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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, NUTRITIONAL COMPOSITION, *IN VITRO* ANTIOXIDANT CAPACITY, AND ACUTE TOXICITY OF *Cola acuminata* LEAVES

OKOLIE, N.P.[1] AND *OKUNGBOWA, A.I. [2]
[1]Department of Biochemistry, University of Benin,
[2]Department of Biological Sciences, Benson Idahosa University
*Corresponding author: aokungbowa@biu.edu.ng

Abstract
Plant leaves are known to be abundant in phytochemicals, which are effective protectants of cells against oxidative stress, a leading cause of many diseases. The leaves of Cola acuminata (CA) have been used in herbal medicine to manage gastrointestinal infections, upper respiratory tract infections, and anaemia in adults and children. Fresh leaves of Cola acuminata were air-dried, ground into powder, then macerated in methanol for 72 hours, while stirring intermittently. The mixture was then filtered to obtain the crude extract by concentration. The nutritional composition, qualitative and quantitative phytochemical constituent, in vitro antioxidant capacity, as well as acute toxicity of the methanol leaf extract of CA were evaluated following standard methods. Proximate analysis revealed that the plant extract contained substantial amounts of proteins, lipids, carbohydrates, fibre, and ash, with a low moisture content. Qualitative phytochemical analysis reveals appreciable amounts of flavonoids, cardiac glycosides, saponins, alkaloids, terpenoids, coumarins, tannins, phenols, and reducing sugars. In vitro antioxidant analysis indicates that CA has a high total antioxidant capacity and effectively scavenges DPPH radical. Acute toxicity studies show that the leaf extract had LD_{50} values >5,000 mg/kg body weight, with no sign of toxicity or mortality. This study therefore supports the claims of the efficiency of Cola acuminata methanol leaf extract in combating disease states in humans.

Keywords: *Cola acuminata, Antioxidants, Nutritional, Herbal medicine, Acute toxicity*

Introduction

Cola acuminata (P. Beauv.) Schott and Endler is an evergreen, medium sized tree having low grey or dark green branches, green leaves, and whitish flowers (Facciola, 1998). It is popularly known as a kola nut but commonly called 'evbee' in Edo, 'Oji' in Igbo, 'obi' in Yoruba, and 'goro' in Hausa (Ugwuowo *et al*., 2021). The genus *C. acuminata* comprises approximately 125 native species found in Africa's tropical rainforests. In traditional medicine, dehydrated cola nuts are ground into powder and mixed with honey to treat cough (Adebayo and Oladele, 2012). Mboto (2009) reported proof of improved recovery in a combination regimen of *Vernonia amygdalina,* honey, and

Garcinia kola in the treatment of chronic ulcers, fresh injuries, and male circumcision related wounds.

Several researches have reported the medicinal efficacy of *C. acuminata* nuts. They contain large amounts of caffeine, and theobromine and are therefore used as stimulants. The caffeine in their nuts expand the bronchia, thus constitute an effective remedy for asthma and whooping cough. *C. acuminata* also improves alertness and physical energy, elevates mood, reduces appetite and serves as a sex enhancer (Lowe *et al*., 2014). The nut extracts of *C. acuminata* are antiparasitic especially against trichomoniasis infection in women (Adebayo and Oladele, 2012; Kanoma *et al*., 2014). Studies by Oboh *et al*., (2014) show that the seed extracts of *C. acuminata* inhibited the activities of neurotransmitters, acetylcholinesterase and butyrylcholinesterase in a dose dependent fashion. The extracts of *C. acuminata* also prevented oxidative stress induced neurodegeneration due to its high radical scavenging and Fe^{2+} chelating activities of phytochemicals. Lowe *et al*. (2014) reported that n-hexane extract of the nut killed 100% of the breast and prostate (DU-145 and PC3) cancer cell lines. Another research showed the cytotoxicity of *C. acuminata* in cell lines of breast cancer (MCF-7 and DA-MB 468) and prostate cancer (LNCaP). The reduction of viability of these cell lines is via the mechanism of apoptosis (Edrini *et al*., 2011; Fortenort *et al*., 2007).

However, despite the wide application of this leaf in folk medicine for the treatment of spleen injury, low packed cell volume, cough, and diarrhoea, there is little scientific evidence giving credence to its effectiveness in disease states. This research, therefore, seeks to evaluate the nutritional, antioxidant, phytochemical properties, and acute toxicity of the leaves.

Materials and Methods

Plant Materials

Freshly harvested leaves of *C. acuminata* were obtained 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.

Preparation of Methanol Extract

The leaves were dried openly and milled into powder using a blender. Five grams of powdered sample were macerated in 2,500 mL methanol for 72 hours at room temperature with continuous stirring at 24-hour intervals. A muslin cloth was then used to obtain the filtrate. A concentrated crude extract was obtained with a rotary evaporator at 45°C.

Proximate Analysis

The methods of the Association of Official Analytical Chemists method (AOAC, 2000) was employed for the analysis of proximate composition

Phytochemical Analysis

Phytochemical screening was conducted using the method outlined in literature (Enabulele and Ehiagbonare, 2011) to qualitatively determine the presence or absence of the following secondary metabolites: Flavonoids, Alkaloids, Saponins, Cardiac glycosides, Tannins, and reducing sugars.

Ethical Approval

The Faculty of Life Science Research Ethics Committee (FLSREC) of the University of Benin gave the animal study ethical clearance and issued an ethical approval number, FLSRE-2023-008.

Source of Experimental Animals

Male albino rats of the Wistar strain (100-120 g), bred in the animal houses of the departments of Biochemistry and Anatomy, University of Benin, Benin City, Edo State, were used for this research.

Acute Toxicity Study

An acute toxicity study was done following Lorke's method. Nine animals were used for the first phase. They were grouped into three consisting three animals per group, and received extract doses, 10,100, and 1000mg/kg respectively. In order to track their behaviour and identify any instances of fatality, the animals were keenly observed for a whole day. The second stage involved the use of three animals. They received doses of extract (1,600, 2,900, and 5,000 mg/kg) and were separated into three groups of one animal each.

Quantitative Phytochemical Screening of Leaf Extract

Determination of Total Flavonoid Content

Total flavonoids were measured following the procedure outlined by Ebrahimzadeh *et al*. (2008) 0.5 mL of the extract (1 mg/ mL in methanol) was combined with 1.5mL of methanol and 0.1 mL of 10% aluminium chloride, and 2.5mL of distilled water. This was left to stand for half an hour at room temperature after which absorbance was read at 415nm with the aid of a spectrophotometer. A standard curve was calibrated with quercetin using graded concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml. The extrapolated values from the standard curve were given as milligrams quercetin equivalent (QE) per gram extract.

Determination of Total Phenolic Content

Total phenolics were estimated as described by Folin and Ciocalteu (2007). Exactly 0.5mL of the extract (1 mg/ mL in methanol) was mixed 2.5mL of Folin-Ciocalteu reagent (0.1 %v/v) and 2 mL of 7.5% Sodium Carbonate. The mixture was kept on the bench for 30 minutes. With a spectrophotometer, the absorbance at 760nm was determined. The standard curve was calibrated using gallic acid at graded concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml. The extrapolated results from the standard curve were represented as milligrams gallic acid equivalent (GAE) per gram of extract (mgQE/g extract).

In vitro Antioxidant Activity Determination

Total Antioxidant Capacity (TAC) Assay

The phosphomolybdate method, according to Prieto *et al*. (1999) was followed to evaluate the Total Antioxidant Capacity (TAC) of *C. acuminata* methanol leaf extract. Three millilitres of the reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) were combined with one millilitre of an aliquot (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) of the leaf extracts in test tubes. They were covered with aluminium foil, then incubated for 90 minutes at 95°C in a water bath. Absorbance of each solution at 695 nm was measured against a blank after the reaction mixture had been kept to cool on the bench. Three millilitres of the reagent solution together with the proper amount of the dissolving solvents were included in the blank. The identical incubation conditions as the test samples were used for this. The standard reference compound used to compare the extracts' activity was

ascorbic acid. Three duplicates of the test were conducted.

DPPH Radical Scavenging Activity

The method outlined by Brand-Williams *et al*. (1995) was applied for the evaluation of DPPH radical scavenging activity. About 3.9 mL of 0.15 mM DPPH in ethanol together with about 0.1 mL of the extract and fractions (100 mg/mL) was combined. After a thorough mixing, the solution was left to stand on the bench for half an hour in the dark. The standard control used was ascorbic acid, in quantities similar to the test samples. The tests were done in triplicate. As a control, a blank solution comprising 0.1 mL of ethanol and 3.9 mL of DPPH was employed. Absorbance was read at 517 nm using a spectrophotometer. The equation below was followed to estimate DPPH scavenging activity (Jamous *et al*., 2015).

% Radical Scavenging Activity

$$= \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} x100$$

Data Analysis

All values were expressed as mean ± standard deviation with the Microsoft Excel version 2504.

Results and Discussion

Proximate Content of Cola acuminata Methanol Leaf Extract

Table 1 shows the nutritional composition of *Cola acuminata* methanol leaf extract. It reveals that the leaves contain proteins, lipids, carbohydrates, and minerals. It also contains an appreciable quantity of fibre and low moisture content.

Carbohydrates, proteins, lipids, fibre, and minerals are important components of food that must be present in an appropriate ratio to confer health benefits on individuals. While proteins serve several biochemical functions, including enzymatic, structural, transport, storage, etc, carbohydrates and lipids provide the needed energy for cellular functions. Fibre aids easy digestion through the digestive system, and minerals are important for proper absorption of nutrients, as well as cofactors for some enzymes. They are also useful in health and disease states to improve immune function. The results for proximate analysis (Table 1) show that CA methanol leaf extracts contain minerals, proteins, lipids, fibre, and carbohydrate with low moisture content. Low moisture content of the leaves reduces the chances of being spoiled by microbes (Adeyeye and Adejuyo 1994), thus elongating the shelf life. Unuigbe *et al*. 2019 and Evuen and Kpomah 2023 have reported similar findings.

Table 1: Proximate composition of *Cola acuminata* methanol Leaf extract

Nutrient	***Cola acuminata* (%)**
Ash	6.40 ± 0.12
Protein	6.12 ± 0.01
Lipids	0.13 ± 0.05
Fibre content	1.21 ± 0.02
Moisture content	2.05 ± 0.03
Carbohydrate	84.09

Values are mean ± standard deviation (n = 3)

Qualitative Phytochemical Analysis of Cola acuminata methanol Leaf extract

Phytochemicals are important plant secondary metabolites that have become the focus of medicinal plant research, due to their efficacy in scavenging free radicals in cells, which may result in oxidative stress. Their basic roles in plants are to act as regulators of several physiological processes, secondary messengers for biochemical pathways, and inhibitors of cell cycle (Imam, *et al*., 2017).

Table 2 shows the qualitative phytochemical constituent of *C. acuminata* methanol leaf extract and reveals that the leaves contain ample phytochemicals, which is similar to findings reported by other researchers Okungbowa *et al*. (2017); Sharma and Kumar (2019), Zailani *et al*. (2020); Mordi *et al*. (2021)). Alkaloids are nitrogenous compounds found in both marine and terrestrial organisms. They constitute nearly 20% of secondary metabolites occurring in plants (Henrich *et al*., 2021). Their pharmacological activities include antibacterial, analgesic, antimalarial, anti-inflammatory, hypoglycemic, anticancer, antiarrhythmic, and psychotropic effects (Ferreira, 2022). Zilani *et al*. (2020) extracted alkaloids, phenolics, and flavonoids from the leaves of *C. acuminata,* which they confirmed to effectively treat malaria-infected mice. Alkaloid concentration in the leaves of *C. acuminata* is very high, indicating the efficacy of the extract as cytotoxic, anti-viral, and anti-bacterial (Bribi, 2018).

The abundance of alkaloids found in the leaves of *C. acuminata* indicates potential health benefits. Saponins are bioactive secondary metabolites with a bitter taste, found in plants and some marine animals. They have a lipophilic sugar moiety associated with a lipophilic aglycone. They are useful as expectorants, emulsifiers, stabilizers, and foaming agents. They are known to lower cholesterol, aggregate platelets, neutralize lead poisoning, and exhibit anticancer properties Timilsena *et al*., 2023). Although beneficial to health, high amounts of saponins cause gastroenteritis (Awe and Sodipo, 2001). Saponin content of *C. acuminata* was found to be low in quantity, making their consumption safe.

Tannins are commonly present in fruits, nuts, and vegetables and contribute significantly to the colours and flavour in them. Research has proven that they exhibit antiviral, antibacterial, anti-tumour, antifungal, anticancer, and antidiabetic properties. They also reduce blood pressure thereby lowering the risk of cardiovascular anomalies (Cosme *et al*., 2025).

Cardiac glycosides are plant secondary metabolites, but may also be present in amphibians. They block the sodium potassium ATPase of the heart, with a positive inotropic effect (Botelho *et al*. 2019) significantly impacting on the cardiovascular system.

Terpenoids are structurally composed of numerous isoprene units. They play important roles in plant growth and response to environmental stimuli. Terpenoids are effective against malaria, oxidative stress, inflammation, cancer, pain, and neurodegenerative diseases (Boroushaki *et al*., 2016, Yang *et al*., 2020).

Coumarins are organic plant compounds known to have biological and pharmacological effects on human health. They curtail the overwhelming consequences of free radicals, microorganisms, and inflammation. In addition, they possess antihypertensive, anti-inflammatory, anticoagulant, antimicrobial, anticonvulsant, and neuroprotective capacity. Derivatives of coumarins may also influence signaling pathways in cells (Flores-Morales *et al*., 2023).

Reducing sugars function in plants as an energy source, regulatory molecules in plant metabolism, growth, disease resistance, and stress. They also aid the synthesis of secondary metabolites which

confer medicinal properties on plants (Khatri and Cchetri, 2020).

Table 2: Qualitative Phytochemical Analysis of *C. acuminata* methanol leaf extract

Phytochemicals	**Result**
Flavonoids	+++
Cardiac glycosides	++
Saponins	++
Steroids	-
Terpenoids	+++
Alkaloids	+++
Coumarins	++
Tannins	+++
Reducing sugars	+++

Key: ++ =Strongly present ++ = Moderately present + = Slightly present - = Absent

Table 3: Quantitative phytochemical determination of total flavonoid and phenolic contents of *Cola* acuminata methanol leaf extract.

Phytochemical	*Cola acuminata*
Total flavonoids (mg QE/gram extract)	1.730 ± 0.090
Total phenolics (mg GAE/gram extract)	0.014 ± 0.001

*Values are expressed as mean ± SE (n=3), GAE = Gallic Acid Equivalent, QE = Quercetin Equivalent.

Quantitative Phytochemical Screening of Leaf Extract

Table 3 reveals the results for the quantitative phytochemical analysis of the methanol extract of *C. acuminata* leaf extract. The extract had a high number of total flavonoids and total phenolics. In combating diseases, flavonoids possess antioxidant and anti-inflammatory effects (Lesjak *et al*., 2019; Guelfi *et al*., 2023). They are the class of polyphenols with the highest popularity and are available in numerous plants species. They may be categorized as hydrophilic, and lipophilic chelators. The lipophilic chelators enhance iron absorption, while reducing its excretion, and improve tissues deposits of excess iron. This makes them a good choice for managing anaemia resulting from to iron deficiency. The hydrophilic chelators inhibit iron absorption, induce the elimination of excess iron, and improve anti-inflammatory and antioxidant effect without having other complications (Kontoghiorghes and Kontoghiorghe, 2020).

Flavonoids, saponins, alkaloids, terpenoids, coumarins, and reducing sugars are compounds in plant extracts that indicate the plant has antioxidant potential in scavenging free radicals. The findings reported by Khatri and Cchetri (2020); Agidew, (2022), Sharma and Kumar (2019) and Mandal (2023) are in tandem with the current research.

In vitro Antioxidant Capacity of Cola acuminata Leaves

Fig. 1 shows results for *In vitro* Total antioxidant capacity of *C. acuminata* leaves. This reveals that *C. acuminata* had high total antioxidant capacity than when compared with vitamin C, the standard drug. Antioxidants are compounds that scavenge free radicals and pro-oxidants by several mechanisms, including iron chelation, donation of protons, etc. Plant phytochemicals have been shown to effectively curtail the effects of free radicals in biological systems, thereby preventing the numerous diseases associated with oxidative stress. This makes antioxidants key targets for drug design. Total antioxidant capacity analysis in this study (Fig. 1) shows that the methanol leaf extract of *C. acuminata* serves as reservoir of antioxidants effective in the prevention and management of common diseases.

DPPH radical scavenging assay is the most generally accepted antioxidant assay used to assess foods and herbal medicine because its procedure is easy and fast with a dependable outcome. The DPPH scavenging potential of bioactive compounds present in leaf extract directly depends on their ability to donate hydrogen, thus conferring antioxidant status (Jimoh *et al*., 2010). *C. acuminata* in this study has a high total antioxidant capacity (Figure 1) and scavenges DPPH radical effectively (Figure 2) when compared with vitamin C, the standard drug. The total antioxidant capacity of CA may be attributed to the numerous antioxidant compounds present in the leaves, as shown by the results of phytochemical analysis. The extract also contains antioxidant compounds that confer its capacity to scavenge the DPPH radical. The results are similar to those of Khatri and Cchetri (2020). Fig. 2. reveals that *C. acuminata* scavenges DPPH radical when compared with vitamin C.

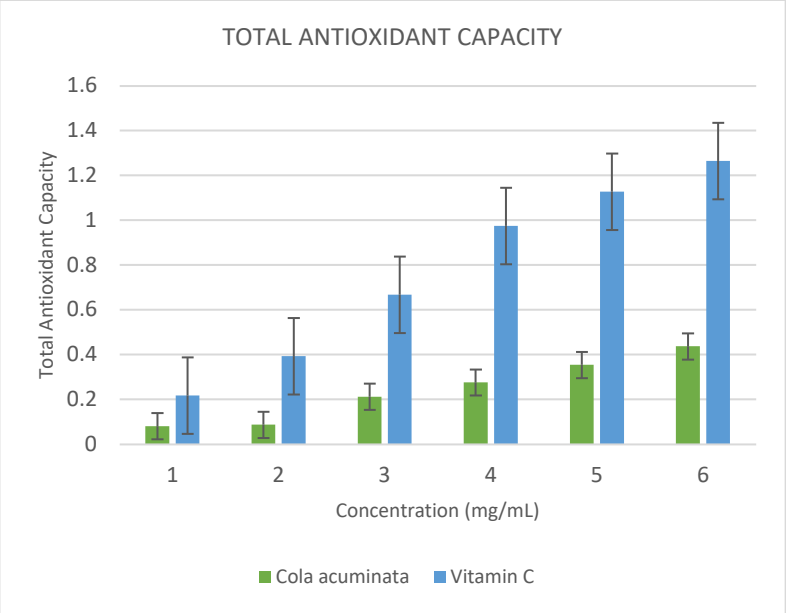

Fig. 1: Total Antioxidant Capacity of *Cola acuminata* Leaves

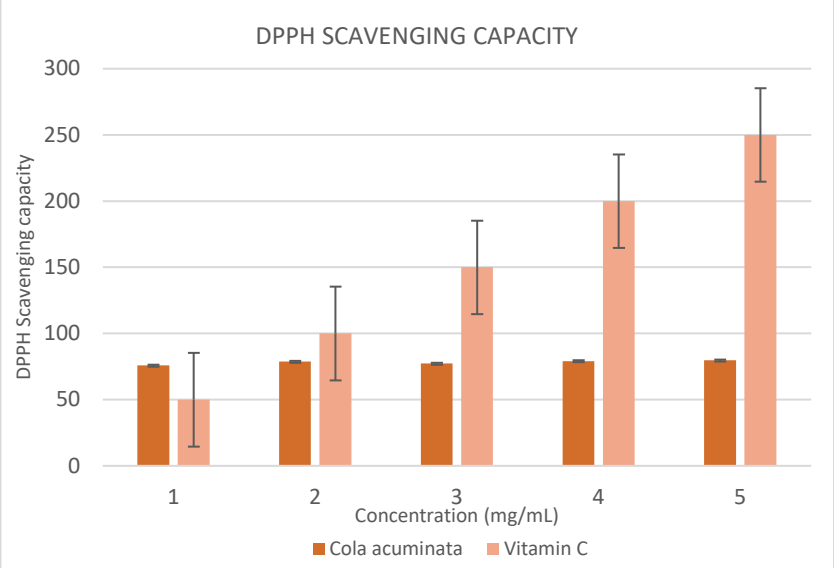

Fig. 2: DPPH Scavenging capacity of *Cola acuminata* Leaves

Acute Toxicity of Cola acuminata Methanol Leaf Extracts in Albino Wistar Rats

Table 4 shows results for acute toxicity testing. There were no signs of toxicity or mortality observed within twenty-four hours. The LD_{50} for both *C. acuminata* methanol leaf extracts were greater than 5,000mg/kg body weight of albino rats. Acute toxicity studies ascertain the short-term toxic effects of a drug on an organism. In this study (Table 4), *C. acuminata* methanol leaf extract was non-toxic, and no mortality was recorded at all doses within twenty-four hours. Alaebo *et al*. (2022) reported similar observations with *Dennettia tripetala* methanol leaf extract.

Table 4: Acute toxicity of *Cola acuminata* of Methanol Leaf Extracts in albino Wistar rats

Extracts	Phase 1 Dose (mg/kg Body weight)	Mortality	Phase 2 Dose (mg/kg Body weight)	Mortality
Methanol Extract of *Cola acuminata*	10	0/3	1600	0/1
	100	0/3	2900	0/1
	1000	0/3	5000	0/1

Conclusion

Cola accuminata methanol leaf extract from this study has proven to contain useful nutrients and phytochemicals. It scavenges DPPH radicals, has good total antioxidant capacity, and is non-toxic. These results support the use of the leaves by traditional medicine practitioners. It is therefore a good choice for the design of novel drugs.

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