International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Total phenolic and flavonoid contents of C*ola acuminata* extract and evaluation of sub-acute toxicity on the hematological parameters of Wistar rat

Chigozie Celestina Ezeagha [1, *], Chioma Mmesoma Ifeakandu [1], Chinenye Henrietta Nedum [3], Onumsinachukwu Consolata Orji [1], Emmanuel Chuks Oranu [1], Ngozi Ukamaka Madubogwu [2] and Ejike Victor Otitochukwu [1]

[1] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University Igbariam, Anambra State, Nigeria.*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University Igbariam, Anambra State, Nigeria.*
[3] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.*

International Journal of Science and Research Archive, 2026, 19(01), 1119-1127

Publication history: Received on 17 March 2026; revised on 25 April 2026; accepted on 27 April 2026

Article DOI: https://doi.org/10.30574/ijsra.2026.19.1.0844

Abstract

Cola acuminata, also known as kola nut, is a West African evergreen tree with notable medicinal properties. Traditionally, its seeds have been used for their therapeutic effects, including antioxidant activity.

In this study, *Cola acuminata* leaves were collected, dried, and extracted using a cold maceration method with methanol. To assess the extract's safety, a subacute toxicity test was conducted on 24 Wistar rats over 14 days. The rats were divided into four groups and given varying doses of the extract: control (distilled water), low (250 mg/kg), medium (500 mg/kg), and high (1000 mg/kg). After the test period, the rats were fasted and euthanized, and blood samples were analyzed for toxicity through a full blood count test. The antioxidant properties of the extract were evaluated by measuring total phenolic and flavonoid contents. The total phenolic content was determined using the Folin-Ciocalteu reagent method, with results expressed as gallic acid equivalents (GAE) per milliliter, while flavonoid content was measured using the aluminum chloride colorimetric method with quercetin as the standard and expressed as quercetin equivalents (QE) per milliliter.

Results indicated that the *Cola acuminata* leave extract was non-toxic at all tested doses compared to the control. It exhibited a low total phenolic content of 28.48 GAE mg/mL but a high total flavonoid content of 146.67 QE mg/mL, suggesting potential antioxidant property. Thus, *Cola acuminata* leaves extract is non-toxic and demonstrates strong potential antioxidant property.

Keywords: *Cola acuminata;* Total Phenolic Contents; Flavonoid Contents; Sub-Acute Toxicity; Hematology

1. Introduction

Medicines for humans are based on natural compounds derived from plants, animals, and minerals. [1, 2] There is currently a demand for medicinal plants, and there is a growing acceptance of them. Throughout human history, medicinal plants have been utilized to treat illnesses. Today, a tenth of plant species, or more than 50,000 species, is utilized in pharmaceutical and cosmetic products. [1] The term medicinal plant is referred to as wide range of plants with therapeutic properties. These plants have a wealth of chemicals that can be utilized to create new drugs. Different

* Corresponding author: Chigozie Celestina Ezeagha

kinds of seeds, roots, leaves, fruits, bark, flowers, and even the entire plant are among the parts of medicinal plants that can be employed. The majority of medicinal plants' active ingredients are employed as medical agents because they have either direct or indirect therapeutic effects. Certain molecules known as active compounds are created and stored in the bodies of these plants, and they have physiological impacts on living things. Compounds originating from plants can significantly improve diseases like cancer that are difficult to cure. [1, 3]

Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines. [4] Medicinal plants are employed worldwide as a supplemental or alternative therapy, Research and development of drugs requires an understanding of these therapeutic plants including toxicological and pharmacological analysis. [5]

Chemicals for harm and safety are evaluated using both acute and sub-acute toxicity testing. These studies, which assess the effects of single or repeated exposure on animals, are frequently employed in risk assessment and identification processes pertaining to the manufacture, handling and application of chemicals. Subacute toxicity test uses multiple doses of a drug or chemical to evaluate the possibility of long-term effects on target organs, whereas acute toxicity test uses a single dosage of a chemical to evaluate the animal level of toxicity. [6, 7]

Cola acuminata is a medicinal plant commonly known as kola nut. It's a tropical evergreen tree that is indigenous of West Africa. For generations, people have utilized *Cola acuminata* seeds for their therapeutic qualities. It contains theobromine, caffeine and other methylxanthines which have a stimulant effect on the nervous systems, these substances can lessen weariness, promote alertness and enhance cognitive performance. [8]

Cola acuminata extract contains a high concentration of flavonoids and phenolic chemicals which has been linked to possible health benefits. On the other hand, nothing is known about the full profile of phenolic and flavonoid content in *Cola acuminata* extract and how it affects hematological parameters after sub-acute exposure. Thus, the purpose of this study is to evaluate the sub-acute toxicity, total phenolic content, and total flavonoid content of *Cola acuminata* extract on Wistar rat hematological parameters.

The aim of this study is to evaluate the total phenolic content, total flavonoid content and sub-acute toxicity of *Cola acuminata* extract on hematology parameters of Wistar rat. In particular, the aim is to quantify the amount of flavonoids and phenolic compounds in the extract and look into any possible negative effects on the platelet count, hemoglobin level, hemoglobin count, red blood cell count, and white blood cell count in Wistar rats after sub-acute administration.

The investigation into the total phenolic and flavonoid content of *Cola acuminata* extract is of paramount importance due to the well-documented antioxidant properties of these compounds. Phenolics and flavonoids are recognized for their ability to neutralize free radicals, thereby reducing oxidative stress and preventing cellular damage. By quantifying these components in *Cola acuminata*, the study provides valuable insights into the potential health benefits and therapeutic applications of the extract. This information is crucial for developing natural antioxidant therapies, particularly in the context of chronic diseases where oxidative stress plays a key role, such as cardiovascular diseases, diabetes, and cancer. [9, 10, 11]

Additionally, the subacute toxicity evaluation of *Cola acuminata* extract on the hematological parameters of Wistar rats is essential for determining the safety profile of the extract. Hematological parameters, including red and white blood cell counts, hemoglobin levels, and platelet counts, are critical indicators of overall health and can reveal toxic effects on the bone marrow, liver, and other organs. By conducting a sub-acute toxicity study, this research aims to identify any potential adverse effects associated with the extract's consumption, ensuring its safety for potential therapeutic use. This aspect of the study is particularly significant for informing dosage guidelines and risk assessment for human applications.

The combined analysis of total phenolic and flavonoid content along with sub-acute toxicity evaluation offers a comprehensive understanding of both the beneficial and potential adverse effects of *Cola acuminata* extract. This holistic approach not only underscores the extract's antioxidant potential but also ensures that its application in traditional and modern medicine is safe and effective. By bridging the gap between phytochemical analysis and toxicological assessment, this study contributes to the growing body of knowledge required to support the use of *Cola acuminata* in health and disease management, potentially leading to new, natural therapeutic options. [12]

2. Materials and method

2.1. Materials

Test tubes, test tube racks, spatula, measuring cylinder (various sizes), micropipette, beakers (various sizes), stirrer, glass funnel, mortal & pestle, syringe, rat cage, dissecting kit, EDTA tubes, oral galvage. Peak Visible Spectrophotometer (Model 721, China), Centrifuge, Incubator, G&G Analytical weighing balance (Model JJ224BC), automated analyzer, Digital thermostatic water bath, Rotary evaporator, Laboratory oven, Refrigerator, Folin-ciocalteu phenol reagent, Na_2CO_3, $NaNO_2$, $AlCl_3$, NaOH, distilled water, chloroform, methanol.

2.2. Animal

A total of twenty-four rats were used during the course of the experiment. The rats used were purchased from the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Chukwuemeka Odumegwu Ojukwu University, Igbariam campus. The animal was allowed to acclimatize to the environment for 1 week. They were housed in a clean and well-ventilated cage and fed with feed and water *ad libitum.* The animals were handled in compliance with the national institute of health (NIH) guidelines for the care and use of lab animals for research purposes (Pub No 85-23. Revised 1985) Ethical permit number PHACOOU/ARE/2024/015 was obtained from faculty of pharmacy COOU animal Research Ethics Committee.

2.3. Plant Collection

Cola acuminata leaves sample were obtained from Umuoru-Uga, Aguata local government in Anambra State. The leaf sample were identified and authenticated by a taxonomist Dr. Onyinye Okonkwo in the department of Pharmacognosy and Traditional medicine, Faculty of Pharmaceutical Science, Chukwuemeka Odumegwu Ojukwu University, Igbariam. The leaves were air dried and pulverized with a grinding machine.

2.4. Extraction Procedures

The powdered *Cola acuminata* leaves (100 g) was weighed and placed into a beaker and 500 ml of methanol was added to the beaker. The resulting mixture was stirred and left to stand for 24 hours. The content of the beaker was filtered after 24 hours. The extraction was repeated using 500 ml of the solvent. After 24 hours, the mixture was filtered and the combined extract was concentrated using a rotatory evaporator. The dried extract was stored in a labeled sample bottle.

2.5. Sub-acute Toxicity

The animals were grouped into four (4) groups with each group having a total number of six (6) rats. The weight of each rat was determined each week and the extracts were administered orally based on their new weight. Four doses were used to carry out the test which is the vehicle (distilled water) in which the extract was dissolved in, high dose (1000 mg/kg), medium dose (500 mg/kg), low dose (250 mg/kg). It was administered as:

- Group1— vehicle
- Group2— low dose
- Group 3—-medium dose
- Group 4—high dose

Each of these doses was given to the rats every day for 14 days and at the end of the 14 days the rats were fast overnight and sacrificed.

2.6. Sample collection

At the end of the study, animals were deeply anaesthetized with chloroform and were dissected to expose the visceral. With the aid of needle attached to 5 ml syringes, blood samples were collected from the inferior vena cava and were delivered into EDTA and mixed gently to avoid clotting.

2.7. Determination of hematological parameters using MIDRAY BC-2800 auto hematological analyzer

The EDTA tubes containing blood were rocked for 15 minutes using an electrical multi-functional mixer which ensures proper mixing of the blood elements. Then the hematological parameters were analyzed using Midray BC 2800, a hematological analyzer.

Each sample was analyzed for full blood count. A volume of 18 µL of sample was taken through probe. The aspirate key was pressed for 2-3 seconds and 18 µL of sample was taken by the analyzer, and diluent was used to separate the cells in the blood as well as to create a conducive environment for cell counting. The red blood cells were lysed before the WBC was counted. All samples were run at the temperature of 24^0C [13].

2.8. Determination of Total Phenolic Content:

Folin-Ciocalteu reagent method described by Yadav[14] was used to determine the amount of phenolics present in the samples. The reaction mixture contained 0.2 ml of Folin-Ciocalteu phenol reagent (diluted 20% v/v), 1 ml of 7.5% solution of Na_2CO_3 solution, 0.4 ml of the extract and 0.8 ml of distilled water. The mixture was heated for 30 min at 40^0C in water bath and was allowed to cool. Absorbance was taken at 760 nm using U.V Spectrophotometer. All the tests were performed in triplicate and results were determined from garlic acid calibration curve and expressed as gallic acid equivalent (GAE mg/ml). A calibration curve was prepared with 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml. A test tube containing distilled water and all reagents (without sample) served as blank. Total phenolic content of samples was estimated from garlic acid calibration curve.

2.9. Determination of Total Flavonoid Content:

Total flavonoid content was estimated by the aluminum chloride colorimetric method described by Muthukumaran[15] using quercetin as standard.
125 µl of sample was added to 75 µl of 5% $NaNO_2$. The mixture was allowed to stand for 6 min. Then, 150 µl of 10% $AlCl_3$ was added and incubated for 5 minutes. This was followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500µL with distilled water. After 15 min of incubation the absorbance was measured at 510 nm using U.V spectrophotometer. The total flavonoids content was expressed as mg quercetin/ml of sample. A calibration curve was prepared with 3.90, 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml. A test tube containing distilled water and all reagents (without sample) served as blank. Total flavonoid content was estimated from quercetin calibration curve.

3. Results

Table 1 Result of Extraction

Extract	Quantity of plant(g)	Weight of extract(g)	Yield (%)
Cola acuminata leave methanol	100g	28.38g	28.38%

Table 2 Effect of *Cola acuminate* leaves extract on %weight gain

				BODY WEIGHT (g)			
GROUP	Dose	S/N	BT	WEEK 0	WEEK 1	WEEK 2	WT GAIN (%)
1	Control (Distilled water)	1	1	95	119	131	27.48
		2	2	98	132	149	34.23
		3	3	114	149	154	25.97
		4	4	63	88	105	40.00
		5	5	104	129	144	27.78
2	Low Dose (250mg/Kg) of *Cola acuminate*	6	1	95	128	141	32.62
		7	2	96	124	134	28.36
		8	3	89	115	121	26.45
		9	4	90	127	124	27.42
		10	5	76	109	141	46.10
3	Medium Dose (500mg/Kg)	11	1	113	154	157	28.03

	Of *Cola acuminate* extract	12	2	87	125	133	34.59
		13	3	85	126	133	36.09
		14	4	91	130	138	34.06
		15	5	107	143	151	29.14
4	High Dose (1000mg/Kg) Of *Cola acuminate*	16	1	73	99	113	35.40
		17	2	103	133	144	28.47
		18	3	97	131	143	32.17
		19	4	87	114	125	30.40
		20	5	82	110	126	34.92

3.1. Result on hematological parameters

Table 3 Effect of *Cola acuminata* on Full Blood Count

Group	WBC (10^9/L)	HBG (g/dl)	RBC (10^{12}/L)	HCT (%)	MCV (fL)
Control	14.86 ± 3.48	16.32 ±1.64	7.68 ± 0.53	47.86 ± 4.07	62.30 ± 1.82
250 mg/kg	14.62 ±4.93^{ns}	14.46 ± 3.55 ^{ns}	6.97 ± 1.72 ^{ns}	43.84 ± 11.15 ^{ns}	62.90 ± 1.60 ^{ns}
500 mg/kg	17.40 ±2.88 ^{ns}	16.28 ± 0.59 ^{ns}	7.94 ± 0.19 ^{ns}	48.64 ± 1.58 ^{ns}	61.34 ± 1.87 ^{ns}
1000 mg/kg	12.50 ±3.50 ^{ns}	12.54 ± 3.86 ^{ns}	6.09 ± 1.92 ^{ns}	37.60± 11.95 ^{ns}	61.88 ± 2.32 ^{ns}

Values are presented as mean ± Standard deviation. ^{ns}P>0.05 Not statistically significantly different from control group**. WBC** (White blood cell), **HBG** (Hemoglobin), **RBC** (Red blood cell)**, HCT** (Hematocrit/packed cell volume), **MCV** (Mean corpuscular volume).

Table 4 Effect of *Cola acuminata* on Full Blood Count

Group	MCH (pg)	MCHC (g/dl)	RDW-CV (%)	RDW-SD (fL)
Control	20.74 ± 0.65	33.42 ± 0.52	16.28 ± 1.36	33.62 ± 1.72
250 mg/kg	32.70 ± 27.00 ^{ns}	33.00 ± 0.57 ^{ns}	15.52 ± 1.03 ^{ns}	32.34 ± 1.97 ^{ns}
500 mg/kg	20.34 ± 0.36 ^{ns}	33.18 ± 0.45 ^{ns}	16.18 ± 0.32 ^{ns}	32.16 ± 1.84 ^{ns}
1000 mg/kg	20.60 ± 0.66 ^{ns}	33.42 ± 0.64 ^{ns}	15.90 ± 1.43 ^{ns}	33.20 ± 1.68 ^{ns}

Values are presented as mean ± Standard deviation. ^{ns}P>0.05 Not statistically significantly different from control group**. MCH** (Mean corpuscular hemoglobin), **MCHC** (Mean corpuscular hemoglobin concentration), **RDW-CV** (red cell distribution width - coefficient of variation), **RDW-SD** (red cell distribution width - standard deviation).

Table 5 Effect of *Cola acuminata* on Full Blood Count

Group	PLAT (10^9/L)	MPV (fL)	PDW (%)	PCT (%)
Control	557.40 ± 67.69	8.54 ± 0.25	14.68 ± 0.19	0.47 ± 0.05
250 mg/kg	447.80 ± 100.52 ^{ns}	8.22 ± 0.30 ^{ns}	14.48 ± 0.27 ^{ns}	0.37 ± 0.09 ^{ns}
500 mg/kg	519.20 ± 31.52 ^{ns}	8.48 ± 0.16 ^{ns}	14.66 ± 0.05 ^{ns}	0.44 ± 0.33 ^{ns}
1000 mg/kg	498.00 ± 185.93 ^{ns}	8.46 ± 0.15 ^{ns}	14.66 ± 0.09 ^{ns}	0.42 ± 0.16 ^{ns}

Values are presented as mean ± Standard deviation. ^{ns}P>0.05 Not statistically significantly different from control group. There was dose dependent reduction in platelet count, but was not statistically significant when compared to control. **PLAT (**platelet), MPV **(**Mean platelet volume), **PDW (**platelet distribution width), **PCT** (Plateletcrit).

Table 6 Results of Antioxidant Assay

Samples	Total Phenolic Content (GAE mg/ml)	Total Flavonoid Content (gQE/ml)
Cola *acuminata*	28.48	146.67

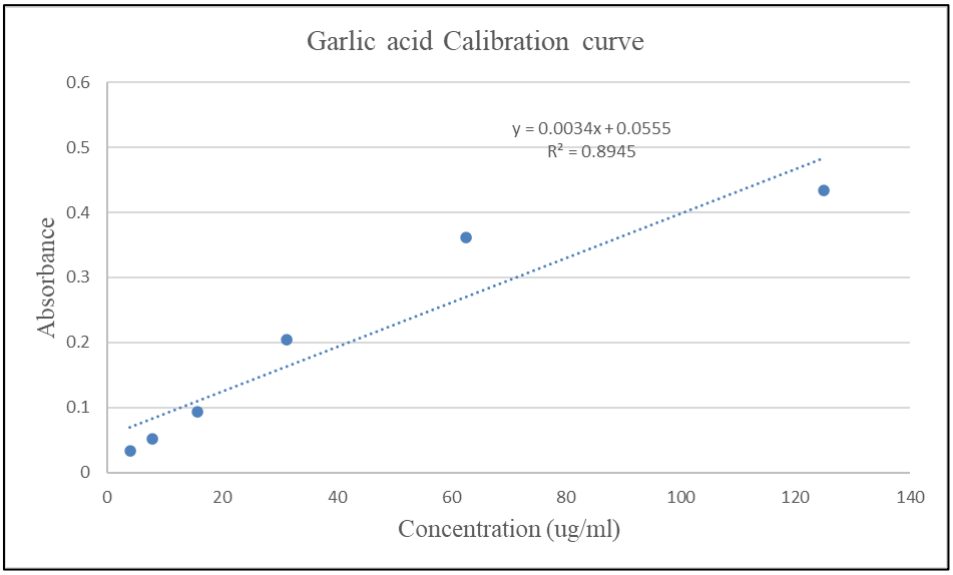

Figure 1 A graph of absorbance against Concentration (ug/ml) for Garlic acid Calibration Curve

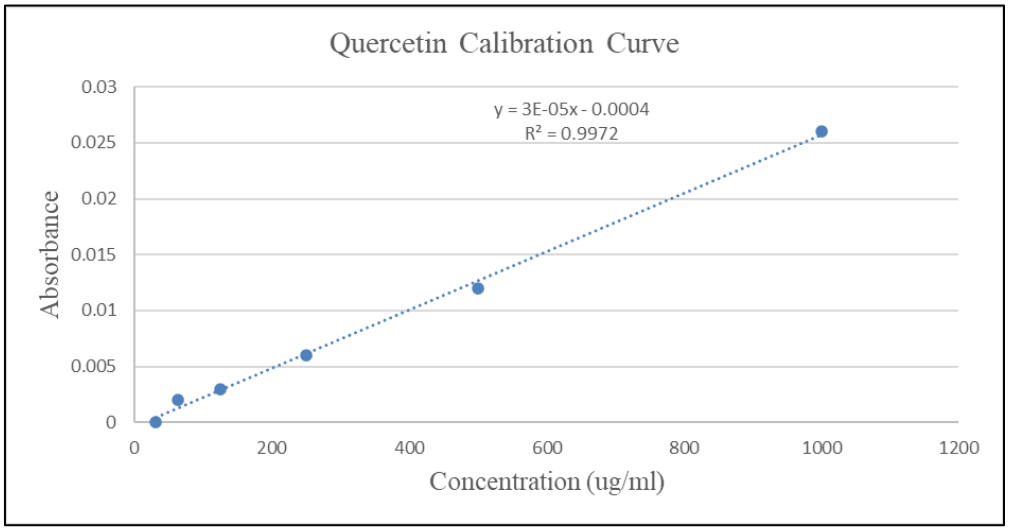

Figure 2 A graph of absorbance against Concentration (ug/ml) for Quercetin Calibration Curve

4. Discussion

Based on percentage weight gain of the rats, the rats in each group had a significant increase in weight gain from week 1 to week 2 suggesting that the *Cola acuminata* leaves extract did not affect the eating ability of the rats and neither did it cause any harm or sickness which would have drastically cause a decrease in their weight gain.

This study evaluated the sub-acute toxicity of *Cola acuminata* leaves extract using a total number of 24 rats for 14 days. The toxicity effect of the plant was evaluated by comparing the hematological parameters of the test group to the control. The blood is a crucial component of the body that helps to transport food, drug and other substances throughout the body system therefore blood profile typically gives an insight on how the body reacts to trauma, lesions and stress. From

the result obtained the extract showed no significant difference in WBC (^{ns} p> 0.05) when compared to the control. This means that there was no difference between the WBC of the control group which took only the vehicle (distilled water) and the test group that was treated with the extract, this result contradicts the report of [16] which says that methanol extract of *Cola acuminata* cause a significant increase on WBC count of the rat. The WBC helps the body to build immunity and defend body against any toxic substances or microbes so a significant decrease in WBC above the normal range indicates the presence of toxicity, infection or lesion. The most reliable indicators for diagnosis of anemia are the red blood Cell, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and hematocrit which did not show any significant difference (^{ns} p >0.05) when compared to the control. This means that the extract did not cause anemia to the rat, this also indicate that the extract did not cause any internal bleeding, deficiencies in iron and vitamins and showed no signs of adverse effect related to ulcer, cancer or hemorrhoids. The hemoglobin is a protein in the red blood cells that carries oxygen from the lungs to the body's tissues, the extract showed no significant difference in HGB (^{ns} p >0.05), thus there was no interference of the extract with oxygen transportation. The hemoglobin is also a reliable indicator in diagnosis of anemia. There was no significant difference (^{ns} p >0.05) in platelet (PLAT), PDW, MPV and PCT when compared to the control thus the extract did not affect the platelet formation or function. Also, there was no significant difference (^{ns} p >0.05) in RDW-CV, RDW-SD when compared to the control. Therefore, the leave of *Cola acuminata* can be said to be relatively non-toxic to the rat.

This study also determined the Total phenolic and flavonoid content of *Cola acuminata* leaves extract using Folin-Ciocalteu phenol reagent method and Aluminium chloride colorimetric method respectively. Phenolics and flavonoids are class of plant compounds or phytochemicals known for their potential health benefit and diverse biological activities which include; modulation of inflammatory pathways (Anti-inflammatory), inhibition of bacteria, virus and fungi growth (Anti-microbial) [17,18,19], inhibition of cell proliferation (Anti-cancer) and neutralization of free radicals thereby reducing oxidative stress (Anti-oxidant) [20,19]. Many research works have shown that antioxidant activity of a plant is proportional to the content of phenolic and flavonoid content [21, 22]. This is as a result of the hydroxyl group found in the structure of flavonoids (flavones, flavonols), and phenolics (lignans, gallic acid) [23, 24]. Therefore, the presence of phenolics and flavonoids in leaves extract of *Cola acuminata* suggest a potential antioxidant activity and the higher the flavonoid and phenolic content the higher the potential anti-oxidant, anti-microbial and anti-inflammatory activities. From the result the Total phenolic content test gave a quantitative value of 28.48 GAE mg/ml while the Total flavonoid content test gave a quantitative value of 146.67 mg QE/ml, this shows that 1ml of *Cola acuminata* leaf contains 28.48 mg of phenolic compounds and 146.67 mg of flavonoid compounds.

5. Conclusion

Based on the research study carried out on the plant, *Cola acuminata* (leaves) commonly known as kola nut has shown to have no toxicity effect on the animal. It also demonstrates the potential antioxidant property of *Cola acuminata* (leaves) due to its high flavonoid content and phenolic content which has the ability to ameliorate the damages caused by free radicals.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge Dr. Earnest Erhirhie for his immense contribution to this work.

Disclosure of conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethical approval

The animals were handled in compliance with the national institute of health (NIH) guidelines for the care and use of lab animals for research purposes (Pub No 85-23. Revised 1985) Ethical permit number PHACOOU/ARE/2024/015 was obtained from faculty of pharmacy COOU animal Research Ethics Committee.

References

[1] Jamshidi-Kia Fatemeh, Zahra Lorigooini, Hossein Amini-Khoei. Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacol*.2018; 7(1):1-7.

[2] Ezeagha CC, Duru CP, Agbasi IS, Orji OC, Ogbuebuna JO, Nedum CH, Anozie AN, Chukwudulue UM (2024) Nutritional properties of *Nauclea latifolia* root and leave. *GSC Biological and Pharmaceutical Sciences.* 2024; 26(01): 130–138.

[3] Ezeagha CC, Nnaoma-Gabriel A, Orjiakor J, Orakwe J, Otarigho BE, Oranu EC. Determination of inhibitory activities of the constituents of *Curcuma longa* (l) against *Streptococcus pneumoniae* by molecular docking simulation. *Magna Scientia Advanced Biology and Pharmacy* 2025, 16(01): 016–027.

[4] Nwokorie, C.C., Nwachukwu N.C., Ezeanokete C.C, Ike C.C. The Phytochemical and Antimicrobial Analysis of *Pterocarpus santalinoides* Plants; *Asian Journal of Science and Technology,* 2015; 6(05): 1411-1418.

[5] Oranu EC, Ezeagha CC, Otitochukwu EV, Orji OC, Chukwudulue UM, Madubogwu NU, Anozie AN. Evaluation of antidiarrheal activity of ethanolic leaf extract of *Ficus sur* (Moraceae). *Magna Scientia Advanced Biology and Pharmacy.* 2025; 16(01): 001–006.

[6] Ghizlane Nouioura, Meryem Tourabi, Adel Tahraoui, Karima El-yagoubi, souad Maache, Hinde Elfatemi, Badiaa Lyoussi, El Houssine Derwich, (2023). Assessment of the acute and subacute toxicity of the aqueous extract of Moroccan Ferula communis fruit in a mouse model 2023

[7] Chukwudulue UM, Nwosu EC, Iwuala UF, Mbionwu O, Ezechinwoye U, Aniemeka KK, Ezeagha CC, Oranu E, Anyanwu OO, Erhirhie EO. Toxicity Evaluation of Methanol Leaf Extract of *Psydrax manensis* (Aubrevilla and Pellegrin) Bridson in Wistar Albino Rats. *African Journal of Pharmaceutical and Herbal Research (AJPHR)*. 2025; 1 (1): 47-67.

[8] Müller, W. E., Rolli, M., Schäfer, C., Hafner, U., Fichte, K., Zöller, M., &Wollert, U. Effects of caffeine on the antagonized contraction by adenosine of vascular smooth muscle. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 2007; 374(2): 129-139.

[9] Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability*. American Journal of Clinical Nutrition*, 2004; 79(5): 727-747.

[10] Chukwudulue UM, Nnamdi CI, Chukwukeme PO, Nnajiofor EI, Ezeagha CC, Ogbuebuna JO, Oranu EC, Anyanwu OO. Phytochemistry and antimicrobial properties of *Psydrax manensis* leaf. *World Journal of Biology Pharmacy and Health Sciences*. 2023; 16(03): 147–154.

[11] Ezeagha CC, Orji OC, Ogbuebuna JO, Anozie AN, Nedum CH. Phytochemical Screening and Evaluation of Antioxidant Properties of *Nauclea latifolia* Root Smith. (Rubiaceae). *World Journal of Pharmaceutical Research*. 2023; 12(12): 24-30.

[12] Pietta, P. G. Flavonoids as antioxidants. Journal of Natural Products, 2000; 63(7): 1035-1042.

[13] Paulinus Mfon Udoudoh, Daniel Ejim Uti, Emmanuel E. Edet, Sunday Nkereuwem Etukudoh, Blessing Godwin Ukwak, Uket Nta Obeten and Grace UfedoUmoru. Synergistic anti-diabetic activity of *Gongronema latifolium* and *Telfairia occidentalis* leaves extracts on hepatic function and hematological indices in wistar rats. *Asian J. Biol. Sci.,* 2020; 13: 42-52

[14] Yadav, R.N.S. and Agarwala, M. Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 2011; 3(12): 10-14.

[15] Muthukumaran P., Saraswathy N., Aswitha V., Balan R., Gokhul V.B., Indumathi. P. and Yuvapriya.S. Assessment of total phenolic, flavonoid, tannin content and phytochemical screening of leaf and flower extracts from *Peltophorum pterocarpum* (DC.) Backer ex K.Heyne: a comparative study. *Pharmacognosy Journal*. 2016; 8(2):140-143.

[16] Norman Chinweike Asogwa, Chukwuka Christian Onyeka, Elijah Chibuezeodii, Felicia Nkechi Ekeh, Chika Bright Ikele, Bernard Obialo Mgbenka and Joseph Effiong Eyo. Heamatological and Cortisol Dynamics in Albino Rats Stimulated by Intake of Methanol Extract *Cola acuminata. World journal medical science,* 2014; 10(4):384-391.

[17] Okoli JN, Okolo CC, Anyanwu OO, Oranu EC, Ezeagha CC, Obidiegwu OC, Okoye NN, Okoye FBC. Antimicrobial activity of secondary metabolites produced by Colletotrichum species, an endophytic fungus on *Vernonia amygdalina* Del (fam. Asteraceae). World Journal of Biology Pharmacy and Health Sciences. 2023; 14(01): 031–042.

[18] Chidera V. Eboh, Chukwubuikem C. Okolo, Ogechi O. Anyanwu, Nkeoma N. Okoye, Chigozie C. Ezeagha and Festus B.C. Okoye (2022). Metabolites of *Colletrotricum* species, an endophytic fungus isolated from *Vernonia amygdalina Del* possess antimicrobial and antioxidant activities. *GSC Biological and Pharmaceutical Sciences,* 20(03), 148–159.

[19] Ezeagha CC, Ogbuebuna JO, Anozie AN, Oranu, E.C., Nedum, C.H. Determination of the Phytochemical and Antimicrobial properties of *Nauclea latifolia* Root Smith. (Rubiaceae). GSC Biological and Pharmaceutical Sciences. 2023; 23(02): 182–188.

[20] Oranu EC, Okeke NP, Ezeagha CC, Otarigho BE, Orakwe JC, Meko AO. In silico evaluation of phytocompounds as potential inhibitors of trypanosoma brucei. Open Access Research Journal of Biology and Pharmacy. 2025; 15(02): 019-027.

[21] Muhammed Ma'ruf., Bachri,M. S., & Nurani, L.H. Phytochemical Screening Analysis and Determination of Total Flavonoids and Total Phenolics Content of Ethanol Extract of Sungkai Leaf (*Penorema canescens* Jack) from Samarinda City. *Jurnal Mandala Pharmacon Indonesia*, 2023; 9(2): 262-272.

[22] Oranu EC, Charles MJ, Ezeagha CC, Otarigho BE, Orakwe JC, Meko AO .Virtual screening of phytochemicals for the identification of novel anti-*Leishmania donovani* Agents. *International* Journal of Biological and Pharmaceutical Sciences Archive. 2025; 10(02): 186–195.

[23] Ezeagha CC, Ajaghaku DL, Ajaegbu EE, Ngwoke KG, Osadebe PO, Okoye FBC. Molucidine and desmethylmolucidine as immunostimulatory lead compounds of morinda lucida (Rubiaceae). Tropical Journal of Natural Product Research. 2021; 5(5): 977–982.

[24] Oranu EC, Ezeagha CC, Madueke AJ, Otarigho BE, Orjiakor J, Etuka IK. Molecular Docking and Virtual Screening of Boswellia dalzielii Phytochemicals as Potential Inhibitors of Rabies lyssavirus. GSC Biological and Pharmaceutical Sciences. 2025; 33(02): 267–279.