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GC-MS, HPLC AND SUB-ACUTE TOXICOLOGICAL ASSESSMENT OF *Cola acuminata* METHANOL LEAF EXTRACT

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Abstract
Plant leaves are known to be rich in a wide range of bioactive compounds with diverse biological properties, making them valuable in drug discovery and development. This study investigated the bioactive constituents and sub-acute toxicity profile of Cola acuminata leaf extract. Fresh leaves were air-dried, ground into powder, and macerated in methanol for 72 hours. The mixture was filtered, and the crude extract was concentrated using a rotary evaporator at 40°C. Twenty-eight male albino Wistar rats were divided into seven groups (four rats per group). Groups 2 to 7 received oral doses of the extract at 10, 100, 1000, 1600, 2900, and 5000 mg/kg respectively, while group 1 received normal saline, all for 14 days. After treatment, animals were sacrificed under light anesthesia. Blood was collected via cardiac puncture, and liver and kidney tissues were harvested for biochemical and hematological analysis. GC-MS and HPLC analyses were performed to identify phytochemical constituents. Liver and kidney markers such as ALP, ALT, AST, urea, and creatinine showed significant changes at higher doses ($p<0.05$), although hematological parameters (HBG, PCV, RBC, PLT, WBC) remained unaffected. No mortality was observed. GC-MS identified 17 bioactive compounds, including β-Longipinene, Pentadecane, cetene, 1-octadecene, neophytadiene, 1-eicosene, oxazepam, etc while HPLC detected flavonoids like flavone, narigenin, catechin, anthocyanin, tannin, among others. The results suggest that while Cola acuminata leaf extract is rich in beneficial phytochemicals, prolonged use at high doses may cause liver and kidney dysfunction.

Keywords: *Bioactive compounds, Cola acuminata, GC-MS, HPLC, Phytochemicals, Antioxidants*

Introduction

Cola acuminata (P.Beauv.) Schott and Endler, tree is an evergreen, medium sized tree, having low grey or dark green branches, very green leaves and whitish flowers. It is popularly known as kola nut but commonly called 'evbee' in Edo, 'Oji' in Igbo, 'obi' in Yoruba and 'goro' in Hausa (Ugwuowo *et al*., 2021). The genus *Cola* (Malvaceae), native to tropical West Africa, comprises five edible species—*C. nitida*, *C. acuminata*, *C. ballayi*, *C. verticillata*, and *C. sphaerocarpa. Cola nitida* and *Cola acuminata* are more widely utilized than other species.

While cola nuts are globally recognized for their stimulant properties—owing to caffeine (1.25–2.4%), theobromine, theophylline, tannins, saponins, and flavonoids—the medicinal use of *C. acuminata* extends to its other plant parts (Jacob *et al*., 2024). Additionally, seed extracts exhibit high polyphenol content and strong in vitro antioxidant activity (Jacob *et al*., 2024).

Several pharmacological studies provide evidence supporting the traditional therapeutic claims of the seeds. Aqueous seed extracts demonstrated inhibitory activity against acetylcholinesterase (AChE, $IC_{50} \approx 14.6$ µg/mL) and butyrylcholinesterase (BChE, $IC_{50} \approx 96.2$ µg/mL), reducing oxidative stress in rat brain homogenates. This suggests potential in managing neurodegenerative disorders like Alzheimer's disease (Oboh *et al*., 2014). Antidiabetic and antioxidative activity: In alloxan-induced diabetic rats, young leaf extracts significantly lowered blood glucose in a dose-dependent manner and enhanced antioxidant enzyme activities (glutathione, superoxide dismutase), comparable to glibenclamide (Victoria *et al*., 2023).

Antimicrobial effects: Seed extracts displayed measurable antimicrobial activity against both bacterial (e.g., *Staphylococcus*, *Bacillus*) and fungal strains, while leaf extracts performed moderately. Both also displayed notable antioxidant properties via DPPH and ABTS assays (Victoria *et al*., 2023). Memory enhancement synergy: Combined administration of *C. acuminata* seed extract with *Spondias mombin* leaf extract improved cognitive performance and countered scopolamine-induced oxidative stress in rats (Ishola *et al*., 2018).

Traditional healers in West Africa have employed the leaves to alleviate respiratory conditions, treat anemia, diarrhea, and splenic impairment. Recent phytochemical investigations show that aqueous leaf extracts are rich in cardiac glycosides, saponins, terpenoids, tannins, and reducing sugars, along with significant levels of minerals like magnesium, potassium, phosphorous, and iron, suggesting both nutritional and health benefits (Okungbowa *et al*., 2025).

Despite this growing body of preclinical evidence supporting its antioxidant, neuroprotective, antidiabetic, and antimicrobial potentials, comprehensive scientific studies validating the traditional uses of *C. acuminata* leaves are still lacking. Accordingly, this study aims to deepen understanding of its bioactive profile by systematically evaluating phytochemical constituents of leaf extracts and assessing their antioxidant activities in vitro. This will help build a stronger scientific foundation for the ethnomedicinal application of *C. acuminata* leaves.

Materials and Methods

Preparation of Cola acuminata Methanol Leaf Extract

The *Cola acuminata* leaves were harvested 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. They were air-dried and ground into powder from which 500g was macerated in 2.5L of methanol for 72 hours while stirring at

intervals. Thereafter, muslin cloth was used to sieve the mixture and obtain the filtrate, which was concentrated with a rotary evaporator at 40ºC and further dried in an oven to obtain the crude extract.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the leaves was performed on an Agilent 6890 gas chromatograph (GC) which was interfaced to an Agilent 5973N Mass Spectrometer (MS) and fitted with a META X5 coated fused capillary column Length: 30m, Diameter: 0.25mm with a film thickness of 0.25µm; and a maximum temperature, 325 °C.

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC analysis of the extracts was done with a RESTEK 15METER MXT-1 HPLC system using methanol AT 5PSI as carrier.

Determination of in vitro Antioxidant Activity:

Total Antioxidant Capacity (TAC) assay**:**

The Total Antioxidant Capacity (TAC) of *Cola acuminata* leaf extract was determined by the phosphomolybdate method according to Prieto *et al*. (1999). 1 ml of aliquot (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) of the leaf extracts were mixed with 3mL of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) in test tubes. The tubes were capped with aluminum foil and incubated in boiling water at 95°C for 90 min. The reaction mixture was allowed to cool at room temperature and the absorbance of the solution was measured at 695nm against a blank. The blank contained 3ml of the reagent solution and the appropriate volume of the dissolving solvents. This was incubated under the same conditions as the test samples. Ascorbic acid was used as the standard reference compounds to compare the activities of the extracts. The test was done in triplicates.

DPPH Radical Scavenging Activity:

About 0.1 mL of the extract and fractions (100 mg/ mL) was added by 3.9 mL of 0.15mM DPPH in ethanol. The solution then was mixed vigorously and allowed to stand in the dark for 30 mins at room temperature. All tests were carried out in triplicate and ascorbic acid was used as a standard control at concentrations comparable to the test samples, a blank solution containing 0.1 mL ethanol with 3.9 mL DPPH was used as a control. Absorbance was measured at 517 nm using a spectrophotometer. The DPPH scavenging activity was calculated according to the equation (Jamous *et al*., 2015).

$$\%\text{Radical Scavenging Activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} x100$$

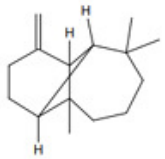
Sub-acute Toxicity Studies

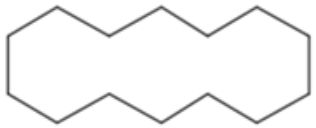
Twenty-eight male albino Wistar rats were grouped into seven groups of four rats each, per extract. Each extract was administered separately with graded doses as follows: 10,100, 1000, 1600, 2900, and 5000mg/kg for groups 2-7 respectively, while group 1 received normal saline. Thereafter, animals were sacrificed and blood was collected, liver, kidney and spleen were harvested for biochemical and hematological analysis.

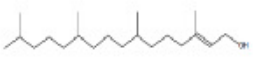
Results

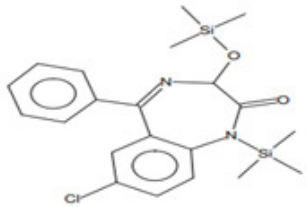
Table 1 shows results for the GC-MS analysis *Cola acuminata* leaf extract. Several bioactive compounds were found to be present in the leaves.

Table 1. GC-MS analysis of *Cola acuminata* Leaves

PEAK NO.	RETENTION TIME (MIN.)	% COMPOSITION	NAME OF COMPOUND	STRUCTURE	MOLECULAR WEIGHT	BIOLOGICAL ACITIVITY	REFERENCES
1	6.149	0.420	β-Longipinene	$C_{15}H_{24}$ 	204	Sesquiterpene/ antimicrobial and anti-insecticidal activity antioxidant and anti-inflammatory	Shukurova *et al*., 2020; Santana *et al*., 2012.
2	6.275	0.606	Pentadecane	$C_{15}H_{32}$ 	212	Alkane, hydrocarbon/anti-inflammatory, antipyretic and analgesic	Chuah *et al*., 2018; Okechukwu, 2020.
3	6.538	3.292	Cetene	$C_{16}H_{32}$ 	224	Alkene, hydrocarbon/antibacterial, antioxidant	Yogeswari *et al*.,2012; Elgorban *et al*., 2019
4	6.825	1.047	Heptadecane	$C_{17}H_{36}$	240	Alkane, hydrocarbon/ antibacterial	Keke *et al*., 2023; Togashi *et al*., 2007.

5	6.893	1.629	Cyclotetradecane	$C_{14}H_{28}$ 	196	Cycloalkane, hydrocarbon/ antimicrobial	Keke *et al*., 2023; Chuah *et al*., 2018.
6	7.065	2.625	1-Octadecene	$C_{18}H_{36}$ 	252	Alkene, hydrocarbon/ Antibacterial, antioxidant and anticancer activity	Keke, *et al*., 2023; Belakhdar *et al*., 2015.
7	7.174	3.896	Neophytadiene	$C_{20}H_{38}$ 	278	Diterpene, an anti-inflammatory agent, an antimicrobial agent, antifungal and antioxidant properties, cardioprotective, inhibition of cyclooxygenase or lipoxygenase	Rajeswaran and Rajan, 2025

8	7.231	1.357	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ 	296	Diterpene alcohol/ Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, antimalaria, precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 and modulates transcription.	(Banakar and Jayaraj, 2018),
9	7.391	4.148	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ 	270	Alkane, Anti HIV, Antioxidant, Antibacterial, Antimicrobial , Cytotoxic effect, Antimicrobial, Antimalarial,	Banakar and Jayaraj, 2018.
10	7.654	2.034	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether	$C_{26}H_{58}O_2Si_2$ 	458	Alkane, antifungal, antioxidant, anti-inflammatory, analgesic, and antipyretic	Okechukwu, 2020; Ahsan *et al*., 2017.

11	7.889	3.677	Phytol	$C_{20}H_{40}O$ 	296	Antioxidant and antimicrobial activities	Gollo *et al*., 2020; El-sayed and Ismail, 2022
12	8.015	1.950	Oxazepam, 2TMS derivative	$C_{21}H_{27}ClN_2O_2Si_2$ 	430	Diterpenoid/Antimicrobial, Anti inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, Resistant gonorrhea, Joint dislocation, Headache, Hernia, Stimulant and antimalaria	Banakar and Jayaraj, 2018.
13	8.696	0.949	1-Eicosene	$C_{20}H_{40}$ 	280	Antioxidant and Insecticidal Activities	Ganesh and Mohankumar, 2017.
14	9.468	7.500	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$ 	390	Antimicrobial activity	Ganesh and Mohankumar, 2017.

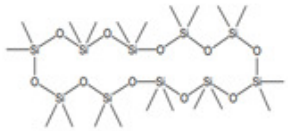
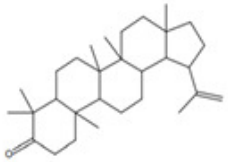
15	10.275	2.393	Eicosamethyl-cyclodecasiloxane,	$C_{20}H_{60}O_{10}Si_{10}$ 	740	Organic compound phthalate, antimicrobial, cytotoxic, anticancer antibacterial, larvicidal	Lofty *et al.*, 2018; Javed *et al.*, 2022.
16	10.767	2.960	Eicosane	$C_{20}H_{42}$ 	282	Antimicrobial activity	Ganesh and Mohankumar, 2017.
17	21.141	12.279	Lup-20(29)-en-3-one	$C_{30}H_{48}O$ 	424	Anti-inflammatory, analgesic, antipyretic, wound healing, antioxidant	Balachandran *et al.*, 2023; Okechukwu, 2020.

Table 2 shows results for the HPLC analysis of the leaves of *Cola acuminata*. Phytochemicals of biological importance were detected in the leaves. Catechin had the highest concentration, followed by tannins.

Table 2. High Performance Liquid Chromatography (HPLC) analysis of *Cola acuminata* Leaves

S/N	Component (*Cola acuminata*)	Retention	Area	Height	Concentration (µg/mL)
1	Kaemferol	0.280	3426.8154	117.436	4.2254
2	Steroid	2.390	12252.8106	301.042	10.5084
3	Epihedrine	4.120	6344.5478	157.568	2.7206
4	Catechin	6.016	18153.9630	442.688	28.1631
5	Anthocyanin	7.470	8442.8722	206.427	7.2409
6	Dihydrocytisine	10.366	19598.0759	476.646	16.7936
7	Aphyllidine	12.970	6238.3258	152.341	7.0277
8	Cyanogenic glycoside	15.460	4967.5726	121.273	4.4824
9	Aphyllidine	17.966	11339.3364	276.425	7.6359
10	Narigenin	20.313	12756.4948	307.631	10.9404
11	Tannin	22.730	9573.3711	233.187	25.3877
12	Flavonones	25.650	10008.8176	245.115	17.1678
13	Ammodendrine	27.536	11458.0104	280.295	1.9287
14	Flavone	29.860	5478.6156	133.724	9.3973
15	Proanthocyanidin	32.996	14337.0482	348.877	8.8967
16	Ribalinidine	34.600	6059.7940	147.836	7.5207
17	Spartein	36.876	6988.5601	170.310	8.9904
18	Oxalate	39.200	10234.8247	249.264	22.8219
17	Sapogenin	42.276	3473.1416	85.310	4.0338
18	Phytate	44.170	10509.6912	256.782	7.7220

Sub-Acute Toxicity of Cola acuminata Methanol Leaf Extracts on some Liver Function Markers

Table 3 reveals the results for ALP, ALT, AST and Albumin. Albumin, ALP and ALT activities in *Cola acuminata* extract treated group reveal that there was no significant difference in the low dose group compared to the control, but higher doses induced a significant ($p<0.05$) higher activity compared with the control. On the other hand, the extract induced a significant ($p<0.05$) reduction in AST activity across all the groups.

Table 3: Effect of *Cola acuminata* methanol leaf extract on Plasma ALP, ALT and AST

Group	ALP (U/L)	ALT(U/L)	AST(U/L)	ALBUMIN (g/L)
Group 1 Control (Normal saline)	223.00±5.24[a]	38.00±1.41[a]	43.50±3.54[a]	39.10±4.00[a]
Group 2 (10 mg/kg body weight	224.00±5.65 [a]	39.04±3.84[a]	14.33±8.08[b]	40.13±3.57[a]
Group 3 (100 mg/kg body weight)	222.00±3.46 [a]	42.12±2.14[a]	15.33±6.00[b]	37.67±2.41[a]
Group 4 (1,000 mg/kg body weight)	236.00±0.00[b]	32.01±2.82[a]	10.00±3.00[b]	36.20±0.00[a]
Group 5 (1,600 mg/kg body weight)	189.00±4.94[b]	64.36±3.29[b]	22.00±2.10[b]	36.50±3.39[a]
Group 6 (2,900 mg/kg body weight)	206.00±4.94[b]	42.32±3.00[a]	12.66±7.37[b]	40.66±3.21[a]
Group 7 (5,000 mg/kg body weight)	224.00±5.65 [a]	57.23±2.30[b]	13.33±8.50[b]	39.60±5.43[a]

Values are mean ± SD n=4 values with different alphabet are significantly ($p<0.05$) different.

Sub-Acute Toxicity of Cola acuminata Methanol Leaf Extracts on Some Kidney Function Markers

The results for the sub-acute toxicity testing of *Cola acuminata* methanol leaf extract on plasma urea and creatinine concentrations in albino Wistar rats are presented in table 4. There was no significant difference in urea and creatinine concentrations of the low dose and high dose *Cola acuminata* methanol leaf extract treated groups when compared with the control.

Table 4: Effect of *Cola acuminata* methanol leaf extract on Plasma Urea and Creatinine

Group	UREA (mg/dL)	CREATININE (mg/dL)
Group 1 Control (Normal saline)	105.00±1.41[a]	2.14±0.36[a]
Group 2 (10 mg/kg body weight	89.74±4.51[a]	2.12±1.62[a]
Group 3 (100 mg/kg body weight)	85.74±4.51[a]	4.01±4.3[a]
Group 4 (1,000 mg/kg body weight)	92.01±0.00[a]	2.08±0.00[a]
Group 5 (1,600 mg/kg body weight)	116.61±4.07[a]	1.92±0.91[a]
Group 6 (2,900 mg/kg body weight)	91.25±7.57[a]	4.28±2.43[a]
Group 7 (5,000 /kg body weight)	100.93±4.06 [a]	1.49±1.48[a]

Values are mean ± SD n=4 values with different alphabet are significantly ($p<0.05$) different

Sub-Acute Toxicity of Cola acuminata Methanol Leaf Extracts on some Hematological indices

Results for some hematological indices in *Cola acuminata* methanol extract treated groups are presented in table 5. *Cola acuminata* methanol leaf extract showed no significant difference in the PCV, WBC, RBC, HBG and PLT concentrations across all the groups when compared with the control.

Table 5: Effect of *Cola acuminata* methanol leaf extract on some Hematological indices

Group	PCV (%)	WBC(U/l)	RBC(U/l)	HBG(d/L)	PLT(U/l)
Group 1 Control (Normal saline)	64.80±9.87	5.50±2.80	9.68±1.40	14.60±2.95	366.00±137.00
Group 2 (10 mg/kg body weight	56.40±2.47	5.60±0.91	6.11±4.53	13.60±1.81	771.00±182.00
Group 3 (100 mg/kg body weight)	57.60±4.00	5.60±1.91	9.00±0.28	14.70±0.63	160.00±72.00
Group 4 (1,000 mg/kg body weight)	58.80±0.00	5.70±0.00	8.80±0.00	15.20±0.00	693.00±0.00
Group 5 (1,600 mg/kg body weight)	50.40±3.74	7.50±3.04	7.58±0.32	13.50±0.63	338.00±253.00
Group 6 (2,900 mg/kg body weight)	54.00±7.21	6.20±4.71	8.29±1.29	17.60±4.34	645.00±144.00
Group 7 (5,000 mg/kg body weight)	76.0±8.15	8.60±5.50	11.95±0.72	20.00±2.91	289.00±1.31

Values are mean ± SD n=4 values with different alphabet are significantly ($p<0.05$) different.

Discussion

GC-MS

A number of bioactive compounds having biochemical and structural importance were found in the leaves of Cola acuminata. GC-MS results show the presence of terpenes, including β-Longipinene, neophytadiene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and Oxazepam 2TMS derivative. These compounds demonstrate several beneficial properties, such as antioxidants, antifungal, antimicrobial, and anti-inflammatory activities. Additionally, CA contain other bioactive compounds like eicosane, known for its anti-inflammatory, antioxidant, antitumor, immunostimulant, anticancer, and lipoxygenase-inhibitor properties. Moreover, Bis(2-ethylhexyl) phthalate, pentadecane, tetradecane, hexadecane, cetene, heptadecane, and octadecane exhibit antioxidant, antimicrobial, and anti-inflammatory effects (Banakar and Jayaraj, (2017 and 2018); Yogeswari *et al*. (2012); Elgorban *et al*. (2018); Kim *et al*. (2013); Pratama *et al*. (2019)).

Several bioactive compounds with notable biochemical and structural significance were identified in the leaves of Cola acuminata. GC-MS analysis revealed the presence of various terpenes, including β-Longipinene, neophytadiene, phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, lup-20(29)-ene-3-one, and nerol methyl ether. These compounds are known to possess antioxidant, antifungal, antimicrobial, and anti-inflammatory properties.

In addition to terpenes, Cola acuminata also contains other biologically active compounds such as eicosane, which

has been reported to exhibit a wide range of pharmacological activities, including anti-inflammatory, antioxidant, antitumor, immunostimulant, anticancer, and lipoxygenase-inhibitory effects.

Furthermore, hydrocarbons such as Bis(2-ethylhexyl) phthalate, pentadecane, tetradecane, hexadecane, cetene, heptadecane, and octadecane were also identified. These compounds have been associated with antioxidant, antimicrobial, and anti-inflammatory activities (Banakar and Jayaraj, 2017, 2018; Yogeswari *et al*., 2012; Elgorban *et al*., 2018; Kim *et al*., 2013; Pratama *et al*., 2019).

HPLC

HPLC analysis revealed that the leaves of Cola acuminata contain a diverse range of bioactive phytochemicals, including flavonoids, catechins, proanthocyanidins, and anthocyanins—well-known plant-derived antioxidants. The extract also showed high concentrations of tannins, flavones, flavanones, steroids, naringenin, cyanogenic glycosides, anthocyanidins, and sparteine.

Flavonoids, in particular, represent the most abundant class of polyphenolic compounds in plants. They are responsible for the pigmentation of many plant tissues and play key roles in plant physiology, including protection against environmental stressors. Functionally, flavonoids act as chemical messengers, regulators of plant growth, and inhibitors of the cell cycle (Imam *et al*., 2017). Their pharmacological significance is well-established, with extensive evidence supporting their anti-inflammatory and antioxidant activities (Rathee *et al*., 2009).

Sub-acute Toxicity

Urea and creatinine are critical biomarkers for assessing kidney function. The slight elevation in creatinine levels observed may be indicative of glomerular inflammation or interstitial nephritis, as noted by Kodner and Kudrimoti (2003). The findings of this study align with previous reports by Alaebo *et al*. (2022), Ogbonna *et al*. (2020), and Salawu *et al*. (2019), but contrast with those of Mordi *et al*. (2021). Although the methanol leaf extract of Cola acuminata was not overtly nephrotoxic, it altered kidney enzyme activities, suggesting that prolonged exposure to high doses may contribute to kidney damage.

Albumin, a major plasma protein synthesized in the liver, plays essential roles in the transport of hormones, vitamins, and ions, and in maintaining fluid balance. It serves as a useful marker of both liver and kidney function (Ogbonna, 2020). Alongside enzymes such as ALT, AST, and ALP, albumin levels are commonly assessed to evaluate liver health (Sun *et al*., 2019). Elevations in these markers typically reflect hepatocellular damage, allowing leakage from hepatocytes into circulation and impairing liver function.

In this study, plasma albumin concentrations remained statistically unchanged across all treatment groups compared to the control. Similar outcomes were reported by Alaebo *et al*. (2022), Salawu *et al*. (2019), and Ogbonna *et al*. (2020) at 100 and 200 mg/kg extract doses. However, those studies observed significant reductions ($p<0.05$) in serum albumin at 400 mg/kg, contrary to the present findings.

Plasma ALP activity showed no significant differences at lower doses of Cola acuminata methanol leaf extract compared to the control. However, higher doses led to a significant ($p<0.05$)

reduction in ALP activity, with the lowest value observed at 1,600 mg/kg. Interestingly, the 1,000 mg/kg dose caused a significant increase ($p<0.05$) in ALP activity. Similar alterations in liver and kidney enzymes have been reported by Salawu *et al*. (2019) and Ogbonna *et al*. (2020) in response to plant extracts.

ALT activity increased in the low-dose groups but did not differ significantly from the control. In contrast, higher doses (1,600 and 5,000 mg/kg) led to significant ($p<0.05$) increases in ALT activity. The 1,000 and 2,900 mg/kg groups exhibited ALT levels comparable to the control, with the 1,000 mg/kg group showing a non-significant reduction.

AST activity was significantly ($p<0.05$) reduced across all treatment groups, with the most pronounced decline observed at the 1,000 mg/kg dose. These findings differ from those reported by Obasi et al. (2022), likely due to differences in plant species or experimental conditions.

Heamatology Studies

The methanol leaf extract of Cola acuminata did not cause statistically significant changes in hematological parameters, including packed cell volume (PCV), white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HBG) concentration, and platelet (PLT) count across all treatment groups compared to the control. However, the extract did induce non-significant alterations in these values.

Specifically, PCV values decreased in all groups except the group administered 5,000 mg/kg body weight. WBC counts exhibited slight increases across all groups, while RBC levels showed a general decline. Hemoglobin concentrations remained largely comparable to the control. Platelet counts varied, with the highest value recorded in the group given 10 mg/kg and the lowest in the 100 mg/kg group. Although these changes were not statistically significant, the observed trends suggest that the extract may influence hematological parameters over a long period of exposure. Such effects may be attributed to interactions between phytochemicals in the extract and blood biomolecules, potentially modulating their synthesis, degradation, or function.

Given these findings, caution is advised when consuming Cola acuminata extracts, especially in individuals without underlying health conditions. Continued use, particularly at higher doses, may pose risks by subtly altering hematological homeostasis, which could have long-term physiological implications.

Conclusion

This study demonstrates that the methanol leaf extract of *Cola acuminata* is a viable source of antioxidant phytochemicals, which are key components in traditional medicine. The findings provide scientific evidence supporting its medicinal potential and relative safety when used at low to moderate doses over an extended period. However, caution is warranted with prolonged administration at high doses, as this may lead to liver and kidney dysfunction. Overall, the results validate the traditional use of *Cola acuminata* and highlight the importance of dose regulation to ensure therapeutic safety.

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