Nigeria. Identity of the seeds was authenticated at the Taxonomy Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Experimental animals: Adult male albino rats (*Rattus norvegicus*, Wistar strain, 180-200 g) used for this study were purchased from the Genetics and Animal Breeding House, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They had *ad libitum* access to commercially available rat chow and water for the duration of the investigation.

Preparation of *C. acuminata* extract: The methanolic extract of *C. acuminata* was prepared as described earlier (Asogwa *et al*., 2014). Briefly, 2 kg of the seeds of *C. acuminata* was floor-dried and pulverized with an electric blender. 50 g of the powder was put into a conical flask to which 200 ml of absolute methanol was added. The mixture was allowed to stand for 24 hr. It was then filtered using a clean muslin cloth. The extract was concentrated to dryness using a rotary evaporator. The concentrated extract was used to prepare various concentrations administered to the experimental animals.

Quantitative phytochemical analysis: Detailed quantitative phytochemical determinations were previously described: alkaloids (Henry, 1973), tannins (Dawra *et al*., 1988), saponins (Brunner, 1984), flavonoids (Zhishen *et al*., 1999), terpenoids (Łukowski *et al*., 2022), steroids (Birner, 1969), and total cyanide (Haque & Bradbury, 2001).

Determination of lethal dose (LD_{50}): The lethal dose (LD_{50}) of the extract was determined according to the method of Lorke (1983). Briefly, a preliminary test was done using 10, 100, and 1000 mg/kg body weight of the extract (n=5 mice/group). When no mortality was recorded after 24 hr, a new set of mice administered 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg of the extract (n=5 mice/dose). The number of dead mice were recorded for 24 hrs, while a probit curve was plotted to deduce the LD_{50} of the extract.

Experimental design: A total of 60 adult male albino rats were randomly divided into four groups (0, 100, 150, and 200 mg/kg, 3 replicates/group, n=5/replicate), each group with 3 replicates (n=5/replicate). The rats were maintained at optimum laboratory conditions (25^{o}C, 12L/12D photoperiod) with *ad libitum* access to food and water. Rats were weighed using a sensitive balance before extract administration and after every 7 days following administration.

Collection of blood sample: Blood samples were collected from the orbital sinus before the commencement of extract administration and every other 7 days. Blood was allowed to clot and centrifuged at 1200 rpm to separate the serum. Serum samples were stored frozen if not analysed immediately (Machado *et al*., 2009). All Biochemical analyses were performed at Shalom Diagnostic Laboratories, Nsukka Local Government Area, Enugu State, Nigeria.

Determination of total cholesterol concentration: Total plasma cholesterol levels were determined as previously described (Kishi *et al*., 2002) using a commercially available diagnostic kit (Randox, Germany). This method was based on the principle that cholesterol esters are hydrolysed by cholesterol esterase to free cholesterol and fatty acids, while free cholesterol is oxidized to cholest-4-ene-3-one and hydrogen peroxide, which in the presence of phenol and amino-4-antipyrin forms a red complex whose optical absorbance is measured at 550 nm.

Statistical Analysis

The data obtained were subjected to a Two-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS ver. 20 for Windows, IBM Statistics, USA). Post hoc tests (Duncan) were utilized to identify significant changes in the mean body weight and total cholesterol of both the control and treated groups. Statistical significance was set at $p \leq 0.05$, and the results were presented as mean ± standard error of the mean (SEM).

.

RESULTS

Quantitative phytochemical composition of the methanolic extract of *C. acuminata:* Our phytochemical analyses revealed in qualitative and quantitative terms, the presence of saponins, alkaloids, flavonoids, tannins, terpenoids, and cyanides (Table 1). Additionally, the concentration of alkaloid was only lower than those of flavonoids and tannins, and with a negligible amount of cyanide.

Table 1:
Phytochemical compositions of the methanolic extract of *C. acuminata*

Phytochemicals	Qualitative	Quantitative (mg/100 g)
Saponins	+	0.31±0.004
Alkaloids	++	3.06±0.0032
Flavonoids	++	3.90±0.0027
Tannins	++	3.64±0.0036
Terpinoids	+	1.32±0.002
Steroids	+	1.51±0.003
Cyanide	+	0.31±0.004

Notes. +: present, ++: moderately present. Each determination was performed in triplicates (n = 3). Table shows a higher amount (mg/100 g) of flavonoids, tannins, and alkaloids than saponins, terpinoids, and steroids. Quantitative values are Mean±SEM.

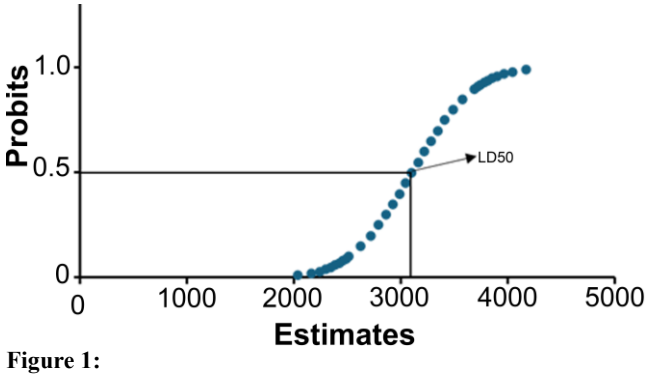

Figure 1:
Dose-response curve of the methanolic extract of *C. acuminata*. Adult male mice (n = 3 for each of the 10, 100, 1000, 1600, 2900, and 5000 mg/kg doses) were used for the LD_{50} determination. Rectangular tracing shows the estimated dosage that would cause a 50% mortality of the test mice.

Lethal dose (LD_{50}) of the methanolic extract of *C. acuminata*: The acute toxicity test of the methanolic extract of *C. acuminata* revealed an LD_{50} of 3082.81 mg/kg of body weight (Figure 1).

Effects of the methanolic extract of *C. acuminata* on body weight: The administration of graded doses of *C. acuminata* caused a time-dependent increase in body weights of rats in the 100 mg/kg group on day 28, while rats that received 200 mg/kg experienced a time-dependent reduction in body weight starting from day 7 until day 28 (Figure 2, replicates/group, n = 5/replicate, Two-way ANOVA, $p \leq 0.05$). Similarly, there was a dose-dependent reduction in body weight of rats given 200 mg/kg of extract on day 21, and rats that received 150 and 200 mg/kg on day 28 (Figure 2, replicates/group, n = 5/replicate, Two-way ANOVA, $p \leq 0.05$).

Effects of the methanolic extract of *C. acuminata* on serum total cholesterol concentration: The study recorded a significant time-dependent elevations in serum total cholesterol concentrations in the 100 and 150 mg/kg treatments on Days 14 and 21 compared with baseline values (Figure 3, replicates/group, n = 5/replicate, Two-way ANOVA, $p \geq 0.05$). However, no significant (Figure 3, replicates/group, n = 5/replicate, Two-way ANOVA, $p \leq 0.05$) elevations in serum cholesterol concentration were recorded in the 200 mg/kg group except on day 7 of extract administration (Figure 3, replicates/group, n = 5/replicate, Two-way ANOVA, $p \leq 0.05$). On the other hand, it was only on day 7 that a significant dose-dependent elevations in total serum cholesterol was recorded (Figure 3, replicates/group, n = 5/replicate, Two-way ANOVA, $p \leq 0.05$).

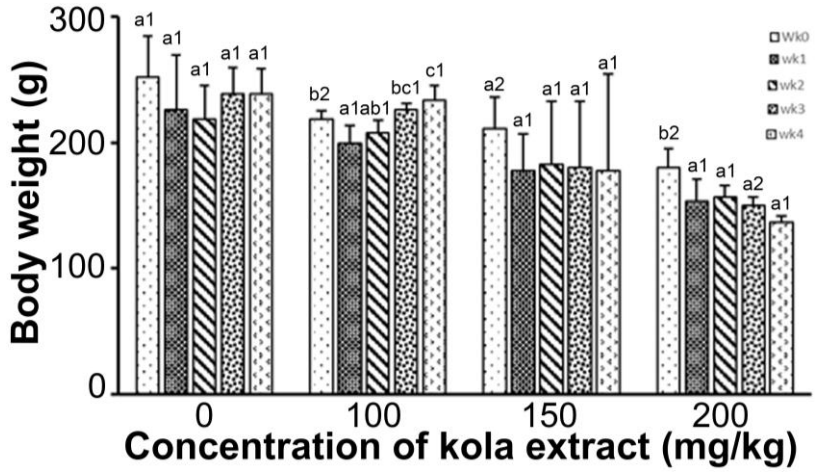

Figure 2:
Effects of a 28-day administration of the methanolic extract of *C. acuminata* on the body weight of adult male albino rats. Different bars with different alphabets within a treatment group are significantly different (3 replicates/group, n = 5/replicate, One-way ANOVA, $p \leq 0.05$). The same bars with different numbers across the treatment groups are significantly different (3 replicates/group, n = 5/replicate, One-way ANOVA, $p \leq 0.05$). Plot shows clear dose-dependent reductions in the body because of extract administration. Plotted values are mean±SEM. Error bars represent SEM.

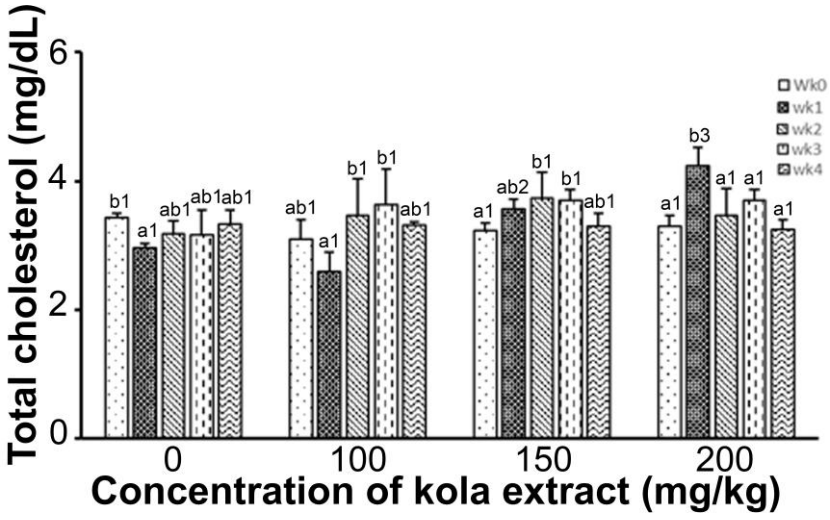

Figure 2:
Changes in total plasma cholesterol levels of adult male albino rats administered graded doses of the methanolic extract of C. acuminata for 28 days. Plotted values are mean±SEM. Error bars represent SEM. Top right: legends. Different bars with different alphabets within a treatment group are significantly different (3 replicates/group, n = 5/replicate, One-way ANOVA, $p \leq 0.05$). The same bars with different numbers across the treatment groups are significantly different (3 replicates/group, n = 5/replicate, One-way ANOVA, $p \leq 0.05$). Plot depicts a gradual dose-dependent elevation in total plasma cholesterol levels in the treated groups compared with the control (0 mg/kg).

DISCUSSION

This study investigated the proximate composition and impacts of chronic sublethal doses of the methanolic extract of *C. acuminata* on the body weight and some lipid profile biomarker of adult male albino rats for 28 days. Our findings reveal that the extract contains varying amounts of alkaloids, tannins, flavonoids, terpenoids, steroids, and cyanide. In addition, at the highest dose administered, methanolic extract of *C. acuminata* strongly reduced the body wight of albino rats. Additionally, the two lowest doses of the extract caused a significant time-dependent increase in the serum cholesterol concentration relative to baseline values.

Although we found similar phytochemical constituents in the kola seeds used in this study, there are obvious variabilities in the specific amount of each phytochemical present. For example, whereas we found that flavonoids and tannins were the most abundant phytochemicals followed by alkaloids and tannins, Osaro *et al*. (2024) reported that tannins and alkaloids followed by saponins and flavonoids. On the other hand, Uwabunkeonye *et al*. (2015) reported higher amounts of tannins and phenols compared with alkaloids and flavonoids. These findings demonstrate that although *C. acuminata* contains a lot of beneficial phytochemicals, the presence of methylxanthine alkaloids points to the fact that potentially unrecorded harmful effects may be associated with *C. acuminata* consumption. The discrepancies between our findings and previous studies could be due to differences in farming, soil, humidity, post-harvest handling, and method and length of storage (Touati *et al*., 2014; Kapcum & Uriyapongson, 2018; DeBenedictis *et al*., 2023; Tedeschi *et al*., 2023).

The effects of methanolic extract of *C. acuminata* on body weight and lipid profile indices recorded in this study compares largely with previous investigations in humans and rodents. For instance, intraperitoneal injections of 20 and 30 mg/kg caffeine extract of Cola nitida into adult male albino rats resulted in a significant reduction of body weight compared with the control and 10 mg/kg injected group. Similarly, Umoren *et al*. (2009) and Salahdeed *et al*. (2009) reported that supplementing rat diet with different concentrations of *C. nitida* extract, or the caffeine extracted thereof, resulted to a significant weight reduction in the exposed rats. Even though these studies employed C. nitida, another species of *C. acuminata*, and that the routes of administration differed, the observation that kola or its extracts have weight-reduction potential is consistent with our current findings. It is possible that *C. acuminata* extract may have modulated the activity of the feeding/satiety center that could have led to changes in feeding habit, ultimately resulting to apparent changes in body weight. Although the likely extract-related changes in feed consumption were not recorded in this study, previous studies showed that kola or caffeine ingestion caused a significant change in dietary intake in both humans and rats. The possible impacts of *C. acuminata* on food and feeding habits remain to be ascertained.

Earlier investigations found that diet supplementation with *C. nitida rubra* (15 g/ 100 g of rat feed) significantly elevated total cholesterol concentration, whereas a higher supplementation (30 g/100 g of rat feed) significantly reduced total cholesterol levels compared with control rats (Nku *et al*., 2014) These findings are consistent with the results of the current study, which show that lower concentrations of the methanolic extracts of *C. acuminata* (100 and 150 mg/kg) let to a significant increase in total plasma cholesterol compared to control rats. In contrast to the significant reduction in total cholesterol levels recorded in rats fed diets supplemented with a higher amount of *C. nitida* (Nku *et al*., 2014), the highest concentration of *C. acuminata* (200 mg/kg) used in our study did not result in significant changes in total plasma cholesterol levels. This suggests that there may be a concentration threshold required to produce significant alterations in cholesterol metabolism. In a human-based study, chronic kola nut consumers (aged 55.3±9.15 yrs) were found to have significantly higher serum total cholesterol levels compared with age-matched non-consumers (Ewenighi *et al*., 2016). Since other studies suggested weight-reducing potentials for flavonoids and terpenoids, we predict that the observed impact of the methanolic extract of *C. acuminata* could be the result of synergistic interactions among essential phytochemicals. Future investigations should explore this.

Although the lipotropic potentials of caffeine were proposed over a century ago (Heppel *et al*., 1947), the consumption of caffeine and caffeine-containing products remain unabated. As we progress through a fast-paced and increasingly complex world, there is projected increase in stress and stress-related complications owing to rising job demands, anxiety, depression, and social isolation. To counteract the attendant adverse consequence, people would usually seek over-the-counter energy boosters and sleep-influencing drugs. We believe that kola nut and other kola-containing products would also be highly sort after. Therefore, we recommend a cautious use of *C. acuminata* in its various formulations, especially when stress, age, physical activities, and existing health conditions may be of major concerns. Since the proximate and phytochemical compositions of kola nut depend on a variety of factors, including cultivars, season, soil type, farming system, method and length of storage (Touati *et al*., 2014; Kapcum & Uryapngson, 2017; DeBenedictis *et al*., 2023; Tedeschi *et al*., 2023), a periodic assessment of the potential impacts of commercially available kola nuts on various biological indices has become crucial.

Acknowledgements

We sincerely thank the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka for providing a conducive environment, and the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for identifying the C. acuminata used for this study

REFERENCES

Adesida, F. A., Oluyole, K. A., Agulanna, F. T., Oladokun, Y. O. M., Adelusi A. A., Agboola, L.O., Ogunwolu, Q. A., Ujunwa, E. I. and Mustopha, F. B. (2021). Economic evaluation of kola-nut processing and marketing in South-West, Nigeria. Moor J. Agri. Res. 22: 149 -158.

Ajiboye, A. and Afolayan, O. (2009). The impact of transportation on agricultural production in a developing country: a case of kola nut production in Nigeria. Int. J. Agric. Econ. Rural Dev. 2: 49-57.

Asogwa, C. N., Onyeka, C. C., Odii, E. C., Ekeh, F. N., Ikele, C. B., Mgbenka, B. O. and Eyo, J. E. (2014). haematological and

cortisol dynamics in albino rats stimulated by intake of methanolic extract of Cola acuminata. World J. Med. Sci. 10(4): 384-391.

Beattie, G. B. (1970). Soft drinks flavours. Their history and characteristics. I. Cola or 'Kola' flavours. The Flavour Industry, pp. 390-394.

Birner, J. (1969). Determination of total steroid bases in Solanum species. J. Pharm. Sci. 58(2): 258-259.

Brunner, J. H. (1984). Direct spectrophotometric determination of saponin. Anal. Chem. 42: 1752-1754.

Dawra, R. K., Makkar, H. P. S. and Singh, B. (1988). Protein-binding capacity of microquantities of tannins. Anal. Biochem. 170: 50-53.

DeBenedictis, J. N., de Kok, T. M. and van Breda, S. G. (2023). Impact of processing method and storage time on phytochemical concentrations in an antioxidant-rich food mixture. Antioxid. 12: 1252.

Erinfolami, A., Eegunranti, A., Ogunsemi, O., Oguntuase, A., Akinbode, A. and Erinfolami, G. (2011). Prevalence and associated risk factors of kola nut chewing among secondary school students in Osogbo, Nigeria. Mental Illness, 3: e6.

Ewenighi, C., Dimkpa, U., Onoh, L., Onoh, G., Ezeugwu, U. and Dibia, J. (2016). Chronic kola nut consumption and its effect on uric acid level and lipid profile. J. Mol. Pathophysiol. 5(2): 23-27.

Haque, M. R. and Bradbury, J. H. (2001). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chem. 77:107-114.

Heppelm, L. A., Porterfield, V. T. and Peake, E. G. (1947). The lipotropic activity of caffeine, theobromine and theophylline. Arch. Biochem. Biophys. 15: 439-443.

Igbinovia, E. N. S., Ugwu, A. C., Nwaopara, A. O., Otamere, H. O. and Adisa, W. A. (2009). The effects of Cola acuminata on arterial blood pressure. Pak. J. Nutr. 8(2): 148-150.

Ikegwuonu, F. I., Aire, T. A. and Ogwuegbu, S. O. (1981). Effects of kola-nut extract administration on the liver, kidney, brain, testis and some serum constituents of the rat. J Appl. Toxicol. 1(6): 292-294.

Jacob, D. E., Nelson, I. U. and Izah, S. C. (2023). Cola acuminata: phytochemical constituents, nutritional characteristics, scientific validated pharmacological properties, ethnomedicinal uses, safety considerations, and commercial values. Herbal Medicine Phytochemistry. Springer Nature Switzerland AG, pp 141–179.

Kapcum, C. and Uriyapongson, J. (2018). Effects of storage conditions on phytochemical and stability of purple corn cob extract powder. Food Sci. Technol. Campinas. 38(1): 301-305.

Kishi, K., Ochiai, K., Ohta, Y., Uemura, Y., Kanatani, K., Nakajima, K. and Nakamura, M. (2002). Highly sensitive cholesterol assay with enzymatic cycling applied to measurement of remnant lipoprotein-cholesterol in serum. Clin. Chem. 48(5): 737-741.

Lorke, D. (1983). A new approach to practical acute toxicity testing. Arch. Toxicol. 54(4): 275-287.

Łukowski, A., Jagiełło, R., Robakowski, P., Adamczyk, D. and Karolewski, P. (2022). Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. Plant Sci. 314: 111090.

Machado, M., Antunes, W. D., Tamy, A. L. M., Azevedo, P. G., Barreto, J. G. and Hackney, A. C. (2009). J. Exerc. Sci. Fit. 7(2): 91-97.

Obidike, I. R., Aka, L. O. and Ezema, W. S. (2011). Effects of caffeine extract from kola nut on body weight, hematology, sperm reserve and serum enzyme activities in albino rats. Comp. Clin. Pathol. 20: 625-630.

Ogutuga, D. B. A. (1975). Chemical composition and potential commercial uses of kola nut, Cola nitida, Vent. (Schott and Endlicher). Ghana J. Agric. Sci. 8: 121-125.

Osaro, I. E., Chinedu, I. and Yakubu, O. E. (2024). Proximate and phytochemical constituents of ethanolic extract of seeds of Cola acuminata and Cola nitida. AJSTEA. 2(2): 129-144.

Purseglove, J. W. (1997). Tropical crops cotyledons. Longman.

Purseglove, J.W. Tropical Crop Di-cotyledons, John Wiley & Sons Inc, New York, 1968.

Quarcoo, T. (1969) Development of kola and its future in Nigeria. Proceedings of the Agricultural Society of Nigeria, Volumes 6.

Quarcoo, T. (1973). A handbook on kola. CRIN, Ibadan, Nigeria, 90pp.

Salahdeen, H. M. and Alada, A. R. A. (2009). Effects of caffeine and ethanolic extract of kolanut on glucose uptake in the canine hindlimb at rest and during contraction. Nig. J. Physiol. Sci. 24(1):33-45.

Tedeschi, P., Brugnoli, F., Merighi, S., Grassilli, S., Nigro, M., Catani, M., Gessi, S., Bertagnolo, V., Travagli, A., Caboni, M. F. and Cavazzini, A. (2023). The effect of different storage conditions on phytochemical composition, shelf-life, and bioactive compounds of voghiera garlic PDO. Antioxid.12(2): 499.

Touati, N., Tarazona-Díaz, M. P., Aguayo, E. and Louaileche, H. (2014). Effect of storage time and temperature on the physicochemical and sensory characteristics of commercial apricot jam. Food Chem., 145: 23-27.

Umoren, E. B., Osim, E. E. and Udoh, P. B. (2009). The comparative effects of chronic consumption of kola nut (Cola nitida) and caffeine diets on locomotor behaviour and body weights in mice. Nig. J. Physiol. Sci. 24(1): 73-78.

Uwabunkeonye, O. C., Ezeabara, C. A., Horoiheoma, C., Denis, U. C. and Aziagba, B. O. (2015). Comparative phytochemical and proximate compositions of Cola acuminata (P. Beauv.) Schott and Cola nitida (Vent) Schott and Endl. Plant. 3(3): 26-29.

Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4): 555-559