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Research Article

Multi-instrumental analysis of bioactive compounds and antioxidant properties in *Rauvolfia vomitoria* Afzel. leaves extract

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Abstract: This research investigated the phytochemical components and antioxidant properties of the ethanol leaves extract fraction (C5), which was obtained by macerating the leaves in 80% ethanol, fractionating the extract via column chromatography, and further purifying it using TLC. The resulting purified fraction was characterized through UV–Vis, FTIR, and GC–MS analyses. Qualitative phytochemical screening of fraction C5 confirmed the presence of flavonoids, alkaloids, terpenoids, saponins, and cardiac glycosides. UV-Vis spectroscopy revealed an absorption maximum at 406 nm, suggesting extended conjugated systems such as oxidized polyphenolics or carotenoid derivatives. FTIR analysis showed characteristic bands, including O-H stretching at 3200–3600 cm^{-1} (indicative of phenolic and hydroxyl groups), aromatic C=C stretching at 1450–1600 cm^{-1}, aliphatic C-H stretching at 2850–2950 cm^{-1}, and C-O stretching at 1000–1300 cm^{-1} (related to ethers, alcohols, or ester linkages). GC-MS profiling identified 14 constituents, including terpenoids, methoxylated phenylpropanoids, phytosterols, fatty acids, and the therapeutically significant indole alkaloid reserpine. *In-vitro* antioxidant assays showed clear, concentration-dependent activity in all three methods: at 2.0 mg/mL, fraction C5 produced 71.4% DPPH inhibition, 76.8% ABTS inhibition, and 72.5% ferric reducing activity, whereas gallic acid was significantly more potent at every concentration ($p < 0.001$), both exhibited a similar linear dose–response. These findings offer scientific support for the traditional medicinal use of *Rauvolfia vomitoria* Afzel. leaves and highlight its potential as a natural source of antioxidants for managing oxidative stress-related conditions.

INTRODUCTION

The growing global impact of oxidative stress–related disorders, such as cardiovascular diseases, neurodegenerative conditions, and cancer, has heightened scientific interest in plant-derived antioxidants as potential therapeutic alternatives (Iqbal *et al.*, 2024). Chronic oxidative stress is known to initiate persistent inflammation, a crucial factor in the onset of diabetes, cancer, cardiovascular, neurological, and pulmonary diseases (Pizzino *et al.*, 2017). Compared

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to synthetic antioxidants, plant-based compounds are increasingly favoured due to their diverse biological activities, lower toxicity levels, and safer therapeutic profiles (Jack *et al.*, 2020). This shift in preference has rekindled scientific interest in medicinal plants with established ethnopharmacological uses and unexplored phytochemical potential.

Rauvolfia vomitoria Afzel (Apocynaceae), commonly referred to as African serpentwood, is one such plant. İt is widely distributed across tropical Africa and has a traditional history of use for managing hypertension, fever, malaria, and neurological disorders (Iwu *et al.*, 2021). While earlier research has focused on root alkaloids such as reserpine and related indole derivatives (Zeng *et al.*, 2017), there is still limited knowledge regarding the chemistry and bioactivity of its leaves. Significantly, harvesting leaves is more sustainable than collecting roots and may yield different classes of secondary metabolites with therapeutic potential. Ajayi (2021) examined the phytochemical profile using Soxhlet extraction and GC-MS analysis but did not assess antioxidant properties or carry out systematic fractionation studies.

The choice of extraction method and solvent is critical for recovering phytochemicals and assessing their bioactivity. Maceration serves as an effective preliminary technique, while methods such as column chromatography and GC-MS facilitate fractionation and compound identification. Antioxidant activity is generally assessed through in vitro assays; methods like DPPH and FRAP offer complementary insights into radical scavenging and reducing potential (Clark *et al.*, 2026; Ndukwe *et al.*, 2020).

Despite its importance in traditional medicine, comprehensive studies on the phytochemistry and antioxidant properties of *Rauvolfia vomitoria* leaves are still lacking. This study aims to characterize the phytochemical composition of the ethanol extract from the leaves and evaluate its antioxidant capacity using *in vitro* assays. The results will provide scientific validation for its traditional use, expand phytochemical knowledge of African medicinal plants, and support the search for sustainable natural antioxidants with pharmaceutical relevance.

MATERIAL and METHODS

Chemicals and Reagents

Mayer's reagent, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), ethanol, methanol, chloroform, ethyl acetate, gallic acid, trichloroacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), acetate buffer (pH 3.6), iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), n-hexane, dimethyl sulfoxide (DMSO), and helium gas (99.999% purity).

Plant Material

Leaves of *Rauvolfia vomitoria* were gathered from cultivated plots in Obiaruku, Delta State, Nigeria, for this study. Their identification was verified at the Department of Botany, at the University of Calabar, where a voucher specimen (Ref. No. 2025/CAL/HRB 81, dated 14/03/2025) was deposited in the herbarium. The leaves were rinsed with distilled water to eliminate surface contaminants (Clark & Ekpekpo, 2025) and then shade-dried at room temperature (25 ± 2 °C) for three weeks to protect heat-sensitive phytochemicals (Clark & Omo-Udoyo, 2021). The dried leaves were ground into a fine powder using a Thomas Wiley Mini-Mill (Model 3383-L10) and stored in a tightly sealed container until further analysis.

Extraction Technique

The extraction procedure was adapted from established maceration protocols for the recovery of plant secondary metabolites (Abubakar & Haque, 2020; Ndukwe *et al.*, 2020), with the solvent ratio, contact time and agitation frequency modified as described below. The powdered leaves of *Rauvolfia vomitoria* (500 g) were extracted with 80% ethanol (2.0 L) using cold maceration in amber glass containers at room temperature (25 ± 2 °C) for 48 hours, with gentle

agitation every 12 hours. The mixture was then filtered through Whatman No. 1 filter paper; the marc was re-extracted twice with fresh 80% ethanol (1.5 L each for 24 hours each under the same conditions). The combined filtrates were concentrated using a rotary evaporator (Heidolph Laborota 4002) at 40 °C under reduced pressure to obtain the crude ethanol extract. This extract was then stored in amber vials at 4 °C until it was needed for analysis.

Column Chromatography

A portion of the crude extract (5.0 g) was dissolved in a small amount of dichloromethane, adsorbed onto silica gel (10 g, 60-120 mesh), and then evaporated to dryness. The dry sample was applied to a silica gel column (4.0 × 30 cm, approximately 226 g of silica) that had been pre-equilibrated with *n*-hexane. Stepwise elution was performed using n-hexane (100 mL), followed by *n*-hexane–chloroform mixtures in varying ratios (95:5, 90:10, 80:20 and 70:30, v/v; each with a volume of 150 mL), then chloroform (200 mL). This was followed by chloroform-ethyl acetate mixtures (90:10, 80:20 and 70:30, v/v; also at 150 mL each) and then pure ethyl acetate (200 mL), followed by ethyl acetate–methanol mixtures (95:5, 90:10, 80:20, v/v; 100 mL each). Fractions (25 mL) were collected and monitored by TLC on silica gel 60 F_{254} plates using hexane–ethyl acetate (70:30, v/v) and ethyl acetate–methanol (95:5, v/v) as mobile phases. Spots were visualized under UV light at wavelengths of 254 and 365 nm as well as with vanillin spray reagent. Fractions displaying similar TLC patterns were combined to produce eight pooled fractions labeled C1–C8. Fraction C5, which was eluted with chloroform, showed a prominent spot at Rf = 0.57 (in *n*- hexane–ethyl acetate, 70:30, v/v) and was chosen for further purification. The pooled fractions were concentrated under reduced pressure and subsequently analyzed by UV-Vis and FTIR spectroscopy, while the volatile components of C5 underwent GC-MS analysis.

Phytochemical Screening Tests

Phytochemical screening was performed on the chromatographic fraction of *Rauvolfia vomitoria,* leaves using standard colorimetric tests (Clark *et al.,* 2026; Ndukwe *et al.,* 2020). The tests targeted alkaloids, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides. Results were recorded as positive (+) or negative (−) based on colour changes observed.

Gas Chromatography-Mass Spectrometry (GC-MS)

The bioactive compounds in *Rauvolfia vomitoria.* extract were analysed using an Agilent GC-MS system (GC-7890A/5975C) with an HP-5MS capillary column (30 m × 250 µm × 0.25 µm). Extracts were diluted to 1% (w/v) in their respective solvents (methanol and *n*-hexane), filtered (0.45 µm), stored at 4 °C, and analysed within 24 h. Helium (99.995%) was used as the carrier gas at 1.0 mL/min. A 1 µL sample was injected in splitless mode at 280 °C with a 3-min solvent delay. The oven program was as follows: 50 °C (2 min), ramp to 150 °C at 3 °C/min (hold for 10 min), then to 300 °C at 10 °C/min (hold for 10 min), resulting in a total run time of 63.3 min.

The MS was operated in EI mode (70 eV), scanning m/z 40–500, with source and quadrupole temperatures at 230 °C and 150 °C, respectively. Blanks and standards were run for contamination, carryover, and system suitability, while samples were analysed in triplicate. Compounds were identified by matching against the NIST library (≥80% similarity), comparing retention indices, and manually verifying fragmentation patterns. Peaks considered for analysis had areas >1000 counts, S/N ≥3:1 (identification) or ≥10:1 (quantification), and widths of 0.05–0.5 min. Relative quantification was based on peak area normalization (% total area). Data processing was done with Agilent MassHunter, with manual integration of overlapping peaks and triplicate means ± SD reported for reproducibility.

Antioxidant assays

Free radical scavenging activity of ABTS

Free radical scavenging activity using ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was conducted according to the Ilyasov *et al.* (2020), with some modifications. A 4.95

mmol L^{-1} potassium persulfate solution (m = 0.01338 g/10 mL) was mixed and dissolved in distilled water with 7 mmol L^{-1} ABTS (m = 0.03841 g/10 mL). The ABTS + radical cation solution was prepared by mixing equal volumes of ABTS and potassium persulfate solutions. The mixture was incubated in the dark for 12-16 h at room temperature and stored at 4 °C for up to 7 days.

For the assay, the ABTS + solution was diluted with distilled water to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Sample extracts (0.1 mL) were mixed with 3.0 mL of diluted ABTS + reagent, properly mixed, and incubated at room temperature in the dark. After 6 min incubation, the absorbance was measured at 734 nm. A control was prepared using distilled water instead of the sample extract. Gallic acid was used as a positive control. The percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad \text{Equation 1}$$

Where $A_{control}$ is the absorbance of the control reaction (without sample), and A_{sample} is the absorbance in the presence of the test sample (Equations 1 and 2).

DPPH radical scavenging assay

The DPPH radical scavenging activity was evaluated according to the method of Jack *et al.* (2020) with modifications. A fresh 0.15% (w/v) solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol and protected from light. The solution was adjusted to an initial absorbance of 0.90–1.00 at 517 nm prior to analysis.

For each assay, 1.0 mL of the DPPH solution was mixed with 3.0 mL of the test sample (fraction C5 or gallic acid) prepared in methanol at concentrations ranging from 0.25–2.00 mg/mL. The reaction mixture was vortexed and incubated in the dark at room temperature (25 ± 2 °C) for 30 min to ensure completion of the reaction. Absorbance was measured at 517 nm using a UV–visible spectrophotometer against a methanol blank. A control consisting of 1.0 mL DPPH solution mixed with 3.0 mL methanol (without sample) was prepared under identical conditions. All measurements were performed in triplicate (n = 3).

The percentage radical scavenging activity was calculated as:

$$\% \text{ Scavenging Activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad \text{Equation 2}$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability of the extracts was determined using the method of Jack *et al.* (2020). Briefly, 0.5 mL of the test sample (extract or gallic acid) was mixed with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 minutes. Subsequently, 0.5 mL of trichloroacetic acid (10%) was added, and the solution was centrifuged at 3000 rpm for 10 minutes. A 0.5 mL aliquot of the supernatant was then combined with 0.5 mL of distilled water and 0.1 mL of ferric chloride (0.1%). After incubation at room temperature (25 °C) for 10 minutes, absorbance was measured at 700 nm using a UV spectrophotometer. An increase in absorbance indicated stronger reducing power.

The ferric reducing activity was expressed as percentage reducing power relative to the reference standard (gallic acid) after blank correction and calculated using Equation (3):

$$\% \text{ Reducing Power} = \frac{A_{sample}}{A_{standard}} - \frac{A_{blank}}{A_{blank}} \times 100 \quad \text{Equation 3}$$

where A_{sample} is the absorbance of the extract and $A_{standard}$ is the absorbance of gallic acid at the corresponding concentration, and A_{blank} is the absorbance of the reagent blank. All measurements were performed in triplicate and expressed as mean ± standard deviation (SD).

Statistical Analysis

All experiments were conducted in triplicate (n = 3) and results are presented as mean ± standard deviation (SD). Statistical analyses were performed using MATLAB R2023b (MathWorks, Natick, MA, USA). Concentration–response data for DPPH, ABTS, and ferric reducing activity assays were analyzed using simple linear regression based on the least squares method. The coefficient of determination (R^2) was used to assess goodness of fit.

Prior to analysis, the normality of data distribution was verified using Shapiro-Wilk test ($p > 0.05$ for all groups), confirming the appropriateness of parametric tests. Equality of variances between fraction C5 and gallic acid at each concentration using Levene's test. As variances were found to be equal across all concentration levels ($p > 0.05$). independent two-sample Student's t-tests assuming equal variances (pooled variance approach) were employed to compare the mean percentage inhibition of fraction C5 versus gallic acid at each corresponding concentration. Degrees of freedom were calculated as:

$$df = n_1 + n_2 - 2 \quad \text{Equation 4}$$

where $n_1 = n_2 = 3$, yielding $df = 4$. All tests were two-tailed, and statistical significance was established at $p < 0.05$.

RESULTS

Extraction Yield

The extraction of *Rauvolfia vomitoria* leaves (500 g) using 80% ethanol yielded 52 g of crude extract, corresponding to an extraction yield of 10.4% (w/w).

Phytochemical Screening

Preliminary phytochemical screening of fraction C5 derived from *Rauvolfia vomitoria* leaves revealed the presence of terpenoids, saponins, flavonoids, cardiac glycosides, and alkaloids (Table 1). Tannins were absent in this fraction.

Table 1. Phytochemical profile of *Rauvolfia vomitoria* leaves (fraction C5).

Phytochemicals	TLC C5
Alkaloids	+
Cardiac Glycoside	+
Flavonoids	+
Saponin	+
Terpenoids	+
Tannins	-

Key: + Present - Not present

Chemical Characterization

GC-MS analysis

GC-MS analysis identified 14 compounds representing diverse phytochemical classes (Table 2, Figure 1). The identified compounds were categorized as: phenylpropanoids (14.58%), phytosterols (17.2%), fatty acids and derivatives (29.18%), terpenes (24.26%), fatty alcohols (3.94%), and alkaloids (7.57%). The three most abundant constituents were oleic acid (23.29%), β-sitosterol (14.25%), and benzene-1,2,3-trimethoxy-5-(2-propenyl) (14.58%), collectively representing 52.12% of the total extract composition. Other significant compounds included lopanoic acid (12.91%) and reserpine (7.57%).

Table 2. GC-MS profile of *Rauvolfia vomitoria* (fraction C5).

S.No	Name of Compound	RT (min)	Peak area (%)	Molecular formula	Molecular weight (g/mol)	Class of Compound
1	4-terpinenyl acetate	8.176	4.44	$C_{12}H_{20}O_2$	196.29	Monoterpene acetate ester
2	Cis-10-Nonadecenoic acid	8.795	2.96	$C_{19}H_{36}O_2$	296.49	Unsaturated fatty acid
3	Lopanoic acid	9.281	12.91	$C_{30}H_{50}O_3$	458.72	Triterpenoid acid
4	3,7,11,15- tetramethyl-2-hexadencene -1-01	9.768	3.55	$C_{20}H_{40}O$	296.54	Diterpene alcohol
5	2-pentadecanone, 6,10,14-tri methyl	10.091	3.36	$C_{18}H_{36}O$	268.48	Methylated ketone
6	Benzene 1,2,3-trimethoxy-5-(2-propenyl)	10.269	14.58	$C_{12}H_{16}O_3$	208.25	Phenylpropanoid
7	z-8-methyl-9-tetradecenoic acid	11.110	2.93	$C_{15}H_{28}O_2$	240.38	Branched unsaturated fatty acid
8	Oleic acid	11.204	23.29	$C_{18}H_{34}O_2$	282.46	Monounsaturated fatty acid
9	Behenic alcohol	12.039	1.00	$C_{22}H_{46}O$	326.60	Long-chain fatty alcohol
10	reserpine	12.162	7.57	$C_{33}H_{40}N_2O_9$	608.68	Indole alkaloid
11	Hexadecanol,2-methyl	12.757	2.94	$C_{17}H_{36}O$	256.47	Branched fatty alcohol
12	7-methyl-2-tetradecen-1-01 acetate	13.545	3.13	$C_{17}H_{32}O_2$	268.43	Acetate ester
13	Stigmasterol	20.945	2.93	$C_{29}H_{48}O$	412.69	Phytosterol
14	b-sitosterol	21.803	14.25	$C_{29}H_{50}O$	414.71	Phytosterol

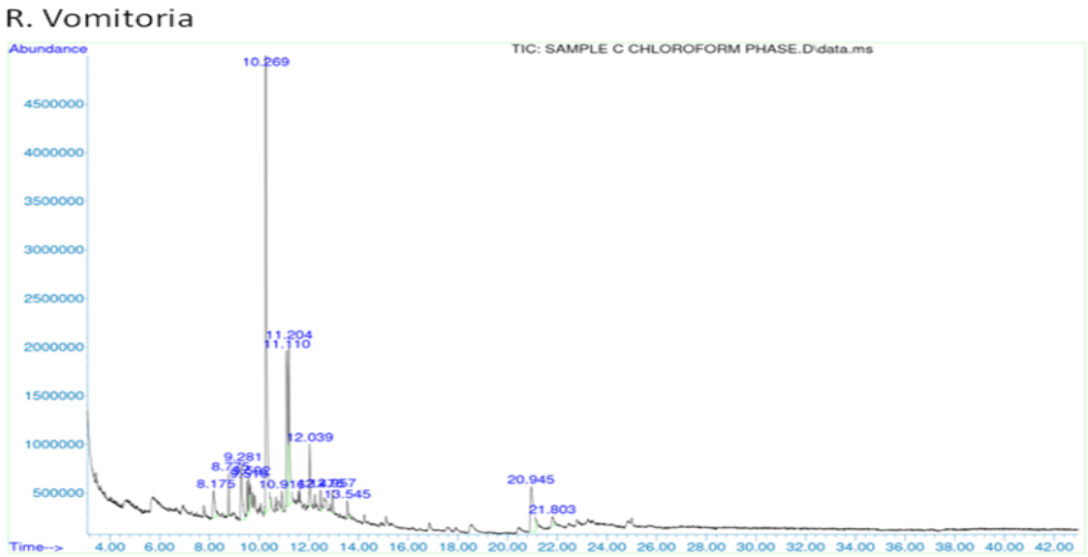


Figure 1. GC-MS chromatogram of *Rauvolfia vomitoria* leaves extract (TLC fraction C5).

UV-visible spectroscopy

The UV-visible spectrum of TLC fraction C5 from *Rauvolfia vomitoria* leaves extract (Figure 2) showed a sharp absorption maximum at 406 nm (Abs = 2.15) with minimal absorption at longer wavelengths (450-800 nm, Abs < 0.5).

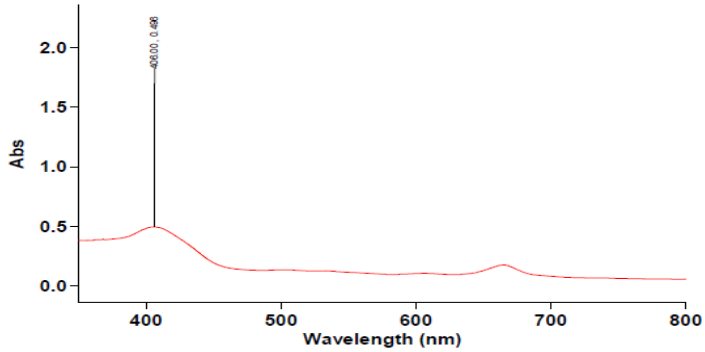


Figure 2. UV-vis spectrum of *Rauvolfia vomitoria* leaves extract (TLC C5).

FTIR spectroscopy

FTIR analysis (Figure 3) revealed characteristic absorption bands at 3356.0 cm^{-1} (broad O-H stretching), 2921.2 and 2851.2 cm^{-1} (C-H stretching), 1654.8 cm^{-1} (C=C stretching), 1427.6 and 1390.1 cm^{-1} (C-H deformation), and 1038.2 cm^{-1} (C-O stretching). No strong carbonyl absorption was observed around 1700-1750 cm^{-1}.

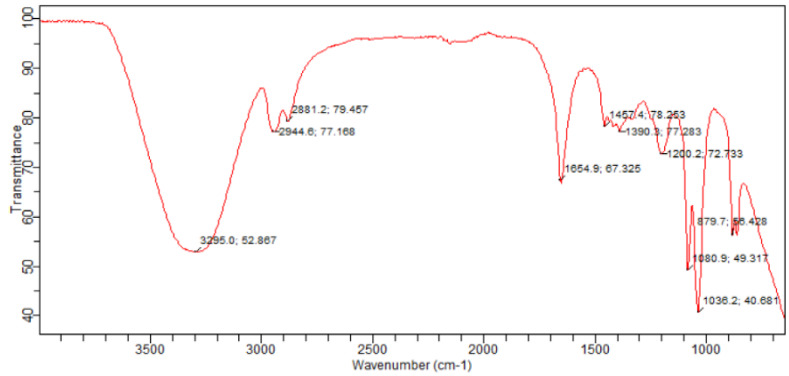


Figure 3. FTIR spectrum of *Rauvolfia vomitoria* leaves extract (TLC C5).

In-vitro Antioxidant Activity

DPPH radical scavenging activity

Both Fraction C5 and gallic acid demonstrated a concentration-dependent DPPH radical scavenging activity, as shown in Table 3 and Figure 4. Fraction C5 showed inhibition values ranging from 28.50 ± 1.2% at 0.25 mg/mL to 71.40 ± 2.0% at 2.00 mg/mL. Gallic acid exhibited higher antioxidant activity, ranging from 45.20 ± 0.8% to 89.60 ± 1.5% at corresponding concentrations. Linear regression analysis showed $R^2 = 0.9091$ for Fraction C5 ($y = 22.87x + 28.91$) and $R^2 = 0.8741$ for gallic acid ($y = 22.07x + 49.03$). The mean differences between gallic acid and Fraction C5 ranged from 16.70% to 20.20%. Independent two-sample t-test analysis (equal variances assumed) revealed that these differences were highly significant at all concentrations ($t = -20.056$ to -12.609; $df = 4$; $p < 0.001$).

Table 3. DPPH radical scavenging activity (% inhibition) of fraction C5 and gallic acid (517 nm)

Concentration (mg/mL)	Fraction (C5 %)	Gallic Acid (%)	Mean Difference (%)	*t*	*df*	*p*
0.25	28.50±1.2	45.20±0.8	16.70	-20.056	4	<0.001*
0.50	42.30±1.5	62.50±1.0	20.20	-19.408	4	<0.001*
1.00	58.70±1.8	78.30±1.2	19.60	-15.693	4	<0.001*
2.00	71.40±2.0	89.60±1.5	18.20	-12.609	4	<0.001*

Notes: Values are mean ± SD, $n = 3$ per group. Mean difference = Gallic acid − Extract. Independent two-sample *t*-test (equal variances). *Statistical significance was set at $p < 0.05$.

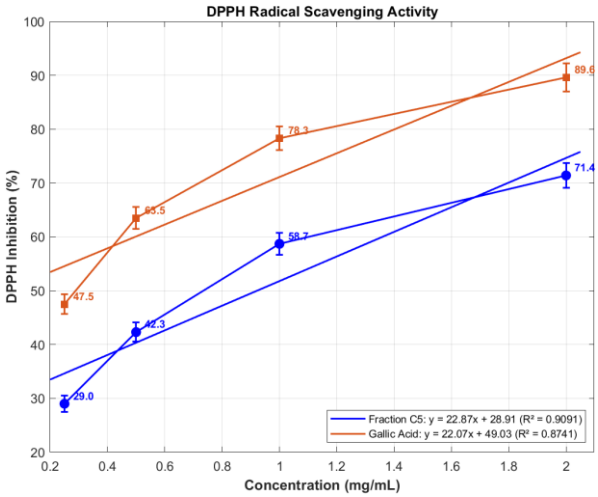


Figure 4. DPPH radical scavenging activity of fraction C5 and gallic acid.

ABTS radical cation scavenging activity

Fraction C5 and gallic acid both showed concentration-dependent ABTS scavenging activity (Table 4, Figure 5). Fraction C5 exhibited inhibition values from 32.40 ± 1.8% at 0.25 mg/mL to 76.80 ± 2.8% at 2.00 mg/mL. Gallic acid showed superior activity, ranging from 52.30 ± 1.2% to 91.40±1.2%. Linear regression revealed $R^2 = 0.9030$ for Fraction C5 (y = 23.75x + 33.41) and $R^2 = 0.8604$ for gallic acid (y = 21.05x + 54.97). The mean differences between gallic acid and Fraction C5 ranged from 14.60% to 20.10%. Independent two-sample t-test analysis (equal variances assumed) showed that these differences were highly significant at all tested concentrations ($t = -15.61$ to -8.04; $df = 4$; $p < 0.001$).

Table 4. ABTS scavenging activity (% inhibition) of fraction C5 and gallic acid (734 nm).

Concentration (mg/mL)	Fraction (C5 %)	Gallic Acid (%)	Mean Difference (%)	*t*	*df*	*p*
0.25	32.40±1.8	52.30±1.2	19.90	-15.61	4	<0.001*
0.50	48.60±2.2	68.70±1.5	20.10	-13.48	4	<0.001*
1.00	64.20±2.5	82.50±1.8	18.30	-10.52	4	<0.001*
2.00	76.80±2.8	91.40±1.2	14.60	-8.04	4	<0.001*

Notes: Values are mean ± SD, $n = 3$ per group. Mean difference = Gallic acid − Extract. Independent two-sample *t*-test (equal variances). *Statistical significance was set at $p < 0.05$.

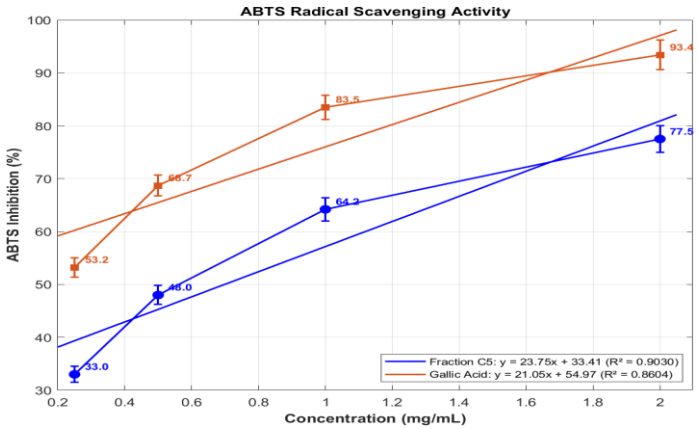


Figure 5. ABTS radical scavenging activity of fraction C5 and gallic acid.

Ferric reducing activity

The ferric reducing activity assay demonstrated concentration-dependent reducing power for both samples (Table 5, Figure 6). Fraction C5 showed absorbance values from 24.30 ± 1.5 AU at 0.25 mg/mL to 72.50 ± 2.5 AU at 2.00 mg/mL. Gallic acid exhibited higher values, ranging from 42.30 ± 1.0 AU to 88.70±1.8 AU. Linear regression analysis showed $R^2 = 0.9363$ for Fraction C5 (y = 26.51x+23.60) and $R^2 = 0.8397$ for gallic acid (y = 23.99x + 45.23). The mean differences between gallic acid and Fraction C5 ranged from 16.20% to 22.70%. Independent two-sample t-test analysis (equal variances assumed) showed that these differences were highly significant at all concentrations ($t = -17.294$ to -9.108; $df = 4$; $p < 0.001$).

Table 5. Ferric reducing activity (% reducing power) of TLC fraction C5 and gallic acid (700 nm).

Concentration (mg/mL)	Fraction (C5 %)	Gallic Acid (%)	Mean Difference (%)	*t*	*df*	*p*
0.25	24.30±1.5	42.30±1.0	18.00	-17.294	4	<0.001*
0.50	38.60±1.8	61.30±1.2	22.70	-18.175	4	<0.001*
1.00	56.80±2.0	79.40±1.5	22.60	-15.658	4	<0.001*
2.00	72.50±2.5	88.70±1.8	16.20	-9.108	4	<0.001*

Notes: Values are mean ± SD, $n = 3$ per group. Mean difference = Gallic acid − Extract. Independent two-sample *t*-test (equal variances). *Statistical significance was set at $p < 0.05$.

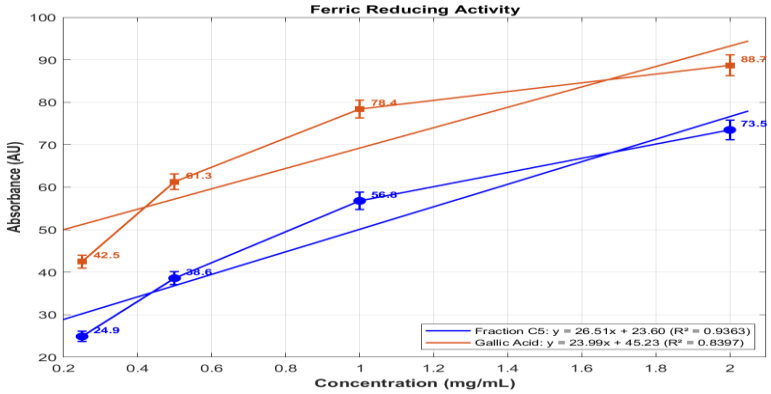


Figure 6. Ferric reducing activity of fraction C5 and gallic acid.

DISCUSSION

The extraction yield of 10.4% (w/w) obtained in this study was achieved under simple room-temperature maceration, indicating that the conditions employed were efficient despite their procedural simplicity. This suggests that extensive heating or sophisticated extraction technologies are not indispensable for effective recovery of bioactives from *Rauvolfia vomitoria*, an important practical advantage for laboratories in resource-limited settings.

The use of 80% ethanol likely contributed substantially to the enhanced recovery of phytoconstituents. Aqueous ethanol mixtures are widely recognized for their ability to extract a broad spectrum of secondary metabolites due to their intermediate polarity, which enables dissolution of both polar and less polar molecules, and have been shown in comparative studies to achieve higher extraction yields than single-solvent systems (El Mannoubi, 2023). In this context, the water fraction facilitates tissue swelling and penetration of plant matrices, improving solvent access to intracellular compounds, while ethanol enhances the solubilization of moderately polar and less polar constituents, thereby maximizing the mass of extractable material (Syafriana *et al.*, 2025). This dual solvation effect is particularly relevant for *Rauvolfia vomitoria*, whose phytochemical profile includes indole alkaloids, flavonoids, phenolic compounds, terpenoids, and other bioactive constituents, such that an 80% hydroethanolic system provides a favorable environment for simultaneous extraction of these diverse classes (Onyeukwu *et al.*, 2025).

Although 70% ethanol is commonly employed in phytochemical extraction due to its effectiveness in recovering a broad range of secondary metabolites in maceration protocols, several studies have demonstrated that slightly higher ethanol concentrations can improve the extraction of alkaloid-rich matrices by enhancing the solubility of moderately polar compounds compared to lower ethanol ratios (Tourabi *et al.*, 2025). Given that *Rauvolfia* species are characteristically rich in monoterpene indole alkaloids, the choice of 80% ethanol in this study likely favored enrichment of these alkaloids alongside associated phenolic constituents without requiring more complex techniques such as Soxhlet or pressurized liquid extraction. It is important to note, however, that extraction efficiency is highly species- and tissue-dependent; variations in secondary metabolite composition, tissue structure, and environmental growth conditions influence solvent–matrix interactions and, consequently, the optimal solvent strength (Lee *et al.*, 2024). Thus, the yield of 10.4% obtained here should be interpreted as evidence of the effectiveness of the specific maceration conditions applied to *Rauvolfia vomitoria* leaves rather than as a universally optimal protocol for all Apocynaceae. From a practical standpoint, these findings indicate that simple maceration using 80% ethanol is a suitable, reproducible and operationally accessible approach for obtaining crude extracts of *Rauvolfia vomitoria*, leaves providing a realistic foundation for subsequent phytochemical and pharmacological investigations in conventional and resource-limited laboratory settings (Abubakar & Haque, 2020).

The phytochemical composition of fraction C5 exhibited both consistency and variation relative to earlier studies on *Rauvolfia vomitoria* leaves. While Iwu *et al.* (2021) identified saponins, flavonoids, alkaloids, tannins, steroids, and triterpenoids, with cardiac glycosides present in trace amounts, Ugwu *et al.* (2022) similarly reported ethanol extracts rich in tannins, phenols, flavonoids, terpenoids, glycosides, and alkaloids in leaves collected from southeastern Nigeria. In contrast, the present fractionation confirmed the presence of flavonoids, alkaloids, and terpenoids in fraction C5, whereas tannins were not detected, indicating a selective enrichment of certain phenolic and alkaloid classes under the partitioning conditions employed. The absence of tannins in this fraction warrants careful interpretation, particularly in light of earlier reports that documented their presence in crude ethanolic leaf extracts (Ajayi, 2021).

Tannins are high-molecular-weight, highly polar polyphenols whose extraction and partitioning are strongly influenced by solvent composition, extraction duration, temperature, and solvent-to-solid ratio. Minor variations in these parameters can significantly affect their solubility, co-extraction with other phenolics, and subsequent detectability after fractionation. In the context of this study, the non-detection of tannins in fraction C5, despite the presence of other phenolics, suggests that the combined maceration and fractionation conditions may have favored lower-molecular-weight phenolics and moderately polar components at the expense of highly polar, polymeric tannins. Supporting this interpretation, some regional investigations on *Rauvolfia vomitoria* from southeastern Nigeria have also reported extract profiles in which tannins and steroids were reduced or undetectable in certain solvent fractions, suggesting that methodological and environmental factors may together account for such discrepancies rather than taxonomic differences alone. Additionally, ecological and geographic influences on secondary metabolite biosynthesis can generate chemotypic variability within the species, so the absence of tannins in fraction C5 does not negate their possible occurrence in other organs, seasons or solvent fractions but rather reflects the selectivity of the current protocol (Lezoul *et al.*, 2020).

Despite this variation, the confirmed presence of flavonoids, alkaloids, and terpenoids in fraction C5 is of substantial pharmacological significance. Flavonoids are well-established radical scavengers that operate via hydrogen atom transfer and single-electron transfer mechanisms and are frequently implicated as key contributors to the antioxidant capacity of plant extracts. Alkaloids contribute to antioxidant defense through transition metal chelation, inhibition of radical chain propagation, and modulation of redox-sensitive signaling pathways, while oxygenated terpenoids may enhance membrane stabilization and modulate enzymes involved in oxidative stress responses. In light of these mechanisms, the collective presence of these bioactive classes in fraction C5 provides a credible mechanistic basis for the antioxidant activity observed in this study and reinforces the ethnomedicinal application of *Rauvolfia vomitoria* leaves in managing conditions associated with oxidative stress, inflammation, and microbial infection (Oborirhovo *et al.,* 2023). These results therefore support the further development of this fraction or related hydroethanolic extracts as affordable antioxidant agents in settings where oxidative stress-related disorders are prevalent.

GC–MS analysis of fraction C5 revealed a complex phytochemical architecture dominated by fatty acids and their derivatives (29.18%), terpenes (24.26%), phytosterols (17.2%), and phenylpropanoids (14.58%). Oleic acid (23.29%), β-sitosterol (14.25%) and benzene-1,2,3-trimethoxy-5-(2-propenyl) (elemicin, 14.58%) together accounted for 52.12% of the total detected composition. This profile reflects the capacity of 80% ethanol to recover moderately polar and lipophilic constituents and aligns with the known chemistry of Apocynaceae species. Notably, the fatty acid pool of fraction C5 consisted exclusively of unsaturated members – oleic acid, cis-10-nonadecenoic acid and z-8-methyl-9-tetradecenoic acid, with no saturated long-chain fatty acids detected under the present chromatographic conditions. Unsaturated fatty acids of this type have been associated with antioxidant, antimicrobial and anti-inflammatory activity in plant matrices, and their abundance here suggests a possible contribution to the lipophilic component of the antioxidant response observed in fraction C5.

The identification of benzene-1,2,3-trimethoxy-5-(2-propenyl) (elemicin) at 14.58% abundance represents a finding of exceptional significance. Elemicin's molecular structure, characterized by three methoxy groups substituted on a benzene ring with an allyl side chain, creates an electron-rich aromatic system that exhibits potent free-radical scavenging capacity through hydrogen atom donation and electron delocalization. Multiple methoxyl (–OCH_3) groups enhance antioxidant activity by stabilizing phenoxy radicals through resonance effects, while ortho-positioned methoxy groups specifically amplify radical scavenging efficiency, as demonstrated in structure–activity studies of methoxylated phenylpropanoids (Asoro *et al.,* 2021). This structural feature is consistent with the intense UV absorption observed at 406 nm and strongly supports the proposal that elemicin is a major contributor to the extract's antioxidant profile.

The detection of reserpine at 7.57% further strengthens the pharmacological relevance of the fraction C5. Reserpine is a well-characterized monoterpene indole alkaloid originally isolated from *Rauvolfia serpentina* and is widely recognized for its antihypertensive and neuroactive properties. Its presence in *Rauvolfia vomitoria* leaf extracts corroborate previous phytochemical investigations demonstrating alkaloid richness in the genus and confirms that the present extraction conditions are capable of recovering key indole alkaloids alongside more lipophilic components (Ajayi, 2021). Beyond its neuropharmacological role, reserpine and structurally related alkaloids have been reported to exhibit antioxidant and antimicrobial activities, suggesting that they may contribute additively to the overall bioactivity of complex plant matrices (Ramu *et al.,* 2021). The co-occurrence of reserpine with phenylpropanoids such as elemicin, long-chain fatty acids, and phytosterols in the current extract therefore raises the possibility of additive or synergistic interactions, in which multiple constituents modulate oxidative and inflammatory pathways through complementary mechanisms. Taken together, the coexistence of these diverse classes of bioactive compounds provides a strong chemical basis for the multi-target pharmacological effects traditionally ascribed to *Rauvolfia vomitoria* leaves and underscores the potential of its hydroethanolic extracts as leads for phytopharmaceutical development.

The UV–visible absorption spectrum provides further insight into the electronic characteristics and likely chromophores present in the extract. The prominent absorption band at approximately 406 nm indicates the presence of conjugated aromatic systems containing electron-donating substituents. Phenolic and methoxylated aromatic compounds typically display absorption in this region due to $\pi \rightarrow \pi^*$ transitions within extended conjugated systems (Ouandaogo *et al.,* 2023). In the context of the present GC–MS profile, the strong absorbance near 406 nm is consistent with the presence of methoxylated phenylpropanoids such as elemicin, whose electron-rich structure lowers the energy gap between molecular orbitals and enhances light absorption in the visible range. This correspondence between UV–Vis and GC–MS data strengthens the assignment of phenylpropanoids as key contributors to both the chromophoric and antioxidant characteristics of the fraction.

The minimal absorption beyond 500 nm suggests that highly conjugated polyene systems such as carotenoids are unlikely to constitute major components of this fraction, as carotenoids typically exhibit characteristic absorption bands extending toward 450–550 nm. This observation indicates that the visible-region color and radical scavenging behavior of the extract arise predominantly from aromatic and phenylpropanoid chromophores rather than from carotenoid-type pigments. Collectively, the spectroscopic and chromatographic data indicate that aromatic phenylpropanoids and related conjugated systems are primary contributors to the extract's chromophoric features and, by extension, to its antioxidant properties.

Taken together, the GC–MS and UV–Vis findings support a multi-component antioxidant system in which phenylpropanoids likely serve as principal radical scavengers via hydrogen atom and electron transfer mechanisms, while fatty acids and phytosterols may contribute indirectly through membrane stabilization and modulation of oxidative processes. Such

mechanistic complementarity may underlie the observed antioxidant activity, reinforcing the concept that whole extracts can exhibit broader functional efficacy than isolated single compounds due to cooperative interactions among constituents. This integrated interpretation of chromatographic and spectroscopic data highlights the practical relevance of using the whole fraction in formulations where robust, multi-pathway antioxidant defense is desirable.

The FTIR spectrum provides molecular-level fingerprinting of the extract by revealing the functional groups that underpin its observed bioactivity. Fourier transform infrared spectroscopy is widely employed in phytochemical studies to characterize functional group composition and to correlate spectral features with classes of secondary metabolites present in plant extracts (Johnson *et al.,* 2020). The overall spectral pattern obtained in this study is consistent with a phytochemically complex matrix containing hydroxylated, aliphatic, and unsaturated constituents, in agreement with the classes inferred from GC–MS analysis.

The broad and intense absorption band observed at 3356.0 cm^{-1} is characteristic of O–H stretching vibrations arising from hydrogen-bonded hydroxyl groups. Such bands, typically spanning the 3400–3200 cm^{-1} region, are widely attributed to phenolic compounds, sterols, fatty alcohols, and other hydroxyl-containing metabolites in plant extracts (Wongsa *et al.,* 2022). The pronounced breadth of this band suggests extensive intermolecular hydrogen bonding, a feature commonly associated with phenolic-rich matrices. Previous FTIR-based studies have correlated strong O–H stretching absorptions with enhanced antioxidant capacity, as hydroxyl groups play a central role in hydrogen atom donation and radical quenching mechanisms (Johnson *et al.,* 2020). In the present context, this feature therefore provides structural support for the antioxidant activity exhibited by the extract and corroborates the UV–Vis and GC–MS indications of phenolic and phenylpropanoid abundance.

The distinct absorption bands at 2921.2 cm^{-1} and 2851.2 cm^{-1} correspond to asymmetric and symmetric C–H stretching vibrations of aliphatic $–CH_2$ and $–CH_3$ groups. These bands reflect the contribution of long-chain hydrocarbons present in fatty acids, sterols, fatty alcohols, and terpenoid constituents, confirming the substantial lipophilic character of the extract. Similar aliphatic C–H stretching features have been consistently reported in FTIR spectra of plant extracts rich in lipid-derived and terpenoid metabolites, where their intensity is taken as evidence of abundant long-chain aliphatic structures (Vittaya *et al.,* 2023). This aligns well with the GC–MS detection of saturated and unsaturated fatty acids and phytosterols and supports their proposed role in modulating membrane-associated oxidative processes.

The absorption band at 1654.8 cm^{-1} is assigned to C=C stretching vibrations, indicative of unsaturation within the extract. In plant-derived matrices, absorptions in the 1640–1670 cm^{-1} region are commonly associated with olefinic double bonds in unsaturated fatty acids as well as aromatic ring vibrations in phenylpropanoid and phenolic compounds (Arya *et al.,* 2025). The presence of such unsaturated systems is functionally significant, as conjugated double bonds enhance antioxidant efficiency by facilitating resonance stabilization of radical intermediates formed during oxidative scavenging processes. FTIR-based correlations between unsaturation features and antioxidant properties have been reported in several phytochemical investigations (Johnson *et al.,* 2020), and the band observed here reinforces the notion that both aromatic and aliphatic unsaturated constituents contribute to the antioxidant profile of Fraction C5.

The prominent band at 1038.2 cm^{-1} corresponds to C–O stretching vibrations arising from multiple oxygen-containing functional groups, including alcohols, esters, and ether linkages. In plant extracts, bands within the 1000–1300 cm^{-1} region are typically attributed to C–O bonds in phenolics, fatty acid derivatives, glycosidic moieties, and methoxylated aromatic compounds (Arya *et al.,* 2025). The presence of this band further supports the abundance of oxygenated secondary metabolites, which are widely recognized as key contributors to antioxidant activity. When considered alongside the O–H and C=C features, this C–O band underscores the

dominance of oxygenated, conjugated structures consistent with the phenylpropanoid and lipid-derived components identified chromatographically.

Notably, the absence of a strong absorption band in the 1700–1750 cm^{-1} region suggests that free carbonyl groups are present only in low concentrations or that their signals are masked by overlapping vibrations from more abundant constituents. Similar observations have been reported in FTIR analyses of complex plant extracts, where dominant hydroxylated and aliphatic components obscure weaker carbonyl absorptions (Johnson *et al.,* 2020). This pattern implies that the antioxidant behavior of the fraction is driven primarily by hydroxylated and conjugated systems rather than by highly carbonyl-rich structures, further emphasizing the central role of phenolics, phenylpropanoids, and unsaturated lipids.

The *in*-vitro antioxidant assays collectively demonstrate that Fraction C5 possesses meaningful radical scavenging and reducing capacity across multiple mechanistic platforms. Although gallic acid exhibited significantly higher activity at all tested concentrations in the DPPH, ABTS, and ferric reducing assays ($p < 0.001$), the progressive narrowing of activity differences at higher concentrations, together with strong linear dose–response relationships ($R^2 = 0.8871$–0.9254), indicates that Fraction C5 produces consistent and concentration-dependent antioxidant effects within the evaluated range. These findings suggest that while the extract is less potent on a per-mass basis than pure gallic acid, it maintains predictable and scalable antioxidant behavior, a desirable characteristic for complex phytochemical formulations (Baliyan *et al.,* 2022), especially in applications where safety and multi-target effects are prioritized over maximal single-molecule potency.

The comparatively steep slopes observed for Fraction C5 in the ABTS and ferric reducing assays are noteworthy. Unlike gallic acid, which represents a single defined antioxidant molecule, Fraction C5 is a heterogeneous mixture of phytochemicals capable of acting through complementary mechanisms. The near-parallel yet slightly steeper dose–response trends may reflect additive or synergistic interactions among constituents, whereby hydrogen atom transfer, single-electron transfer, and chain-breaking mechanisms collectively contribute to total antioxidant capacity. Synergistic enhancement of antioxidant performance has been reported in plant extracts containing mixed phenylpropanoids, sterols, and lipid-derived compounds, where combined effects exceed the expected contribution of individual constituents (Baliyan *et al.,* 2022). While direct synergy was not experimentally quantified in the present study, the observed dose–response behavior is consistent with multi-component antioxidant activity and supports the rationale for using the intact fraction rather than isolated compounds alone.

The GC–MS profile provides a plausible mechanistic framework for these observations. Methoxylated aromatic compounds such as elemicin possess electron-donating substituents capable of stabilizing radical intermediates via resonance, thereby facilitating radical scavenging. Unsaturated fatty acids such as oleic acid contain double bonds that may participate in radical quenching and modulation of lipid peroxidation processes. Phytosterols, including β-sitosterol, have been associated with inhibition of lipid oxidation and membrane stabilization in biological systems, contributing indirectly to antioxidant defense. Additionally, indole alkaloids such as reserpine have been reported to influence cellular redox pathways and modulate antioxidant responses in biological models (Rajurkar & Hande, 2011). The coexistence of these structurally diverse metabolites suggests that Fraction C5 operates through multiple complementary antioxidant pathways rather than relying on a single dominant active compound, a profile frequently observed in complex botanical extracts and advantageous for broad-spectrum oxidative stress management.

The low standard deviations recorded across all assays indicate good experimental reproducibility and measurement precision. Importantly, the use of three complementary antioxidant assays strengthens the validity of the findings. DPPH and ABTS assays evaluate radical scavenging under different radical systems and solvent environments, while the ferric reducing assay assesses electron-donating capacity. The concordance of results across these

mechanistically distinct platforms supports the conclusion that Fraction C5 contains both effective radical scavengers and reducing agents (Chaves *et al.*, 2020). Such cross-assay consistency reduces the likelihood that the observed activity is assay-specific or artefactual and provides a robust basis for considering this fraction as a candidate natural antioxidant source.

Collectively, these results position Fraction C5 as a promising natural antioxidant with reproducible, concentration-dependent activity in vitro. While its potency is lower than that of pure gallic acid, its multi-component composition may offer functional advantages through complementary mechanisms of action and potential synergistic interactions among its constituents. Nevertheless, translation of these findings requires cautious interpretation. Further studies are needed to isolate and quantify key marker compounds, assess potential synergy experimentally, evaluate cytoprotective effects in cellular systems, and determine bioavailability and safety profiles before therapeutic or functional applications can be substantiated (Baskar *et al.*, 2012). This stepwise approach will be essential for moving from *in-vitro* characterization to evidence-based utilization of Fraction C5 in nutraceutical or pharmaceutical contexts.

CONCLUSION

The ethanol leaves extract (fraction C5) of *Rauvolfia vomitoria* contains diverse bioactive constituents, including indole alkaloids, phenylpropanoids, phytosterols, terpenoids, and fatty acids, that collectively contribute to its antioxidant activity. Fraction C5 demonstrated clear, concentration-dependent radical scavenging and reducing capacity in DPPH, ABTS, and ferric-reducing assays, achieving > 70% inhibition at 2.0 mg/mL. Although less potent than gallic acid on a mass basis, its linear dose–response behavior suggests stable and biologically relevant antioxidant potential supported by complementary phytochemical mechanisms. These findings provide experimental support for the traditional use of the plant in oxidative stress–related conditions and align with previous reports of antioxidant activity in the genus *Rauvolfia*. Fraction C5 may serve as a promising natural antioxidant candidate; however further *in-vivo* validation, toxicity profiling, and bioavailability studies are necessary to establish its therapeutic applicability.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

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Authorship Contribution Statement

P.D.C.: Conceptualization, Methodology Design, Manuscript Preparation, and Writing-Original Draft. **M.O.E.:** Laboratory Experiments, Data Collection, and Sample Preparation. **J.O.O.:** Supervision,

Validation, Data Interpretation, and Critical Manuscript Revision. **K.J.A.:** Literature Review and Data Curation **C.I.A:** Investigation, Formal Analysis, Visualization, and Manuscript Editing. **V.O.E.:** Resource Provision, Manuscript Review, and Final Approval.

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