Nigerian Research Journal of Chemical Sciences (ISSN: 2682-6054)
Volume 14, Issue 2, 2026

Phytochemical Profiling and Evaluation of Antioxidant and Anti-Inflammatory Activities of the Leaves of *Cola lepidota* K. Schum

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Accepted: *May 12, 2026.* **Published Online***: May 15, 2026*

ABSTRACT

This study investigated the phytochemical composition, antioxidant capacity, and anti-inflammatory activities of *Cola lepidota* leaves. Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detector (GC-FID) were used to identify bioactive constituents. Antioxidant activity was assessed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH), and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS assays, while the anti-inflammatory activity was evaluated using four *in vitro* models: protein denaturation inhibition, membrane stabilization, proteinase inhibition, and lipoxygenase inhibition assays. The GC-MS analysis revealed 30 distinct peaks with 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester (25.81%) as the most abundant compound, followed by 6-Octadecenoic acid, (Z)- (17.37%) and 1,2-Benzisothiazol-3-amine derivatives (~36% combined). GC-FID identified 25 phytochemicals, with notable concentrations of apigenin (45.07 ppm), artemetin (24.50 ppm), myricetin (19.25 ppm), and flavone (4.70 ppm). The extract showed strong, concentration-dependent radical scavenging activity comparable to standard antioxidants. It also exhibited significant dose-dependent anti-inflammatory effects, with inhibition of protein denaturation (up to ~78%), membrane stabilization (~88%), proteinase activity (~58%) and lipoxygenase activity (~66%). These findings suggest that *Cola lepidota* leaves possess significant therapeutic potential as a natural source of antioxidant and anti-inflammatory agents, supporting its application in pharmaceutical and nutraceutical development.

Keywords: Antioxidant, anti-inflammatory activity, *Cola lepidota*, DPPH, flavonoids, lipoxygenase, phytochemicals.

INTRODUCTION

Inflammation and oxidative stress are closely linked biological processes implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative conditions. Reactive oxygen species (ROS) generated during metabolic processes can induce oxidative damage to lipids, proteins, and DNA, thereby triggering inflammatory responses and tissue injury [1]. Consequently, the search for natural compounds with combined antioxidant and anti-inflammatory properties has gained increasing scientific attention. Plants remain a major source of bioactive compounds with therapeutic potential. Compared to synthetic drugs, plant-derived antioxidants are generally considered safer and may offer fewer side effects, making them attractive candidates for drug discovery and nutraceutical development. *Cola lepidota* commonly referred to as monkey kola, belong to the family *Malvaceae*, subfamily *Sterculioideae*, and traditionally used in ethnomedicine for the treatment of various ailments. The monkey kola varieties include red (*C. lateritia*), yellow (*C. lepidota*), and white (*C. parchycarpa*) types [2]. Ngoka et al [3] reported that *Cola lepidota* is a tropical plant native to the rainforests of West and Central Africa, encompassing nations such as Nigeria, Cameroon, and Gabon. *Cola lepidota* grows up to 18 m tall with a twisted trunk, edible fruit with crunchy and tasty yellow pulp [4][5], roundish pods, and is mostly consumed fresh. It is called “Achicha” or “Ochiricha” in Igbo and “Ndiyah” in Efik and Ibibio [2].

In spite of its traditional applications, there is limited scientific evidence regarding the bioactive constituents and pharmacological properties of the leaves of *Cola lepidota* as most available research focused on other plant parts such as seeds [6], bark, and fruits [7]. Hence the need to systematically evaluate the phytochemical composition and bioactivity of *Cola lepidota* leaves using standard biochemical assays.

The aim of this study is to provide scientific validation for the traditional use of *Cola lepidota* leaves and contribute to the identification of potential natural anti-inflammatory agents.

MATERIALS AND METHODS

Sample Collection and Preparation

Cola lepidota leaves (Plate 1) were collected in a homestead from Amaoba community in Ikwuano *Local Government Area, of Abia State, Nigeria. The plant was identified in the taxonomic unit of* the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture,

Umudike. Voucher specimen was deposited in the Department's herbarium (MOH 00185). The leaves were air-dried for 14 days, and the dried sample was ground into powder with the aid of a blender, sieved and stored in air tight container bottles, and taken to the laboratory for extraction and analysis.

Plate 1: *Cola lepidota* plant

Extraction of Powdered Samples

The powdered sample was subjected to Soxhlet extraction method. 500 mL clean boiling flask was dried in oven at 105 – 110 ^{0}C for about 30 minutes. This flask was transferred into a desiccator and allowed to cool. 10 g of the sample was weighed and poured into the Soxhlet thimble. The extraction thimble was plugged lightly with cotton wool to aid filter the extract. The boiling flask was then filled with about 300 mL of ethanol. The Soxhlet apparatus was assembled and allowed to reflux for about 4 hours at a temperature of 60 ^{0}C. The thimble was then removed with care, thereafter the mixture was poured into a volumetric flask and allowed to cool. The content in the volumetric flask was then transferred into a rotatory evaporator to separate the solvent from the extract.

Extraction of Phytochemicals

About 1 g of the extract was weighed, transferred into a test tube and 25 mL of ethanol was added. The test tube was heated in a hotplate at 60 ^{0}C for 90 min. The tube was washed with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 mL of hexane. After the reaction time, the reaction product contained in the test tube was transferred through a separatory funnel. These

extracts were combined and washed three times with 10 mL of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000 µl of pyridine of which 200 ul was transferred to a vial for analysis.

Phytochemical profiling by GC-MS

The analysis for phytochemicals was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. About 1 microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in a split less mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram [8]. The bioactive compounds extracted from the extracts were identified based on GC-MS retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

Phytochemical profiling by GC-FID

The phytochemical profiling was performed on an Agilent 6890 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m x 250 um x 0.15 um) was used. The injector temperature was 280 ^{o}C with split less injection of 2 µl of sample and a linear velocity of 30 cms^{-1}, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 $mLmin^{-1}$. The oven operated initially at 200 ^{0}C, it was heated to 330 ^{0}C at a rate of 3 $^{0}C\ min^{-1}$ and was kept at this temperature for 5 min while the detector operated at a temperature of 320 ^{0}C. The phytochemicals were determined by the ratio of the area and mass of internal standard, and the area of the identified phytochemicals [9].

DPPH Spectrophotometric Assay

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH (2,2-diphenyl-2-picryl hydrazyl) assay was measured by the method of Mensor [10].

ABTS Scavenging Effect

The antioxidant effect of the leaf sample was studied using ABTS (2,2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of Shirwaikar [11].

Inhibition of Albumin Denaturation

The anti-inflammatory activity of *Cola lepidota* was studied by using inhibition of albumin denaturation technique as reported in previous studies [12, 13] with minor modifications. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction; pH of the reaction mixture was adjusted using small amount of 1M HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660 nm (UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as shown in equation 1:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \quad (1)$$

Heat Induced Haemolysis [13]

The reaction mixture (2 mL) consisted of 1 mL test sample of different concentrations (100 - 500 µg/mL) and 1 mL of 10 % RBCs suspension, instead of test sample, only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for the test sample. The percentage inhibition of haemolysis was calculated as shown in equation 2:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \quad (2)$$

Anti-proteinase Action

The test was performed according to the modified method of Oyedepo and Femurewa [14]. The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL 20 mM Tris HCl buffer (pH 7.4) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37 ºC for 5 min and then 1 mL of 0.8 % (w/v) casein was added. The mixture was incubated for

an additional 20 min. 2 mL of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated as shown in equation 3:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \quad (3)$$

Anti-lipoxygenase Activity [13]

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test sample was dissolved in 0.25 mL of 2 M borate buffer pH 9.0 and added 0.25 mL of lipoxidase enzyme solution (20,000 U/mL) and incubated for 5 min at 25 ^{0}C. After which, 1.0 mL of lenoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percent inhibition was calculated from equation 4:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \quad (4)$$

RESULTS AND DISCUSSION

The GC-MS analysis of the ethanol leaf extract of *Cola lepidota* (Table 1) revealed 30 distinct peaks with 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester (25.81%) as the most abundant compound, followed by 6-Octadecenoic acid, (Z)- (17.37%) and 1,2-Benzisothiazol-3-amine derivatives (~36% combined). This indicates that the leaf extract of *Cola lepidota* is chemically dominated by lipid-derived molecules and synthetic/derivatized aromatic compounds. Chukwuemeka et al [15] reported that the GCMS chromatogram of ethanolic seed extract of *Cola lepidota* contained fourteen fatty acid compounds, of which linoleic acid methyl ester had the largest peak. However, Ogidi et al [6] reported that benzoquinone, 2,3-dimethyl and squalene were the main chemical molecules found in the crude extract of *C. lepidota* seeds.

Fatty acids and their esters are known to exhibit diverse biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects. Oleic acid has been reported for its cardioprotective and anti-inflammatory roles [16, 17]. It was observed that some compounds had multiple peaks. Such compounds include 1-Docosene (7 & 9), 2-Piperidinone, N-[4-bromo-n-butyl]- (11 & 13), 1,2-Benzisothiazol-3-amine, TBDMS derivative (18, 24, 25 & 27). However, the repeated presence of TBDMS derivatives suggests derivatization during GC–MS analysis, not necessarily native compounds. This indicates that sample preparation likely involved silylation,

modifying original molecules. Altogether, the GC–MS results substantiate the traditional use of *Cola lepidota* in African ethnomedicine for managing infections and inflammation, aligning with earlier reports on the therapeutic significance of *Cola* species [18].

Table 1: GC-MS Analysis of Ethanol Extract of *Cola lepidota* Leaves

S/N	Retention Time (min)	% of Total	Identified Phytochemicals
1	10.193	0.25	Hexadecane
2	13.024	0.29	9-Eicosene, (E)-
3	13.171	0.35	1-Octadecanesulphonyl chloride
4	14.710	0.54	Dibutyl phthalate
5	15.771	0.67	1-Octadecene
6	15.898	0.22	Sulfurous acid, butyl tetradecyl ester
7	18.289	0.93	1-Docosene
8	18.402	0.26	1-Octadecanesulphonyl chloride
9	20.609	1.43	1-Docosene
10	20.707	0.33	Docosane
11	21.516	0.39	2-Piperidinone, N-[4-bromo-n-butyl]-
12	21.783	3.18	Bis(2-ethylhexyl) phthalate
13	22.164	0.73	2-Piperidinone, N-[4-bromo-n-butyl]-
14	22.756	1.22	17-Pentatriacontene
15	22.841	0.57	Docosane, 9-octyl-
16	24.857	0.66	Squalene
17	30.006	0.76	4-Cholesten-3-one semicarbazone
18	30.860	0.09	1,2-Benzisothiazol-3-amine, TBDMS derivative
19	30.934	0.02	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
20	31.034	0.05	Erucic acid
21	31.052	0.03	Pentatriacontane, 13-docosenylidene-
22	31.295	2.83	Propanamide, 2,2-dimethyl-N-(4-methylphenyl)-
23	31.335	2.17	Pyrrolidine, 1-methyl-3,2'-spiro-benzo-1,3-dioxolane-
24	33.372	17.43	1,2-Benzisothiazol-3-amine, TBDMS derivative

25	33.448	3.63	1,2-Benzisothiazol-3-amine, TBDMS derivative
26	33.473	1.26	Acetamide, N-(4-fluorophenyl)-2,2,2-trifluoro-
27	33.553	14.67	1,2-Benzisothiazol-3-amine, TBDMS derivative
28	34.901	2.05	Cyclotrisiloxane, hexamethyl-
29	35.384	17.37	6-Octadecenoic acid, (Z)-
30	36.755	25.81	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester

The GC-FID analysis of the ethanol leaf extract of *Cola lepidota* (Table 2) identified 25 phytochemicals, with notable concentrations of apigenin (45.07 ppm), artemetin (24.50 ppm), Myricetin (19.25 ppm), and Flavone (4.70 ppm). The chromatographic analysis revealed a diverse spectrum of polyphenolic compounds, including flavonoids, phenolic acids, stilbenes, and isoflavones, with a total quantified concentration of 112.520 ppm. The identified compounds are dominated by flavonoids, which account for the majority of the total concentration. Apigenin is a well-documented flavone with strong anti-inflammatory, antioxidant, and anticancer properties, primarily through modulation of signaling pathways such as NF-κB and MAPK [19]. Myricetin is a potent antioxidant flavonol with demonstrated free radical scavenging, antidiabetic, and neuroprotective effects [20].

Table 2: GC-FID Analysis of the Ethanol Extract of *Cola lepidota* Leaves

S/N	Retention Time (min)	Amount (ppm)	Identified Phytochemicals
1	3.925	0.929	Catechin
2	4.030	45.066	Apigenin
3	5.172	2.831	Resveratrol
4	5.304	0.409	Coumaric acid
5	6.254	2.129	Genistein
6	6.646	1.883	Epicatechin
7	7.004	4.703	Flavone
8	7.219	0.605	Daidzein
9	7.695	0.148	Epigallocatechin
10	7.999	0.399	Lunamarin

11	8.264	0.409	Daidzin
12	8.401	0.474	Naringin
13	9.016	0.537	Flavon-3-ol
14	9.159	0.291	Butein
15	9.515	24.504	Artemetin
16	9.871	0.550	Vinnillic acid
17	10.156	0.425	Naringenin
18	10.695	0.187	Luteolin
19	11.907	0.312	Kaempferol
20	12.738	4.266	Quercetin
21	14.607	19.251	Myricetin
22	15.115	1.684	Nobiletin
23	17.398	0.158	Baicalin
24	18.351	0.166	Cinnamic acid
25	18.617	0.204	Ferrulic acid
	TOTAL	112.520	

The antioxidant potential of the ethanol extract of *Cola lepidota* leaf was assessed using DPPH and ABTS radical scavenging assays, with BHT - Butylated hydroxytoluene and Gallic acid serving as reference standards. The extract exhibited a steady, concentration-dependent increase in radical scavenging activity with percentage inhibition rising from 66.29% at 40 mg/mL to 92.71% at 160 mg/mL (Table 3). The relatively small difference between 92.71% (extract) and 97.20% (BHT) suggests that the extract contains highly effective natural antioxidants, which may serve as safer alternatives to synthetic compounds. The blank (methanol) showed minimal absorbance (0.083), confirming that the solvent did not interfere with the assay, while the DPPH control validated the baseline radical concentration. The high antioxidant activity is likely due not to a single compound but to synergistic interactions among multiple phytochemicals. Studies have shown that combinations of flavonoids and phenolic acids often produce enhanced antioxidant effects compared to isolated compounds [21]. Although *Cola lepidota* extract possesses strong antioxidant activity, it is less potent than pure gallic acid (Table 4), which is expected given that gallic acid is a highly active isolated compound, whereas the extract is a complex mixture. The

antioxidant activity observed in *Cola lepidota* extract is likely due to its rich composition of phenolic and flavonoid compounds, these classes of compounds have been documented to act as redox mediators, protecting cells from oxidative stress-induced damage [22] and are particularly effective due to their multiple hydroxyl groups and conjugated structures, which enhance radical scavenging ability [23].

Table 3: Antioxidant activity of *Cola lepidota* leaves, using DPPH assay

Concentration of extract (mg/mL)	Absorbance	% Scavenging activity
40	0.735	66.287
80	0.603	73.113
120	0.330	87.229
160	0.224	92.709
Blank (methanol)	0.083	
Blank (positive control DPPH in methanol	1.934	
Reference (BHT) BH	0.116	97.200

Table 4: Antioxidant activity of *Cola lepidota* leaves using ABTS assay

Concentration of extract (mg/mL)	A0	A1	% Scavenging
Cola lepidota			
20	1.922	1.298	32.466
40	1.922	1.127	41.363
80	1.922	0.410	78.668
120	1.922	0.364	81.061
Garlic acid			
20	1.922	0.322	83.247
40	1.922	0.179	90.687
80	1.922	0.097	94.953
120	1.922	0.074	96.149

The anti-inflammatory activity of the leaf extract of *Cola lepidota* was evaluated using four *in vitro* models: protein denaturation inhibition, membrane stabilization, proteinase inhibition, and lipoxygenase inhibition assays. The extract exhibited significant dose-dependent anti-inflammatory effects compared with aspirin (a standard non-steroidal anti-inflammatory drug - NSAID), with inhibition of protein denaturation (up to ~78%) (Table 5), membrane stabilization (~88%) (Table 6), proteinase activity (~58%) (Table 7) and lipoxygenase activity (~66%) (Table 8). This behavior aligns with previous studies showing that plant-derived polyphenols inhibit protein denaturation in a concentration-dependent manner [24]. The concentration-dependent trends observed across all models suggest that the extract's efficacy is directly related to its phytochemical richness, particularly its phenolic and fatty acid content.

Table 5: Anti-inflammatory activities of *Cola lepidota* leaves: Inhibition of albumin denaturation

Extract	Concentration of Extract mg/mL	Absorbance	% Inhibition of Albumin Denature
Cola lepidota	0	1.075	0
	20	0.558	48.093
	40	0.497	53.767
	80	0.350	67.442
	120	0.237	77.953
Aspirin	0	1.075	0
	40	0.293	72.744
	60	0.241	77.581
	80	0.201	81.302
	120	0.102	90.512

Table 6: Anti-inflammatory activities of *Cola lepidota* leaves: Heat induced haemolysis

Extract	Concentration of extract mg/mL	Absorbance	% Inhibition of Heamoglobin
Cola lepidota	0	1.171	0
	40	0.281	76.003
	60	0.174	85.141
	80	0.165	85.909
	120	0.145	87.617
Aspirin	0	1.171	0
	40	0.233	80.102
	60	0.157	86.593
	80	0.134	88.557
	120	0.116	90.094

Table 7: Anti-inflammatory activities of *Cola lepidota* leaves: Anti-proteinase action

Extract	Concentration of extract mg/mL	Absorbance	% Inhibition of Anti-Proteinase
Cola lepidota	0	0.579	0
	20	0.456	21.244
	40	0.351	39.378
	80	0.246	57.513
	120	0.241	58.377
Aspirin	0	0.579	0
	20	0.245	57.686
	40	0.162	72.021
	80	0.123	78.750
	120	0.087	84.974

Table 8: Anti-inflammatory activities of *Cola lepidota* leaf: Anti-lipoxygenase activity

Extract	Concentration of Extract mg/mL	Absorbance	% Inhibition of Anti-lipoxygenase
Cola lepidota	0	0.800	0
	20	0.528	34.000
	40	0.386	51.750
	80	0.342	57.250
	120	0.275	65.625
Aspirin	0	0.800	0
	20	0.367	54.125
	40	0.325	59.375
	80	0.241	69.875
	120	0.168	79.000

CONCLUSION

GC–MS analysis revealed that the extract of the Leaves of *Cola lepidota* K. Schum is predominantly composed of biologically active lipid compounds, with additional contributions from nitrogenous and sulfur-containing constituents. The GC-FID phytochemical profiling showed that the extract is rich in flavonoids and phenolic compounds, with apigenin, artemetin, and myricetin as dominant constituents. The extract demonstrated strong, concentration-dependent free radical scavenging activity, substantial inhibition of protein denaturation, and effective membrane stabilization, alongside moderate proteinase and lipoxygenase inhibitory effects. These findings support the potential application of the extract in pharmaceutical, nutraceutical, and food preservation systems, and justify further studies on its *in vivo* antioxidant and anti-inflammatory effects to fully harness the therapeutic potential of this plant.

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