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ORIGINAL RESEARCH

Phytochemical Characterization, GC–MS Profiling, and *in vitro* Antioxidant evaluation of *Anthocleista djalonensis* Hydroethanolic leaf extract

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Abstract

Background: *Anthocleista djalonensis* A. Chev. is a plant widely employed in West African traditional medicine for the treatment of malaria, diabetes mellitus, jaundice, hepatitis and infectious conditions.

Objectives: To evaluate the phytochemical composition and antioxidant activity of the hydroethanolic leaf extract of *Anthocleista djalonensis*

Methods: The extract was prepared by hydroethanolic maceration and analyzed by gas chromatography mass spectrometry (GC-MS), with total phenolic and flavonoid contents determined spectrophotometrically alongside qualitative and quantitative phytochemical assessment. Antioxidant activity was examined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP), with ascorbic acid as reference.

Results: Seventeen compounds were resolved by gas chromatography mass spectrometry, the volatile fraction dominated by long chain fatty acids. 9-Octadecenoic acid (Z) was the principal constituent, (27.20 % of total peak area). Alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, anthraquinones, and phenolic compounds were detected, with alkaloids yielding the highest quantitative value of 11.70 ± 0.006 %w/w. The extract showed moderate concentration dependent DPPH radical scavenging activity with an IC_{50} of 95.1 µg mL, compared with 18.2 µg mL for ascorbic acid. Total phenolic content (TPC) ranged from 2.15 to 3.59 mg Gallic Acid Equivalents (GAE)g, while total flavonoid content (TFC) ranged from 4.86 to 11.55 mg Quercetin Equivalents (QE) g. Ferric reducing antioxidant power increased from 4.40 to 7.05 mg Ascorbic Acid Equivalents (ASCE)g across concentrations.

Conclusion: The extract exhibited concentration dependent antioxidant activity in vitro, which was modest compared with ascorbic acid and may be associated with its phenolic and flavonoid content.

Keywords: Antioxidant activity, Anthocleista djalonensis, DPPH assay. Ferric reducing antioxidant power, GC–MS, Phytochemical composition

Introduction

Plants remain the principal source of therapeutic agents in primary health care across sub-Saharan Africa, and their chemical constituents continue to yield compounds of established pharmacological utility.[1] Secondary metabolites produced by plants, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, account for the greater part of this therapeutic activity, and several have been translated into clinically used drugs or have provided structural frameworks for synthetic derivatives, including quinine, morphine, and paclitaxel.[2]

Anthocleista djalonensis A. Chev. (Gentianaceae, formerly Loganiaceae) is a medium-sized tree distributed across West tropical Africa, including Nigeria, Guinea, Ghana, Togo, and Cameroon. Decoctions and macerations of the leaves, stem bark, and roots are used traditionally in the management of malaria, diabetes mellitus, jaundice, hepatitis, dysentery, hypertension, fungal infections, and reproductive disorders.[3] Pharmacological studies have reported antidiabetic, antiplasmodial, antibacterial, antifungal, anti-inflammatory, anthelmintic, free radical-scavenging, wound-healing, and cytotoxic activities.[4,5] Known constituents include alkaloids, xanthones, phthalides, iridoid glycosides, monoterpene diols, triterpenes, flavonoids and phenolic compounds.[6,7] Phenolic and flavonoid constituents are recognized as principal mediators of free radical scavenging capacity, thereby limiting oxidative processes.

Oxidative stress, arising from an imbalance between reactive oxygen species and the body's antioxidant defense systems, is implicated in the pathophysiology of several diseases, including diabetes mellitus and liver disorders. [8,9] Excessive oxidative damage can contribute to cellular dysfunction and tissue injury in these conditions. Plant-derived compounds, particularly phenolic compounds and flavonoids, have demonstrated free radical-scavenging activity in experimental systems,[10] making medicinal plants potential sources of antioxidant constituents for further investigation.

The chemical constitution of the hydroethanolic leaf extract of *Anthocleista djalonensis* has not been adequately defined, particularly with respect to its volatile constituents and their relation to antioxidant activity. Existing studies have tended to examine isolated parameters, and a coherent account linking chemical composition to functional activity remains lacking. The present study was therefore undertaken to characterize the phytochemical profile of the

hydroethanolic leaf extract using qualitative screening and gas chromatography mass spectrometry, and to evaluate its antioxidant properties by established in vitro assays, with the aim of relating constituent composition to observed activity.

Methods

Study Design: This study employed an experimental laboratory-based design to investigate the phytochemical constituents and in vitro antioxidant activity of the hydroethanolic leaf extract of *Anthocleista djalonensis* (ADHE). Phytochemical analysis and in vitro antioxidant assays were conducted in the laboratory of the Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria, using standard experimental procedures and available laboratory facilities. The study commenced on 24 January 2026 and was completed on 20 April 2026. The study period covered plant collection and authentication, preparation of the hydroethanolic leaf extract, phytochemical analysis, in vitro antioxidant assays, data analysis, and preparation of the final research report.

Chemicals: All chemicals and reagents used were of analytical grade. Hydroethanol (70% v/v) was used for the extraction of plant material. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, quercetin, potassium ferricyanide, ferric chloride, sodium carbonate, and ascorbic acid were employed for the evaluation of antioxidant activity and quantification of total phenolic and flavonoid contents.

Collection and Extraction of Plant Material: *Anthocleista djalonensis* leaves were collected in March 2026 at Ikenne, Ogun State, Nigeria. The plant was authenticated by a qualified taxonomist in the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria. A voucher specimen was deposited in the departmental herbarium under voucher number PHS/OOU/496. The leaves were air-dried at room temperature and subsequently pulverized into a fine powder. The powdered material (1500 g) was macerated in 70% hydroethanol for 72 hours with occasional shaking. The extract was filtered and the solvent removed under reduced pressure using a rotary evaporator. The crude hydroethanolic extract obtained was weighed to give a residue of 165.47 g, corresponding to a percentage yield of 11.03% w/w, and was stored at 4°C until further analysis.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis: Gas chromatography–mass spectrometry (GC–MS) was employed to screen the extract for volatile bioactive constituents using an Agilent GC-MSD system operated with MassHunter software. Separation was achieved on a fused silica capillary column (CP-Sil SCB; 30 m × 0.25 mm internal diameter × 1 μm film thickness) under the PHYTOSCAN acquisition method, with helium as carrier gas at 1 mL/min. The oven temperature was initially maintained at 90°C for 2 min, then increased to 270°C at 10°C per minute and held isothermally for 15 min. Mass spectra were acquired under electron ionization at 70 eV, with a scan range of 36–600 amu and a scan time of 0.6 s. Compounds were identified by comparison of mass spectral fragmentation patterns against the National Institute of Standards and Technology (NIST) mass spectral library, with retention times cross-referenced against published retention index data where applicable.

Quantitative and Qualitative Phytochemical Screening of Anthocleista djalonensis Hydroethanolic Leaf Extract (ADHE): Phytochemical investigation was carried out on the hydroethanolic leaf extract of *Anthocleista djalonensis* (ADHE) to identify and quantify its principal secondary metabolites. Qualitative phytochemical screening for alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids and phenolic compounds was performed following standard protocols described by Harborne[11] and Kebede et al.[12] Quantitative determination of alkaloids, flavonoids, saponins, tannins, terpenoids and phenolic compounds was subsequently carried out according to the methods described by Edeoga et al. [13] Results of quantitative screening were expressed as percentage yield per gram dry weight of extract (% w/w).

Determination of Antioxidant Properties

Total Phenolic Content (TPC): The total phenolic content of the extract was determined using the Folin-Ciocalteu colorimetric method as described by Singleton and Rossi [14] with slight modifications. The extract was prepared at concentrations of 200, 400, 600, 800, and 1000 μg/mL. Aliquots of 0.5 mL of each concentration were mixed with 5 mL of Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Four millilitres of 1M aqueous sodium carbonate solution was then added to neutralize the reaction, and the mixture was allowed to stand for a further 15 minutes before absorbance was measured at 765 nm against a blank. Total phenolic content was determined

from a gallic acid calibration curve constructed and results were expressed as mg of Gallic Acid Equivalents (GAEs) per gram of the dry extract.

Total Flavonoid Content (TFC): The total flavonoid content of the extract was determined using the aluminium chloride colorimetric method as described by Zhishen et al.[15] One millilitre of the plant extract was transferred into a test tube and 4 mL of distilled water was added. To this mixture, 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added and the mixture was allowed to stand for 5 minutes. Subsequently, 0.3 mL of 10% aluminium chloride ($AlCl_3$) solution was added and the reaction mixture was allowed to stand for further 5 minutes. Two millilitres of 1M sodium hydroxide (NaOH) solution were then added and the final volume was made up to 10 mL with distilled water. The mixture was thoroughly mixed and absorbance was measured at 510 nm using a UV-Visible spectrophotometer against a blank prepared without extract. Total flavonoid content was determined from a quercetin standard curve constructed over a concentration range of 200–1000 µg/mL, and results were expressed as mg quercetin equivalent (QE) per gram extract.

DPPH Free Radical Scavenging Assay: The DPPH free radical scavenging activity of the extract was determined according to the method of Brand-Williams et al.[16] A stock solution of 0.1 mM DPPH was prepared in methanol. The hydroethanolic extract (ADHE) and ascorbic acid (reference standard) were prepared at concentrations of 50, 100, 150, 200, and 250 µg/mL. Aliquots of 3 mL of each concentration were transferred into test tubes and 1 mL of the DPPH solution was added to each, giving a total reaction volume of 4 mL. A control was prepared by replacing the extract with an equal volume of methanol. The mixtures were shaken and incubated in the dark at room temperature for 30 minutes, after which absorbance was measured at 517 nm. Percentage inhibition was calculated as: % inhibition = $[(Ac - As)/Ac] \times 100$, where Ac is the absorbance of the control and As is the absorbance of the sample. Antioxidant activity was expressed as IC_{50} (µg/mL), determined by linear regression analysis of percentage inhibition against extract concentration

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing antioxidant power (FRAP) of the extract was determined according to the method of Benzie and Strain[17] with slight modifications. The FRAP reagent was freshly prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution

in 40 mM HCl, and 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) in a ratio of 10:1:1 (v/v/v), and warmed to 37°C prior to use. The hydroethanolic extract was prepared at concentrations of 20, 40, 60, 80, and 100 µg/mL. Aliquots of 90 µL of each concentration were mixed with 900 µL of FRAP reagent and 90 µL of distilled water, and the mixture was incubated at 37°C for 4 minutes, after which absorbance was measured at 593 nm against a blank and expressed as mg ASCE/g.

Statistical Analysis: All experimental data are expressed as mean ± standard error of the mean (SEM) of three independent replicates (n = 3). IC_{50} values for DPPH radical scavenging activity were determined by linear regression analysis of percentage inhibition against extract concentration using Microsoft Excel 2021.

Results

GC–MS Analysis of Anthocleista djalonensis: GC-MS analysis of the hydroethanolic leaf extract of *Anthocleista djalonensis* afforded seventeen chromatographic peaks (Figure 1), the identities of which were determined by comparison of their mass spectral fragmentation patterns against the NIST14 mass spectral library (Table I). The identified compounds were predominantly long-chain C16–C18 fatty acids and their methyl esters, indicating that the volatile fraction of the extract is largely composed of this lipid class. The principal constituent was 9-Octadecenoic acid (Z)- (oleic acid) at 27.2% of the total peak area, followed by hexadecanoic acid methyl ester (13.2%), methyl stearate (11.6%), cis-13-octadecenoic acid methyl ester (11.5%), and n-hexadecanoic acid (10.0%). These five compounds collectively accounted for approximately 73% of the total peak area. Additional constituents detected in moderate proportions included methyl 5,12-octadecadienoate (4.4%), octadecanoic acid (6.0%), and 9-octadecenoic acid methyl ester (3.5%). The remaining peaks were detected at proportions below 3% of the total peak area.

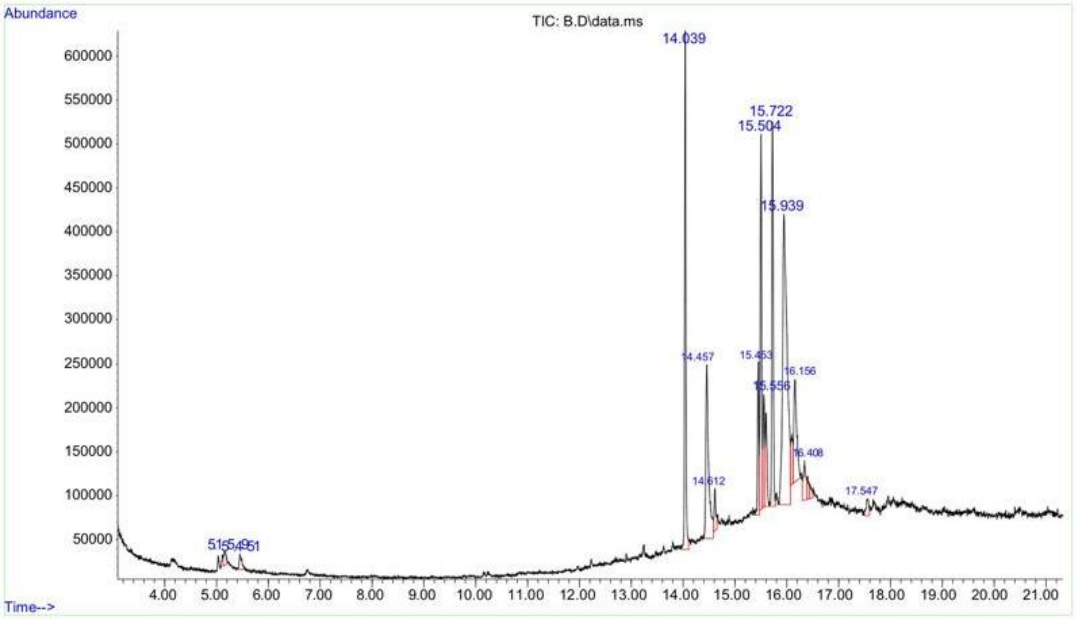

Figure 1: GC–MS chromatogram of the hydroethanolic leaf extract of *Anthocleista djalonensis*

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity of the *Anthocleista djalonensis* hydroethanolic extract (ADHE) was evaluated at concentrations ranging from 50 to 250 µg/mL and compared with ascorbic acid as the reference standard. The percentage scavenging activity of ADHE increased in a concentration-dependent manner, ranging from 48.5 ± 0.1% at 50 µg/mL to 59.1 ± 1.4% at 250 µg/mL. The IC_{50} value of ADHE was 95.1 µg/mL. Ascorbic acid exhibited markedly greater scavenging activity across all concentrations tested, with percentage inhibition ranging from 53.8 ± 0.4% at 50 µg/mL to 72.2 ± 0.7% at 250 µg/mL, and a corresponding IC_{50} of 18.2 µg/mL. The IC_{50} of ADHE was approximately 5.2-fold higher than that of the standard, indicating that the extract possesses moderate free radical scavenging activity relative to ascorbic acid (Table II). Nonetheless, the observed dose-dependent increase in radical scavenging by ADHE is indicative of the presence of antioxidant constituents in the extract.

Table Ia: Bioactive constituents identified in the hydroethanolic leaf extract of *Anthocleista djalonensis* by GC–MS

Peak No.	RT (min)	Compound	Molecular Formula	MW (g/mol)	Corres. Area	Area (%)	Reported Biological Activity	Reference(s)
1	5.159	5-Methylbenzofurazan	$C_7H_6N_2O$	134.14	49787	0.7	Limited pharmacological data available for this specific compound	—
2	5.451	Nonanoic acid	$C_{10}H_{20}O_2$	172.26	58783	0.8	Antimicrobial; skin penetration enhancer; cytotoxic activity	Kabara et al., 1972
3	14.039	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	989414	13.2	Anti-inflammatory (inhibits NF-κB; reduces TNF-α, IL-6, PGE2); antifibrotic; antioxidant	Saeed et al., 2012; Hamed et al., 2020
4	14.457	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	748632	10	Anti-inflammatory (inhibits phospholipase A2, COX-1, COX-2); antibacterial; antifungal; antioxidant	Aparna et al., 2012
5	14.612	Methyl 2,6-dimethyltridecanoate	$C_{16}H_{32}O_2$	256.42	107236	1.4	Branched-chain fatty acid methyl esters exhibit antimicrobial activity against bacteria and fungi; class-level anti-inflammatory effects reported via membrane disruption mechanisms	Kabara et al., 1972
6	15.453	Methyl 5,12-decadienoate	$C_{19}H_{34}O_2$	294.47	331286	4.4	Anti-inflammatory; antioxidant; immunomodulatory; reduces cardiovascular disease risk	Pariza et al., 2001
7	15.504	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	858205	11.5	Anti-inflammatory; antioxidant; antimicrobial; cardiovascular-protective	Gebauer et al., 2011
8	15.556	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$	282.46	263791	3.5	Anti-inflammatory; antioxidant; antimicrobial; cardioprotective	Santa-María Pérez et al., 2023
9	15.601	3-Eicosyne	$C_{20}H_{38}$	278.52	230127	3.1	Biological activity of this compound has not been directly established	—
10	15.722	Methyl stearate	$C_{19}H_{38}O_2$	298.51	869524	11.6	Anti-inflammatory (inhibits pro-inflammatory cytokines); neuroprotective; antimicrobial	Chen et al., 2020

Table Ib: Bioactive constituents identified in the hydroethanolic leaf extract of *Anthocleista djalonensis* by GC–MS

Peak No.	RT (min)	Compound	Molecular Formula	MW (g/mol)	Corres. Area	Area (%)	Reported Biological Activity	Reference(s)
11	15.939	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	2036762	27.2	Anti-inflammatory (inhibits NF-κB via SIRT1; reduces COX-2, TNF-α, IL-6); antioxidant; antimicrobial; anticancer (suppresses HER-2/neu); cardioprotective (reduces LDL oxidation)	Santa-María Pérez et al., 2023; Al-Shudiefat et al., 2013; Menendez et al., 2005
12	16.099	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280.49	157288	2.1	No pharmacological data currently available for this specific compound	—
13	16.156	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	451486	6	Antimicrobial (Gram-positive and Gram-negative bacteria); anti-inflammatory (inhibits TNF-α, IL-6 via NF-κB and MAPK); antifungal	Othman et al., 2015
14	16.34	Oleic acid	$C_{18}H_{34}O_2$	282.46	166364	2.2	Anti-inflammatory; antioxidant; cardioprotective; antimicrobial	Santa-María Pérez et al., 2023; Menendez et al., 2005
15	16.408	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.45	52790	0.7	Long-chain aldehydes reported to possess antimicrobial activity against bacteria and fungi	—
16	16.443	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	43577	0.6	Essential fatty acid; anti-inflammatory; antioxidant; anticancer; immunomodulatory; reduces LDL cholesterol	Pariza et al., 2001
17	17.547	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.46	72731	1	Sex pheromone component; long-chain aldehydes possess antimicrobial properties	—

RT = Retention Time. MW = Molecular Weight. Corresponding Area = absolute integrated area units as generated by the GC-MS detector. Area (%) = percentage of total corrected peak area

Table II: DPPH radical scavenging activity of ADHE and ascorbic acid at varying concentrations

Concentration (µg/mL)	ADHE % Scavenging (Mean ± SEM)	Ascorbic Acid % Scavenging (Mean ± SEM)
50	48.5 ± 0.1	53.8 ± 0.4
100	49.0 ± 0.0	55.1 ± 0.3
150	53.1 ± 1.7	64.6 ± 0.3
200	55.4 ± 0.1	70.7 ± 0.4
250	59.2 ± 1.4	72.2 ± 0.7
IC_{50}	95.1 µg/mL	18.2 µg/mL

Values expressed as Mean ± SEM (n = 3). IC_{50} = concentration required to inhibit 50% of DPPH radicals, determined by linear regression. ADHE = Anthocleista djalonensis hydroethanolic extract

Total Phenolic Content (TPC): The total phenolic content of ADHE increased with increasing extract concentration, ranging from 2.2 ± 0.0 mg of GAE/g at 200 µg/mL to 3.6 ± 0.0 mg of GAE/g at 1000 µg/mL (Table III). The total phenolic content of ADHE was calculated using the linear regression equation obtained from the standard plot of gallic acid: $y = 0.4967x - 0.586$ ($R^2 = 0.9593$). The consistent and progressive increase in phenolic content with increasing extract concentration is indicative of a concentration-dependent relationship and confirms the presence of phenolic compounds in the extract.

Table III: Total phenolic and total flavonoid content of ADHE at varying concentrations

Concentration (µg/mL)	TFC (mg QE/g) Mean ± SEM	TPC (mg GAE/g) Mean ± SEM
200	4.9 ± 0.2	2.2 ± 0.0
400	5.3 ± 0.1	2.6 ± 0.0
600	5.8 ± 0.0	2.9 ± 0.0
800	7.3 ± 0.1	2.9 ± 0.0
1000	11.6 ± 0.0	3.6 ± 0.0

Values expressed as Mean ± SEM (n = 3). TPC = Total Phenolic Content. GAE = Gallic Acid Equivalent. TFC = Total Flavonoid Content. QE = Quercetin Equivalent

Total Flavonoid Content (TFC): The TFC of ADHE increased progressively with increasing extract concentration, ranging from 4.9 ± 0.2 mg of QE/g at 200 µg/mL to 11.6 ± 0.0 mg of QE/g at 1000 µg/mL (Table III). The total flavonoid contents were calculated using the linear regression equation obtained from the standard plot of quercetin: y = 0.0242x +

0.0894, $R^2 = 0.9836$. The mean total flavonoid content across all concentrations tested was 7.0 ± 2.7 mg of QE/g. A notable increase in flavonoid content was observed between 800 µg/mL (7.3 ± 0.1 mg of QE/g) and 1000 µg/mL (11.6 ± 0.0 mg of QE/g), indicating a marked concentration-dependent accumulation of flavonoid constituents at higher extract concentrations. The results confirm the presence of flavonoid compounds in the extract of *Anthocleista djalonensis*.

Ferric Reducing Antioxidant Power (FRAP): The FRAP values of ADHE were calculated using the linear regression equation obtained from the standard plot of ascorbic acid: $y = 0.0160x + 0.2313$ ($R^2 = 0.9421$), where y is the absorbance and x is the ascorbic acid concentration (µg/mL). The FRAP values of ADHE increased in a concentration-dependent manner, ranging from 4.4 ± 0.0 mg of ASCE/g at 20 µg/mL to 7.1 ± 0.0 mg of ASCE/g at 100 µg/mL (Table IV). The mean FRAP value across all concentrations tested was 5.4 ± 1.00 mg of ASCE/g. A near-equivalent FRAP value was observed at 60 µg/mL (5.2 ± 0.0 mg of ASCE/g) and 80 µg/mL (5.2 ± 0.0 mg of ASCE/g), suggesting a transient plateau in reducing activity at these intermediate concentrations, followed by a marked increase at 100 µg/mL (7.1 ± 0.0 mg of ASCE/g) (Table IV).

Table IV: Ferric reducing antioxidant power of ADHE at varying concentrations, expressed as ascorbic acid equivalents

Concentration (µg/mL)	FRAP (mg ASCE/g) Mean ± SEM
20	4.4 ± 0.0
40	5.0 ± 0.0
60	5.2 ± 0.0
80	5.2 ± 0.0
100	7.0 ± 0.0

Values expressed as Mean ± SEM (n = 3). FRAP = Ferric Reducing Antioxidant Power. ASCE = Ascorbic Acid Equivalent

Qualitative Phytochemical Screening of the Hydroethanolic Extract of Anthocleista djalonensis: The qualitative phytochemical analysis of the hydroethanolic extract of *Anthocleista djalonensis* revealed the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones, terpenoids, cardiac glycosides, steroids, and phenolic compounds. Among these, alkaloids, saponins, tannins, anthraquinones, steroids, and phenolic

compounds were abundant (++ve), whereas flavonoids, terpenoids, and cardiac glycosides were present in moderate amounts (+ve) (Table V).

Table V: Qualitative phytochemical profile of the hydroethanolic leaf extract of *Anthocleista djalonensis*

Phytochemical	Test Applied	ADHE
Alkaloids	Dragendorff's; Mayer's; Wagner's	++ve
Flavonoids	Ammonia; H2SO4; Aluminium solution	+ve
Saponins	Frothing test	++ve
Tannins	Ferric chloride test	++ve
Anthraquinones	Borntrager's test	++ve
Terpenoids	Salkowski test	+ve
Cardiac Glycosides	Keller-Killiani test	+ve
Steroids	Liebermann-Burchard test	++ve
Phenols	FeCl3 test	++ve

Note. ++ve = Abundant; +ve = Present; −ve = Absent. ADHE = Anthocleista djalonensis hydroethanolic extract

Quantitative Phytochemical Screening: The quantitative phytochemical composition of the *Anthocleista djalonensis* hydroethanolic extract (ADHE) is presented in Table VI. Alkaloids constituted the most abundant phytochemical class, with a yield of 11.7 ± 0.0% w/w. Saponins were the second most abundant, recording a yield of 1.4 ± 0.0% w/w, followed by tannins (0.9 ± 0.1% w/w), phenols (0.7 ± 0.0% w/w), terpenoids (0.6 ± 0.0% w/w), and flavonoids, which recorded the lowest yield of 0.2 ± 0.0% w/w.

Table VI: Quantitative phytochemical composition of the hydroethanolic leaf extract of *Anthocleista djalonensis*

Phytochemical Constituents	% w/w
Alkaloids	11.7 ± 0.0
Flavonoids	0.2 ± 0.0
Saponins	1.4 ± 0.0
Tannin	0.9 ± 0.1
Terpenoids	0.6 ± 0.0
Phenol	0.7 ± 0.0

Discussion

Seventeen volatile constituents were resolved from the hydroethanolic leaf extract of *Anthocleista djalonensis*, with the chemical profile dominated by long-chain fatty acids and their esterified derivatives. The dominant constituent, 9-Octadecenoic acid (Z), has been reported to attenuate inflammatory signaling principally through SIRT1-mediated suppression of NF-κB, with downstream reductions in COX-2, tumour necrosis factor-alpha, and interleukin-6 expression, and additionally exerts membrane-stabilizing and free radical-scavenging effects.[18] Hexadecanoic acid and its methyl ester have been shown to inhibit phospholipase A_2, COX-1, and COX-2, and have demonstrated broad-spectrum antimicrobial and antioxidant activity across multiple experimental models.[19] Methyl stearate and octadecanoic acid contribute convergent anti-inflammatory and antimicrobial potential through suppression of pro-inflammatory cytokine networks and activity against both Gram-positive and Gram-negative organisms.[20,21] 9,12 octadecadienoic acid, an essential fatty acid identified in the extract, has been associated with lipid regulatory and related bioactivities,[22] while methyl 5,12-octadecadienoate and cis-13-octadecenoic acid are structurally related C18 unsaturated fatty acids with antioxidant and immunomodulatory properties consistent with those reported for this lipid class.[22,23] The presence of these lipid constituents suggests that fatty acids may contribute to the biological activity of *A. djalonensis*, consistent with previous reports on related plant parts.[24]

The hydroethanolic leaf extract of *Anthocleista djalonensis* exhibited concentration-dependent free radical scavenging activity in the DPPH assay, with an IC_{50} value of 95.1 µg/mL. This value indicates moderate antioxidant capacity when compared with standard reference antioxidants such as ascorbic acid, which demonstrated markedly lower IC_{50} values under the same experimental conditions. The IC_{50} obtained in this study is higher than values previously reported by Awah et al.[25] and Erhunse et al.[26], who recorded IC_{50} values of 8.69 ± 0.95 µg/mL and 8.75 ± 0.22 µg/mL for methanol and aqueous leaf extracts of A. *djalonensis* respectively, and by Kone et al.[27], who reported 4.1 µg/mL and 6.5 µg/mL for aqueous and ethanolic preparations respectively. Variations of this nature are widely reported in phytochemical studies,[28] and are largely influenced by solvent polarity, which governs the extraction efficiency of phenolic and flavonoid constituents from plant

tissues, thereby affecting radical scavenging activity.

Total phenolic content (TPC) and total flavonoid content (TFC) in the present extract increased in a concentration-dependent manner. The mean TPC of 2.83 ± 0.53 mg GAE/g recorded in the present study is lower than values reported by Ogunboye et al.[29] and Owoade et al.[30], who documented TPC values of 78.71 ± 3.03 mg GAE/g and 68.39 mg GAE/g respectively for methanolic leaf extracts of A. *djalonensis*, and lower than the range of 14.17–46.95 mg GAE/g reported by Sinan et al.[31] for methanolic and aqueous preparations. The present values are more consistent with the values recorded by Kone et al.[27] for aqueous (10.1±0.0 mg GAE/g) and ethanolic (16.9±0.1 mg GAE/g) extracts of the same plant, suggesting that hydroethanolic and aqueous extraction systems yield moderate phenolic concentrations relative to methanol. Phenolic compounds are consistently identified as the primary drivers of antioxidant activity, with flavonoids contributing as an important subgroup, though the magnitude of their individual contribution varies with assay chemistry and the specific flavonoid classes present. With respect to TFC, the present findings are lower than 154.67 ± 1.77 mg QE/g reported by Ogunboye et al.[29] for methanolic leaf extracts and 34.76 mg RE/g documented by Sinan et al.[31] A positive correlation between TPC and TFC values and radical scavenging activity has been documented across multiple assay systems including DPPH, ABTS, and FRAP, with extracts of higher phenolic and flavonoid content generally demonstrating greater antioxidant capacity.[32]

The FRAP assay measures antioxidant capacity by assessing the ability of extract constituents to donate electrons and reduce Fe^{3+} to Fe^{2+}, and is widely used as a comparative screening tool for plant extracts and phytomedicines.[33] ADHE demonstrated concentration-dependent ferric-reducing activity consistent with an increasing availability of electron-donating constituents at higher extract concentrations. The near-identical values recorded at 60 µg/mL and 80 µg/mL, followed by a sharp increase at 100 µg/mL, indicate a saturation point in reducing activity at intermediate concentrations. Higher FRAP values have been reported for A. *djalonensis* extracts from two locations in Ivory Coast,[31] and methanolic leaf extracts of the same species also demonstrated antioxidant activity in both in vitro and in vivo systems,[29] with aqueous and ethanolic preparations similarly showing reducing capacity.[27]

The qualitative phytochemical screening of the hydroethanolic leaf extract of *Anthocleista djalonensis* (ADHE) showed a chemically

diverse secondary metabolite profile, with alkaloids, saponins, tannins, anthraquinones, steroids and phenols occurring in abundant concentrations, while flavonoids, terpenoids and cardiac glycosides were present at moderate levels. These results indicate that the extract is rich in both nitrogenous (alkaloids) and non-nitrogenous secondary metabolites, suggesting a diverse phytochemical composition. The broad-spectrum phytochemical profile is consistent with reports by Chineze et al.[34] who similarly documented alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids and cardiac glycosides across various solvent extracts of A. *djalonensis* leaves. Quantitative determination identified alkaloids as the dominant constituent at 11.70 ± 0.006% w/w, a yield comparable to or exceeding alkaloid concentrations reported in other solvent systems of the same species by Chineze et al.[34] Alkaloids constitute a structurally diverse class of nitrogenous secondary metabolites with antiplasmodial, antimicrobial, anti-inflammatory and analgesic properties. Saponins (1.35 ± 0.004% w/w) and tannins (0.92 ± 0.060% w/w) were recovered as the next most abundant classes, in moderate but notable quantities that align with quantitative reports by Akachukwu et al.[35] on comparable preparations of this species. Saponins are recognized for their membrane-permeabilizing and haemolytic properties, which underlie their established antimicrobial and immunomodulatory activities, while tannins exert biological effects through protein precipitation, enzyme inhibition and astringent mechanisms relevant to antimicrobial and wound-healing applications.[36] Phenols (0.74 ± 0.006% w/w) and terpenoids (0.60 ± 0.001% w/w) were recovered at levels comparable in order of magnitude to those reported by Popoola et al.[37] Their concurrent presence alongside tannins and flavonoids in ADHE is consistent with the antioxidant and enzyme-modulating properties repeatedly attributed to A. *djalonensis* in literature.[31]

The ability of plant-derived antioxidants to counter free-radical-mediated damage has implications for the investigation of dietary and medicinal plants in conditions associated with oxidative imbalance, including diabetes mellitus, cardiovascular disease, cancer and neurodegenerative disorders.[38] Within this framework, the moderate, concentration-dependent radical-scavenging and ferric-reducing capacity of ADHE demonstrated in this study, together with its abundant alkaloid, saponin, tannin and phenolic constituents and its oleic acid-rich lipid fraction with reported anti-inflammatory and antimicrobial activities, provides a plausible phytochemical basis for

the traditional use of A. *djalonensis* in the management of oxidative stress and inflammation-related conditions such as diabetes, hepatic disorders, wounds and febrile illnesses.[29] The present characterization therefore supports the continued rational evaluation of this species as a source of antioxidant and anti-inflammatory lead compounds, and potentially as a nutraceutical or functional-food ingredient. However, these in vitro findings do not establish therapeutic efficacy or safety in humans, and translation to clinical application will require bioactivity-guided isolation of the active constituents, in vivo pharmacological and toxicological evaluation, and standardized extract preparation.

Conclusion

The extract exhibited moderate, concentration-dependent antioxidant activity across four in vitro assay systems, with an IC_{50} of 95.1 µg/mL in the DPPH assay. The co-occurrence of phenolic, flavonoid and lipid constituents with documented radical-scavenging and anti-inflammatory activities relates the extract's composition to its ethnobotanical use. Bioactivity-guided isolation of individual constituents and in vivo pharmacological evaluation are recommended as the immediate next steps toward validating the therapeutic potential of this plant.

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References

1. Babandi A. Contributions of African Medicinal Plants to Modern Drug Discovery. In: Dekebo A, editor. Bioactive Secondary Metabolites from Medicinal Plants of Africa. Singapore: Springer Nature; 2025: 535-575. doi.org/10.1007/978-981-96-8590-5_16

2. Chaachouay N, Zidane L. Correction: Chaachouay, N.; Zidane, L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs Drug Candidates 2024, 3, 184–207. Drugs and Drug Candidates. 2026;5(1):14. doi:10.3390/ddc5010014

3. Ogunboye A. Anthocleista Djalonensis: A Review of Its Ethnobotanical, Phytochemical and Pharmacological Potentials. Medires Publishing LLC, editor. JCMR. 2022;1(1):01–04. doi:10.58489/2836-2330/002

4. Okokon JE, Antia BS, Udobang JA. Antidiabetic activities of ethanolic extract and fraction of *Anthocleista djalonensis*. Asian Pacific Journal of

Tropical Biomedicine. 2012;2(6):461–464. doi:10.1016/S2221-1691(12)60076-8

5. Anyanwu GO, Nisar-ur-Rehman, Onyeneke CE, Rauf K. Medicinal plants of the genus Anthocleista;A review of their ethnobotany, phytochemistry and pharmacology. Journal of Ethnopharmacology. 2015;175:648–667. doi:10.1016/j.jep.2015.09.032

6. Okorie DA. A new phthalide and xanthones from *Anthocleista djalonensis* and *Anthocleista vogelli*. Phytochemistry. 1976;15:1799–1800. doi:10.1016/S0031-9422(00)97499-5

7. Onocha PA, Okorie DA, Connolly JD, Roycroft DS. Monoterpene diol, iridoid glucoside and dibenzo-α-pyrone from *Anthocleista djalonensis*. Phytochemistry. 1995;40(4):1183–1189. doi:10.1016/0031-9422(95)00121-M

8. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative Stress in Liver Pathophysiology and Disease. Antioxidants. 2023;12(9):1653. doi:10.3390/antiox12091653

9. Lenzen S. Oxidative stress: the vulnerable β-cell. Biochem Soc Trans. 2008;36(3):343–347. doi:10.1042/BST0360343

10. Es-Safi NE, Ghidouche S, Ducrot PH. Flavonoids: Hemisynthesis, Reactivity, Characterization and Free Radical Scavenging Activity. Molecules. 2007;12(9):2228–2258. doi:10.3390/12092228

11. Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media; 1998. 504

12. Kebede T, Gadisa E, Tufa A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLOS ONE. 2021;16(3):e0249253. doi:10.1371/journal.pone.0249253

13. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685–688. doi:10.5897/AJB2005.000-3127

14. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 1965;16(3):144–158. doi:10.5344/ajev.1965.16.3.144

15. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64(4):555–559. doi:10.1016/S0308-8146(98)00102-2

16. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Science and Technology. 1995;28(1):25–30. doi:10.1016/S0023-6438(95)80008-5

17. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry. 1996;239(1):70–76. doi:10.1006/abio.1996.0292

18. Carrillo Pérez C, Cavia Camarero M del M, Alonso de la Torre S. Role of oleic acid in immune system; mechanism of action; a review. 2012;27(4):978-990. doi:10.3305/nh.2012.27.4.5783

19. Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz J Microbiol. 2007;38:739–742. doi:https://doi.org/10.1590/S1517-83822007000400028

20. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-Inflammatory Property of n-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. Chemical Biology & Drug Design. 2012;80(3):434–439. doi:10.1111/j.1747-0285.2012.01418.x

21. Othman AR, Abdullah N, Ahmad S, Ismail IS, Zakaria MP. Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from Jatropha curcas L. plant root. BMC Complement Altern Med. 2015;15(1):11. doi:10.1186/s12906-015-0528-4

22. Pariza MW, Park Y, Cook ME. The biologically active isomers of conjugated linoleic acid. Progress in Lipid Research. 2001;40(4):283–298. doi:10.1016/S0163-7827(01)00008-X

23. Santa-María Pérez C, López Enriquez S, Montserrat de la Paz S, Geniz I, Reyes-Quiroz ME,

Moreno M, et al. Update on Anti-Inflammatory Molecular Mechanisms Induced by Oleic Acid. 2023;15(1): 224. https://doi.org/10.3390/nu15010224

24. Philip-Attah T, Olusola O, Bolarinwa A, Akinbuluma M, Omotoso OT. Chemical Characterization of Anthocleista djalonensis A. Chev. and Uvaria chamae P. Beauv. Extracts as Potential Insecticides of Stored Products against Infestation. Asian Journal of Research in Biochemistry. 2025;15:82–91. doi:10.9734/ajrb/2025/v15i6449

25. Awah FM, Tufon E, Uzoegwu PN. Free radical scavenging activity and phenolic contents of Anthocleista djalonensis (Loganiaceae) leaf extract. International Journal of Biological and Chemical Sciences. International Journal of Biological and Chemical Sciences. 2010;4(6). https://doi.org/10.4314/ijbcs.v4i6.64933

26. Erhunse N, Oriakhi K, Orhue N, Omoregie E. Comparative study on phytochemical constituents, antioxidant activity and acute toxicity of extracts of Alstonia boonei de Wild and Anthocleista djalonensis. Journal of Pharmacy & Bioresources. 2016;13:14. doi:10.4314/jpb.v13i1.3

27. Kone M, Coulibaly OS, Kouangbe AM, Ouattara K, Coulibaly A. Evaluation of the antioxidant and antibacterial activity of aqueous and ethanoic extracts of two medicinal plants used to treat diarrhea in Côte d'Ivoire. GSC Biol Pharm Sci. 2022;21(3):014-020. doi:10.30574/gscbps.2022.21.3.0452

28. Dai J, Mumper RJ. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules. 2010;15(10):7313–7352. doi:10.3390/molecules15107313

29. Ogunboye A. Evaluation Of In Vitro And In Vivo Antioxidant Activities of Methanolic Leaf Extract of Anthocleista Djalonensis in Triton Wr-1339 Induced Toxicity. Clinical Endocrinology and Metabolism. 2022;1:01–14. doi:10.31579/2834-8761/003

30. Owoade A, Adetutu A, Ogundipe O, Owoade A. Phytochemical Constituents and Comparative Antioxidative Effects of Some Medicinal Plants. Journal of Complementary and Alternative Medical Research. 2021;121–133. doi:10.9734/JOCAMR/2021/v16i430302

31. Sinan KI, Zengin G, Uba AI, Caprioli G, Angeloni S, Vittori S, et al. Chemical characterization and biological abilities of Anthocleista djalonensis collected from two locations of Ivory Coast. eFood. 2023;4(4):e100. doi:10.1002/efd2.100

32. Kiss A, Papp VA, Pál A, Prokisch J, Mirani S, Toth BE, et al. Comparative Study on Antioxidant Capacity of Diverse Food Matrices: Applicability, Suitability and Inter-Correlation of Multiple Assays to Assess Polyphenol and Antioxidant Status. Antioxidants. 2025;14(3):317. doi:10.3390/antiox14030317

33. Csepregi K, Neugart S, Schreiner M, Hideg É. Comparative Evaluation of Total Antioxidant Capacities of Plant Polyphenols. Molecules. 2016;21(2):208. doi:10.3390/molecules21020208

34. Chineze NB, Ezinne NV, Chinwuba OO, Maduabuchi EP, Christiana AC. Proximate Composition, Comparative Phytochemical Analysis, and HPLC Profiling of Various Solvent Extracts of Anthocleista djalonensis Leaves. Asian Plant Res J. 2024;12(4):35–47. doi:10.9734/aprj/2024/v12i4260

35. Akachukwu D, Opara C, Igwe GD, Onwenaruwa G, Ibeh C. Effect of Treatment of Malaria and Diabetes with Aqueous Extract of Anthocleista Djalonensis in Mice. Nigerian Veterinary Journal. 2024;45(1):1–16. doi:10.4314/nvj.v45i1.1

36. Voko MP, Ogbe AA, Kulkarni MG, Coopoosamy RM, Van Staden J. The Phytochemical Composition and Molecular Mechanisms Involved in the Wound Healing Attributes of Bulbine Species—A Critical Review. Plants. 2025;14(19):3045. doi:10.3390/plants14193045

37. Popoola O. Phytochemical Status of Different Solvent Extracts of Anthocleista djalonensis A. Chev Leaves. International Annals of Science. 2020;8(1):62–69. doi:10.21467/ias.8.1.62-69

38. Muscolo A, Mariateresa O, Giulio T, Mariateresa R. Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. International Journal of Molecular Sciences. 2024;25(6):3264. doi:10.3390/ijms25063264

39. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty Acids and Derivatives as Antimicrobial

Agents. Antimicrobial Agents and Chemotherapy. 1972;2(1):23–28. doi:10.1128/aac.2.1.23

40. Saeed NM, El-Demerdash E, Abdel-Rahman HM, Algandaby MM, Al-Abbasi FA, Abdel-Naim AB. Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. Toxicology and Applied Pharmacology. 2012;264(1):84–93. doi:10.1016/j.taap.2012.07.020

41. Hamed AB, Mantawy EM, El-Bakly WM, Abdel-Mottaleb Y, Azab SS. Putative anti-inflammatory, antioxidant, and anti-apoptotic roles of the natural tissue guardian methyl palmitate against isoproterenol-induced myocardial injury in rats. Futur J Pharm Sci. 2020;6(1):31. doi:10.1186/s43094-020-00044-y

42. Gebauer S, Chardigny JM, Jakobsen M, Lamarche B, Lock A, Proctor S, et al. Effects of Ruminant trans Fatty Acids on Cardiovascular Disease and Cancer: A Comprehensive Review of Epidemiological, Clinical, and Mechanistic Studies. Advances in nutrition (Bethesda, Md). 2011;2:332–354. doi:10.3945/an.111.000521

43. Chen PY, Wu CYC, Clemons GA, Citadin CT, Couto e Silva A, Possoit HE, et al. Stearic acid methyl ester affords neuroprotection and improves functional outcomes after cardiac arrest. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2020;159:102138. doi:10.1016/j.plefa.2020.102138

44. Al-Shudiefat AAR, Sharma AK, Bagchi AK, Dhingra S, Singal PK. Oleic acid mitigates TNF-α-induced oxidative stress in rat cardiomyocytes. Mol Cell Biochem. 2013;372(1):75–82. doi:10.1007/s11010-012-1447-z

45. Menendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/*neu* (*erb*B-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin™) in breast cancer cells with Her-2/*neu* oncogene amplification. Annals of Oncology. 2005;16(3):359–371. doi:10.1093/annonc/mdi090

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