Phytochemical Profiling and Antioxidative Potential of Phenolic-rich Extract of *Cola acuminata* Nuts

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**Abstract:** The unprecedented rise in chronic diseases and severe health risks of prolonged usage of synthetic drugs has opened a window for alternative remedies. This study screened the phytochemical contents and the *in vitro* antioxidant activity of *Cola acuminata* aqueous extract (CAAE). The phytochemical and antioxidative potential of CAAE was determined using standard techniques. The result of the phytochemical analysis shows that the extract contains a high abundance of phenols > alkaloids > flavonoids > reducing sugars. CAAE had high NO and TBARS inhibitory activities with 5.56 and 29.16 µg/ml IC$_{50}$ values, respectively, more effective than the 10.02 and 60.27 µg/ml obtained for the standard drugs, ascorbic acid, and butylated hydroxytoluene, respectively. Also, CAAE showed good DPPH inhibitory activity, with IC$_{50}$ of 6.01 µg/ml, while ascorbic acid had 6.31 µg/ml. The total antioxidant capacity and FRAP activity were maximal at 15.63 and 32.25 µg/ml with an IC$_{50}$ of 4.89 and 32.44 µg/ml, respectively. These results demonstrate that the *Cola acuminata* nut has antioxidative capacity. Thus, *Cola acuminata* could be employed as a medicinal and pharmaceutical agent to scavenge and inhibit oxidative stress caused by free radicals generated in the body during biochemical processes.

**Keywords:** antioxidant; chronic diseases; *Cola acuminata*; free radical; oxidative stress; phytochemicals.

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1. **Introduction**

Chronic diseases ranging from diabetes, cancer, and cardiovascular and neurological diseases have been associated with cellular damage caused by redox equilibrium disruption [1]. Disturbance of redox balance due to excessive reactive oxygen and nitrogen species production creates a pro-oxidative environment leading to oxidative stress [1,2]. Oxidative stress and nitrosative stress result from oxidation reactions and nitrogen reactions, respectively, which initiate harmful chain reactions that damage cellular components [3], leading to organ and tissue damage [4,5], which is the key to the pathophysiology of many illnesses [2]. Ideally, the body is equipped with endogenous antioxidants, including enzymes such as glutathione peroxidase, catalase, superoxide dismutase, and non-enzymatic antioxidants, saddled with the
responsibility of maintaining redox balance [6,7]. However, oxidative stress pathophysiology usually overwhelms the expression and functionality of the endogenous antioxidant network, necessitating the intake of exogenous antioxidants to avert the onset of metabolic diseases [8].

Exogenous antioxidants from dietary sources provide resistance to oxidative impairment by scavenging free radicals, lowering lipid peroxidation, protecting several other biomolecules from oxidative damage [2], and modulating gut activity microbiota or epigenetics [9]. The use of dietary antioxidants is gaining momentum due to the inherent side effects associated with synthetic antioxidants such as allergy, hypersensitivity, asthma, hyperactivity, neurological damage, and cancer [10] and its lower cost and better safety profile. Emerging evidence from several studies has shown that natural antioxidants prevent the onset of oxidative stress-induced diseases ranging from autoimmune, cancer, cardiovascular, and neurological diseases [11-13]. The antioxidative effects of plant-based products are strongly correlated to the presence of bioactive phytochemicals [2].

Phytochemicals are naturally occurring, physiologically active chemical substances found in plants that are proven to treat and ameliorate several diseases [14]. They protect plant cells against pathogenic attachment, pollution, stress, drought, and ultraviolet (UV) exposure [15]. The antioxidative potency of plant-based products is achieved through stimulation of expression of endogenous antioxidants, suppression of effects of singlet oxygen, chelation of metal ions, and reduction of free radicals, which prevents radical chain reactions [16]. Because endemic plants are bound to certain locations, the knowledge of the chemical contents of these plants is vital given that such useful information will be valuable for the synthesis of novel compounds and may serve as a positive source for antioxidants which will ameliorate the sudden rise in chronic diseases [17].

*Cola acuminata* (cola nut), called Oji in Nigeria’s eastern region, is a West African endemic member of the Sterculiaceae family [18,19]. Cola nut is an evergreen plant that grows up to 20 cm in height, with leaves measuring about 30 cm long, while the nut has 3-6 cotyledons (Figures 1S and 2S). In traditional contexts, *Cola acuminata* is chewed as a remedy for whooping cough, fever, asthma, malaria, and as a caffeine-containing stimulant [20]. The leaves, nuts, and stem bark of *C. acuminata* have traditionally been used as a drink or decoction to cure various ailments [19]. It has also been used to treat respiratory infections, hypertension, antiparasitic, and as an aphrodisiac [21]. Owing to the relationship between antioxidants and diseases caused by the harmful effects of free radicals, this study aims to identify and quantify the phytochemicals and the *in vitro* antioxidant activity of *Cola acuminata* aqueous extract.

2. Materials and Methods

2.1. Collection and identification of the plant material.

*Cola acuminata* nuts were purchased from Ogige market in Nsukka Local Government Area of Enugu State, Nigeria. Taxonomic identification of the nut was made at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, by Mr. Felix Nwafor. The taxonomist herbarium has voucher specimens of the nut with the identification number PCG/UNN/0330.
2.2. Preparation of extract.

The *C. acuminata* nuts were washed with clean water and air-dried under a shade. The dried nuts were grounded to a fine powder with a milling machine. The pulverized nut (200 g) was macerated in 1000 ml of distilled water. The extract obtained was filtered with calico and filter paper (Whatman No. 1, pore size 11m). The filtrate concentration was done by lyophilization to get brown slurry-like *Cola acuminata* aqueous extract (CAAE) which was stored in a refrigerator for further use.

2.3. Qualitative and quantitative phytochemical analysis of CAAE.

Qualitative and quantitative screening of phytochemicals in CAAE was carried out using the standard procedure described by Trease and Evans [22] and Harbone [23].

2.4. Nitric oxide (NO) scavenging activity of CAAE.

The method described by Sreejayan and Rao [24] was used to determine NO. Two (2) ml of sodium nitroprusside (10 Mm) in phosphate buffer saline (pH 7.4) was added to 0.5 ml of CAAE and ascorbic acid at different concentrations (15.63, 31.25, 62.5, 125, 250, and 500 µg/ml) in a separate test tube. After 15 min of incubation at 25 °C, 500 µl of Griess reagent (2 % orthophosphoric, 1 % sulphonamide, 0.1 % N-naphthalenediamine dihydrochloride) was added to the test tube, which was also incubated again for 30 min at room temperature. Subsequently, absorbance was taken at 546 nm. This procedure was repeated three times. The scavenging activity was determined as follows:

\[
\text{% Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \%
\]

2.5. Inhibition of lipid peroxidation (TBARS) activity of CAAE.

The TBARS test was conducted according to Banerjee *et al*. [25]. Egg yolk homogenate was used as a lipid-rich media for the TBARS assay. In a test tube, 100 µl of extract (15.63-500 g/ml) and 500 µl of egg homogenate (10 % v/v in phosphate-buffered saline pH 7.4) were mixed and diluted to 1.0 ml with distilled water. Then, 20 µl of L-ascorbic acid and 50 µl of FeSO₄ (0.075 M) were added and incubated at 37 °C for 1 h to initiate lipid peroxidation. Each sample was treated with 0.2 ml of EDTA (0.1 M) and 15 ml of TBA reagent before being cooked for 15 min at 100°C. Centrifugation of the cooled samples lasted for 10 min. After that, the absorbance was measured at 532 nm. The test was done in triplicate. Butylated hydroxytoluene was used as a standard. Lipid peroxidation inhibitory capacity (%) was calculated using the equation:

\[
\text{% Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \%
\]

2.6. 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of CAAE.

The Gyamfi *et al*. [26] technique was used to assess the extract’s DPPH scavenging capability. A volume, 1 ml of CAAE and ascorbic acid in 80 % methanol at different concentrations (15.63-500 µg/ml) in different test tubes was mixed with 0.5 ml of 0.076 mM DPPH in methanol. The mixture was thoroughly mixed and incubated in a dark cupboard at room temperature for 25 min. The control consisted of 1 ml of 0.076 mM DPPH in methanol.
Finally absorbance was determined at 517 nm using methanol as a blank. The experiment was done three times. The following equation was used to compute DPPH radical scavenging activity:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \%
\]

2.7. Total antioxidant capacity (TAC) of CAAE.

Phosphomolybdate procedure [27] was employed to determine the total antioxidant capacity (TAC) of the extract. In separate test tubes, an aliquot (0.1 ml) of the extract and ascorbic acid (15.63-500 µg/ml) was mixed with 1 ml of reagent solution (4 mM ammonium molybdate, 28 mM sodium sulfate, and 600 mM sulphuric acid 1:1:1). The test tubes were covered in aluminum foil and incubated in a water bath for 90 min at 95°C. The absorbance of the cooled test tubes was measured at 765 nm with 1 ml of the reagent solution used as blank. The TAC determination was carried out in triplicates. TAC was calculated as shown below:

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \%
\]

2.8. Ferric reducing antioxidant power (FRAP) of CAAE.

The reducing power of the crude extract was determined as described by Sahreen et al. [28]. A volume, 2.0 ml of the extract was added to 2.0 ml of C₆FeK₄N₆ solution (0.1 percent w/v) and 2.0 ml of phosphate buffer (0.2 M, pH 6.6). The mixture obtained was incubated in a water bath for 20 min at 50°C. After that, 2.0 mL of trichloroacetic acid solution (10% w/v) was added. Finally, a 2.0 mL aliquot of the mixture was combined with 0.4 mL of ferric chloride (prepared with distilled water (2.0 mL) and FeCl₃·6H₂O, a 0.1 percent (v/w) solution). Subsequently, absorbance was read at 700 nm after 10 min. The FRAP test was performed three times, and the results were reported in Gallic acid equivalent (GAE).

2.9. Statistical analysis.

The data was analyzed statistically, and the results were written as means and SD with p < 0.05 set as the least significant criteria. To segregate and analyze differences between means, one-way analysis of variance (ANOVA) and post hoc multiple comparisons were performed with version 23 of IBM Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA). Nonlinear regression analysis was plotted to determine the test substance's IC50 and R square values using version 6.05 of the GraphPad Prism (GraphPad Software, Inc., California, USA).

3. Results and Discussion

3.1. Qualitative and quantitative phytochemicals of CAAE.

The results of the phytochemicals screening revealed the presence of the phytochemicals in the following decreasing order: Phenols > alkaloids > flavonoids > reducing sugar > terpenoids > tannins > glycosides > steroids. The largest phytochemical compound contained in the plant extract was phenols (1735.48 ± 88.40 µg/ml), and the smallest phytochemical extract contained in the plant extract is steroids (0.11 ± 0.36 µg/ml) (Table 1). Phytochemicals have biological and pharmacological efficacy required for optimal health:
Flavonoids and phenols play a vital role as an antioxidant due to their ability to donate electrons/hydrogen from the hydroxyl group found on their phenolic ring [10,29], and this explains why they are currently employed as pharmaceutical agents for several diseases. Alkaloids are useful in antimicrobial, analgesic, and other antispasmodic actions [30]. Tannins have a wide range of pharmaceutical activities, including anti-diabetic [31], antioxidant, anti-inflammatory, anti-ulcers, anti-tumors, and anti-diarrhea activities [32], and can also precipitate proteins, so they have therapeutic value as astringents. Also, terpenoids possess antioxidant, anti-carcinogenic, and anti-inflammatory effects [14]. The biochemical functions of these phytochemicals are shown in their ability to stimulate the immune system, reduce the growth of cancer cells and prevent DNA damage [33]. Hence, these phyto compounds in C. acuminata make it a good candidate for the management/treatment of chronic diseases.

**Table 1. Qualitative phytochemical analysis of CAAE.**

| S/N | Phytochemicals | Availability | Amounts (µg/100g) |
|-----|----------------|--------------|-------------------|
| 1   | Reducing sugar | +++          | 71.16 ± 1.27      |
| 2   | Glycosides     | ++           | 2.67 ± 0.47       |
| 3   | Terpenoids     | ++           | 12.27 ± 1.90      |
| 4   | Steroids       | +            | 0.11 ± 0.36       |
| 5   | Tannins        | ++           | 4.01 ± 0.04       |
| 6   | Phenols        | +++          | 1735.48 ± 88.40   |
| 7   | Alkaloids      | +++          | 129.10 ± 15.68    |
| 8   | Flavonoids     | +++          | 94.04 ± 11.04     |
| 9   | Saponins       | ND           | -                 |

Key: +++ = present in high concentration, ++ = moderately present and ND = not detected

The values are reported as mean ± standard deviation of triplicate determination.

**3.2. Nitric oxide scavenging activity of CAAE.**

Nitric oxide is a cellular signaling molecule that acts as a vasodilator, immune regulator, blood pressure regulator, and inflammatory mediator [34]. However, an elevated level was seen in aberrated immune response, oxidative stress, and inflammatory reactions leading to modification of cellular molecules, which leads to deamination, hydroxylation, lipid peroxidation, and DNA strand breakage [35]. CAAE had a concentration-dependent percentage increase in NO scavenging activity from 56.44 ± 1.43 recorded in 31.25 µg/ml to 70.56 ± 2.01 in 500 µg/ml (Table 2). CAAE had better NO scavenging activity with an IC50 value of 5.56 µg/ml than 10.02 µg/ml recorded for ascorbic acid. Interestingly, a positive correction of 0.903 and 0.993 was obtained for CAAE and ascorbic acid, respectively (Figure 1A). This suggests that CAAE could scavenge NO free radicals produced in various biological reactions, thereby inhibiting its reaction with ROS to form peroxynitrite, an agent of lipid peroxidation [34].

**Table 2. Nitric oxide scavenging activity of CAAE.**

| Conc. (µg/ml) | CAAE (%)   | Ascorbic acid (%) |
|--------------|-----------|-------------------|
| 15.63        | 66.73±0.28| 60.40±0.12        |
| 31.25        | 56.44±1.43| 77.21±1.37        |
| 62.5         | 59.67±3.04| 82.10±0.51        |
| 125          | 61.66±1.14| 84.63±1.78        |
| 250          | 67.90±1.86| 85.05±5.14        |
| 500          | 70.56±2.01| 84.54±4.90        |

Values are reported as mean ± SD of triplicate determination. Mean values in the same column with different alphabets as superscripts compared to the groups are significantly different at p < 0.05.
3.3. *Inhibition of lipid peroxidation (TBARS) activity of CAAE.*

Assessment of the extent of inhibition of lipid peroxidation is one of the models mostly employed to evaluate the antioxidant capacity of a test compound [36]. Results in Table 3, which depicts the percentage inhibition of lipid peroxidation by cola extract and standard drug, butylated hydroxytoluene, show that the highest inhibitory capacity of the test substances was recorded at 62.5 and 125 µg/ml, respectively. Taken together, the overall inhibition by CAAE with IC\textsubscript{50} 29.16 µg/ml was significantly (p < 0.05) higher than that of BHT (IC\textsubscript{50} 60.27 µg/ml). The inhibitory activity was positively correlated with concentration, as revealed by the obtained R\textsuperscript{2} values of 0.890 and 0.955 for CAAE and BHT, respectively (Figure 1B). Reactive oxygen species, particularly polyunsaturated fatty acids, cause membrane damage through lipid peroxidation. The extract’s high lipid peroxidation inhibitory capacity suggests that it could serve as a reducing agent needed to reduce free radicals, thereby terminating radical-induced oxidative damage to the cell membrane, lipids, and lipoproteins, which will mitigate oxidative stress-induced diseases [17]. Conversely, *Cola acuminata* is a good source of natural antioxidants that may be utilized to cure various illnesses caused by free radical peroxidation.

### Table 3. Lipid peroxidation inhibitory activity of CAAE.

| Conc. (µg/ml) | CAAE (%) | Butylated Hydroxytoluene (%) |
|---------------|----------|-------------------------------|
| 15.63         | 33.33 ± 0.19 | 19.73 ± 1.98 |
| 31.25         | 32.10 ± 1.50 | 27.68 ± 1.61 |
| 62.5          | 90.71 ± 1.12 | 29.60 ± 2.61 |
| 125           | 94.06 ± 1.36 | 51.36 ± 0.54 |
| 250           | 70.37 ± 1.43 | 58.32 ± 2.50 |
| 500           | 77.78 ± 2.22 | 74.15 ± 0.97 |

Values are reported as mean ± SD of triplicate determination. Mean values in the same column with different alphabets as superscripts compared to the groups are significantly different at p < 0.05.

3.4. DPPH free radical scavenging activity of CAAE.

DPPH free radical scavenging activity is the most commonly used method for testing the antioxidant capacity of natural and synthetic compounds because it is a quick and simple procedure [37]. The extract exhibited high non-concentration-dependent inhibition of DPPH...
radical, highest at 125 µg/ml (Table 4). CAAE DPPH inhibitory capacity was non-significantly (p < 0.05) higher than that of ascorbic acid, as shown in their IC₅₀ values of 6.01 and 6.31 µg/ml, respectively. The DPPH inhibitory potential of both CAAE and ascorbic acid was positively correlated with concentrations, having R² values of 0.979 and 0.997, respectively (Figure 2A). When DPPH reacts with an antioxidant, the electron is paired off, and the solution of DPPH is decolorized from violet to yellow. The stabilization of DPPH radicals into the hydrazine form depends on the number of electrons taken up. Thus high DPPH scavenging activity of CAAE may be due to the donation of electrons from the multiple phenolic rings of the polyphenols [38,39] in the extract since studies have shown DPPH reduction is highly dependent on the amounts of phenolic compounds [40]. This also demonstrates the extract’s antioxidative capacity.

Table 4. DPPH free radical scavenging activity of CAAE.

| Conc. (µg/ml) | CAAE (%) | Ascorbic acid (%) |
|---------------|----------|-------------------|
| 15.63         | 88.01 ± 2.81 | 62.61 ± 1.97 |
| 31.25         | 89.71 ± 2.02 | 63.19 ± 1.34 |
| 62.5          | 94.04 ± 2.39 | 63.02 ± 0.78 |
| 125           | 97.59 ± 0.54 | 63.30 ± 0.68 |
| 250           | 89.72 ± 1.78 | 64.13 ± 0.72 |
| 500           | 91.49 ± 3.01 | 62.78 ± 0.96 |

Values are reported as mean ± SD of triplicate determination. Mean values in the same column with different alphabets as superscripts compared to the groups are significantly different at p < 0.05.

Figure 2. Nonlinear regression curve for IC₅₀ values of DPPH (Figure A) and TAC (Figure B) inhibitory activities of CAAE.

3.5. Total Antioxidant Capacity (TAC) of CAAE.

The TAC result shows that the various concentrations of CAAE had a non-significantly (p > 0.05) decrease in TAC activity except for 500 g/ml, which showed a significant difference (p < 0.05) when compared to 15.63-250 µg/ml. The highest inhibitory activity of 93 ± 1.63 µg/ml was obtained at the least concentration, 15.63 µg/ml. (Table 5). The IC₅₀ value of CAAE (4.89 µg/ml) was lower than that of the standard ascorbic acid (9.58 µg/ml), which suggests that the extract had better antioxidant capacity (Figure 2B). Antioxidant capacity depends on the presence of its bio-active components. Conversely, the bioactive agents present in the extract donated electrons which reduced Mo (VI) to Mo (V), leading to the formation of the green phosphate Mo (V) complex measured in the TAC assay [16]. Therefore, the high antioxidant capacity of the cola extract is an important indicator of the presence of abundant bioactive antioxidative compounds that will be of immense benefit in the management of oxidative stress-induced chronic diseases.
**Table 5.** Percentage total antioxidant capacity of CAAE.

| Conc. (µg/ml) | CAAE (%)   | Ascorbic acid (%) |
|---------------|------------|-------------------|
| 15.63         | 93.50b ± 1.63 | 65.17a ± 2.27    |
| 31.25         | 93.78b ± 0.16 | 80.44a ± 3.29    |
| 62.5          | 85.70b ± 3.26 | 89.62b ± 0.35    |
| 125           | 90.53b ± 1.00 | 87.33b ± 1.75    |
| 250           | 83.10ab ± 2.15 | 93.21bc ± 3.53  |
| 500           | 72.42a ± 4.28 | 94.51b ± 4.08    |

Values are reported as mean ± SD of triplicate determination. Mean values in the same column with different alphabets as superscripts compared to the groups are significantly different at p < 0.05.

### 3.6. Ferric Reducing Antioxidant Power (FRAP) of CAAE

The presence of reductants, which exert antioxidant action by breaking free radical chains through donating electrons, is quantified with the FRAP test [17]. FRAP is based on the ability of a test substance to donate electrons needed to reduce the Fe$^{3+}$/ferricyanide complex in the test solution to its Fe$^{2+}$/ferrous form [8]. The cola extract’s FRAP result expressed in gallic acid equivalent was maximal at 15.63 µg/ml and 31.25 µg/ml with values 35.76 ± 4.65 and 18.38 ± 1.94 respectively. (Figure 3). The IC$_{50}$ value obtained from the nonlinear regression curve was 32.44 GAE. Besides, a negative correlation with log concentration (R$^2$ = -0.213) was obtained from this study (Figure 4). CAAE iron-reducing capability was proven in this work by its ability to decrease the color intensity of the solution, which depicts the reduction of Fe$^{3+}$ to Fe$^{2+}$ [39]. The Fenton reaction occurs when ferrous ion breaks the lipid membrane, resulting in the formation of alkoxy and peroxyl radicals, which are oxidative damage mediators [38]. A study on selected plants, including oregano, marjoram, lemon balm, and rosemary, reported similar FRAP activity [41]. Invariably, the FRAP potential could be anchored on the rich phytoconstituents present in the plant, which are positively correlated with phenolic and flavonoid content [9].

![Figure 3. Ferric reduces the antioxidant activity of CAAE. Results are expressed as mean ± SD of triplicate determination. When compared, mean values with different alphabets as superscripts are significantly different at p < 0.05.](image-url)

![Figure 4. Nonlinear regression curve for IC$_{50}$ values of FRAP inhibitory activity of CAAE.](image-url)

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4. Conclusions

This study has shown that CAAE possesses phytochemicals that play a vital role in biological systems. It also has good antioxidant potential, needed in the prevention of oxidative stress, which can initiate the onset of chronic diseases. Therefore, *Cola acuminata* could serve as a potential therapeutic agent in the prevention and/or treatment of oxidative diseases.

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Conflicts of Interest

The authors declare no conflict of interest.

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