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Original Paper

GC-MS Analysis, Total Phenolic Content, and DPPH Radical Scavenging Activity of *Ricinodendron heudelotii* (Akpi) Seeds

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ABSTRACT

Background: *Ricinodendron heudelotii* (Akpi) is a medicinal plant traditionally used in West and Central Africa to treat inflammation, malaria, and digestive disorders. While its leaves and bark have been studied, the seeds - particularly non-polar extracts - remain largely unexplored. **Objective:** This study investigated the phytochemical composition, volatile constituents, total phenolic content, and antioxidant activity of the seeds extract. **Methods:** Dried seeds were sequentially extracted with hexane and 70% ethanol. Phytochemical screening was performed using standard qualitative tests. Hexane extract was analyzed by GC-MS to identify volatile compounds. Total phenolic content (TPC) was measured using the Folin-Ciocalteu method. Antioxidant activity was evaluated through DPPH radical scavenging and total antioxidant capacity (T-AOC) assays. **Results:** The ethanol extract tested positive for flavonoids, tannins, saponins, alkaloids, terpenoids, and volatile oils. GC-MS analysis of the hexane extract revealed methyl palmitate (9.71%) and methyl stearate (11.17%) as major constituents. The extract showed a TPC of 29.65 ± 1.18 mg GAE/g, a DPPH IC50 of 64.51 µg/mL (95% CI: 44.28 - 119.0 µg/mL), and a T-AOC of 23.91 ± 0.37 U/mg protein. Ascorbic acid (0.1 mM) exhibited a DPPH IC50 of 14.70 µg/mL (95% CI: 10.41 - 19.83 µg/mL) and a T-AOC of 45.62 ± 2.14 U/mg protein. Pearson correlation analysis revealed a strong positive correlation between DPPH activity and T-AOC for ascorbic acid ($r = 0.9998$, $p = 0.0141$), while the extract showed a moderate non-significant correlation ($r = 0.500$, $p = 0.667$). **Conclusion:** *R. heudelotii* seeds possess significant antioxidant activity, supporting their traditional medicinal use and potential as a natural source of bioactive compounds for nutraceutical applications.

KEYWORDS: *Antioxidants; DPPH; Gas Chromatography-Mass Spectrometry; Methyl Palmitate; Phenols; Phytochemicals; Ricinodendron heudelotii*

1. INTRODUCTION

Obtained from plants, several products remain a significant source of therapeutic agents, particularly as conventional drugs face challenges such as antimicrobial resistance and adverse effects (1). Oxidative stress, caused by an imbalance between free radical production and antioxidant defenses, is implicated in chronic diseases including cancer, diabetes, and cardiovascular disorders (2). This has driven interest in plant-derived antioxidants as safer, affordable alternatives. *Ricinodendron heudelotii* (Baill.) Pierre ex Heckel, commonly known as Akpi, is a fast-growing deciduous tree of the Euphorbiaceae family native to West and Central Africa, ranging from Senegal to Angola (3,4). The seeds are traditionally crushed into paste to thicken soups and sauces, while decoctions of the stem bark and roots are used to treat inflammation, rheumatism, malaria, dysentery, and gastrointestinal disorders (5,6).

Previous studies have demonstrated antimicrobial, anti-inflammatory, and antioxidant properties of *R. heudelotii* leaf and bark extracts (1,5,7). Wanche Kojom et al. (7) reported 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (EC_{50} = 1.68 µg/mL) for the stem bark extract, while Yakubu et al. (5) isolated the ellagitannin corilagin (IC_{50} = 0.003 mg/mL). The presence of various bioactive compounds in medicinal plants, including phenolic acids, flavonoids, and alkaloids, has been extensively documented as contributing to their therapeutic efficacy (24,25). Furthermore, the antioxidant properties of plant extracts have been correlated with

their total phenolic and flavonoid contents in numerous studies, with flavonoids such as silibinin demonstrating potent antioxidant activity through cyclic AMP-phosphodiesterase inhibition (26,27). Phytochemical screening methods for the detection of alkaloids, flavonoids, saponins, tannins, terpenes, and coumarins have been well-established and applied to various medicinal plants (24,28). However, limited research has focused on the seeds of *R. heudelotii*, particularly using quantitative antioxidant assays such as total phenolic content (TPC) and DPPH radical scavenging. Furthermore, the hexane extract, which captures non-polar bioactive constituents, has received minimal investigation.

Therefore, this study aimed to: (1) qualitatively screen the seed extract for secondary metabolites; (2) identify volatile constituents by GC-MS; (3) quantify total phenolic content; (4) evaluate DPPH radical scavenging activity; and (5) assess total antioxidant capacity (T-AOC). The findings will contribute to the phytochemical characterization of Akpi seeds and support their potential as a natural antioxidant source for nutraceutical applications.

2. MATERIALS AND METHODS

Chemicals and Equipment

All chemicals (analytical grade) were purchased from Thomas Baker (India), RCI Labscan (Thailand), CDH (India), and Sigma Aldrich (Germany). Instruments included a rotary evaporator (Buchi, Switzerland), GC-MS system (Agilent 7890A/5975C, USA), UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), and Soxhlet apparatus.

Plant Material and Extraction

Fresh *R. heudelotii* seeds were sourced from Iraqi herbalists who had previously import them from Côte d'Ivoire (October-November 2025), authenticated at Pharmacognosy and Medicinal Plant Department (College of pharmacy, Al-Turath university), and shade-dried for 14 days. Dried seeds (100 g) were sequentially extracted in a Soxhlet apparatus with 400 mL hexane (72 hours, Fraction F1), followed by 400 mL 70% ethanol (72 hours, Fraction F2). The ethanol extract was partitioned with ethyl acetate (1:1) to obtain Fraction F3.

Phytochemical Screening

The ethanol extract (Fraction F2) was subjected to qualitative phytochemical analysis to detect the presence of major secondary metabolite classes. Standard test procedures were employed for flavonoids, tannins, saponins, terpenoids, alkaloids, and volatile oils, following previously described methods (8-13). The detailed test protocols, including reagents and expected positive results, are summarized in table 1.

Table 1: Qualitative phytochemical screening tests and expected results for *R. heudelotii* seed extract

Phchemical	Test (Developer)	Procedure (Brief)	Positive Result
Flavonoids	Alkaline reagent test (8)	1 mL extract + 2 mL 10% ethanolic KOH	Intense yellow color
Tannins	Ferric chloride test (9)	Extract diluted 10× + 2 mL 1% $FeCl_3$	Blue-black color
Saponins	Froth assay (10)	3 mL extract + 10 mL water, shake 15 min	Stable foam >1 cm
Terpenoids	Salkowski test (11)	5 mL extract + 2 mL $CHCl_3$ + 3 mL conc. H_2SO_4	Reddish-brown interface
Alkaloids	Dragendorff's test (12)	2ml Extract + 1ml Dragendorff's reagent	Orange to brown precipitate
Volatile oils	TLC with vanillin-H_2SO_4 (13)	Spot on silica gel, develop, spray reagent	Colored spots

GC-MS Analysis

Hexane extract (F1) was analyzed using an Agilent 7890A GC coupled with 5975C MS (EI mode, 70 eV). Separation was achieved on an HP-5MS column (30 m × 0.25 mm × 0.25 μm). Helium carrier gas at 1.0 mL/min.

Oven program: 60°C (4 min), 3°C/min to 100°C (2 min), 4°C/min to 260°C (5 min). Injector: 260°C, splitless mode. Mass range: m/z 50–550. Identification used NIST library (2011).

Total Phenolic Content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method using the ethanol extract (F2). Briefly, 1mL of extract (1 mg/mL) or gallic acid – the standard solution – (100, 200, 300, 400, and 500 µg/mL) was mixed with 10ml distilled water in a 25ml-volumetric flask and 1ml of Folin-Ciocalteu reagent was added with shaking. After 5 min, 10ml of 7.5% Na_2CO_3 solution were added and the mixture was incubated at room temperature and in the dark for 45 min. Absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalent (GAE) (14).

DPPH Radical Scavenging Assay

The DPPH assay was performed using the ethanol extract (F2). A 0.6 mM stock solution of DPPH radical was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored under refrigeration. Prior to each assay, the stock solution was diluted with methanol while monitoring absorbance at 517 nm until a reading of 0.98 ± 0.02 was achieved. In a test tube, 3 mL of this adjusted DPPH working solution was mixed with 100 µL of the extract (6.25, 12.5, 25, 50 and 100 µg/mL) or ascorbic acid standard. After 30 minutes of incubation in the dark at room temperature, the absorbance was measured at 517 nm (15). The percentage of radical scavenging activity was calculated as: % Scavenging = [(A_control - A_sample) / A_control] × 100; where A_control is the absorbance of the control (100 µL methanol substituted for the sample). IC_{50} values were determined by non-linear regression analysis.

Total Antioxidant Capacity (T-AOC)

T-AOC was measured using a commercial kit (E-BC-K136-S, Elabscience, USA) based on Fe^{3+} reduction to Fe^{2+} and complexation with phenanthroline (absorbance at 520 nm). The ethanol extract (F2) was used. Sample preparation: 0.5 g seed powder was homogenized in PBS (1:9 w/v) and centrifuged (10,000 × g, 10 min, 4°C). Protein concentration was determined by BCA assay. T-AOC was calculated as U/mg protein following the manufacturer's protocol.

Statistical Analysis

All experiments were performed in triplicate (n = 3), and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare means among different concentration groups. A probability value ($p < 0.05$) was considered statistically significant. Non-linear regression analysis for IC_{50} determination was performed using GraphPad Prism 8.0.2. Pearson correlation analysis was used to evaluate the relationship between total phenolic content (TPC) and DPPH IC_{50} values.

3. RESULTS

Phytochemical Screening

Qualitative analysis of the ethanol extract (Fraction F2) revealed the presence of all tested phytochemical classes. As summarized in table 2, the extract tested positive for flavonoids, tannins, saponins, alkaloids, terpenoids, and volatile oils.

Table 2: Phytochemical constituents detected in the ethanol extract of *R. heudelotii* seeds

Phytochemical	Result
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids (Dragendorff)	+
Terpenoids	+
Volatile oils	+

(+) = present

GC-MS Analysis

Analysis of the hexane extract (Fraction F1) by gas chromatography-mass spectrometry led to the identification of two major compounds, as summarized in table 3 and illustrated in Figure 1. Methyl palmitate (9.71% peak area) and methyl stearate (11.17% peak area) were the predominant constituents. Both compounds were confirmed by matching their mass spectra with the NIST library (match quality >95%). The remaining chromatographic peaks (generally <2% peak area each) were minor and were not identified owing to insufficient confidence in their NIST library matches.

Table 3: Chemical composition of *R. heudelotii* seed hexane extract identified by GC-MS.

Peak	RT (min)	Area (%)	Compound	Formula	MW (g/mol)
1	14.87	9.71	Methyl palmitate	$C_{17}H_{34}O_2$	270.45
2	16.75	11.17	Methyl stearate	$C_{19}H_{38}O_2$	298.50

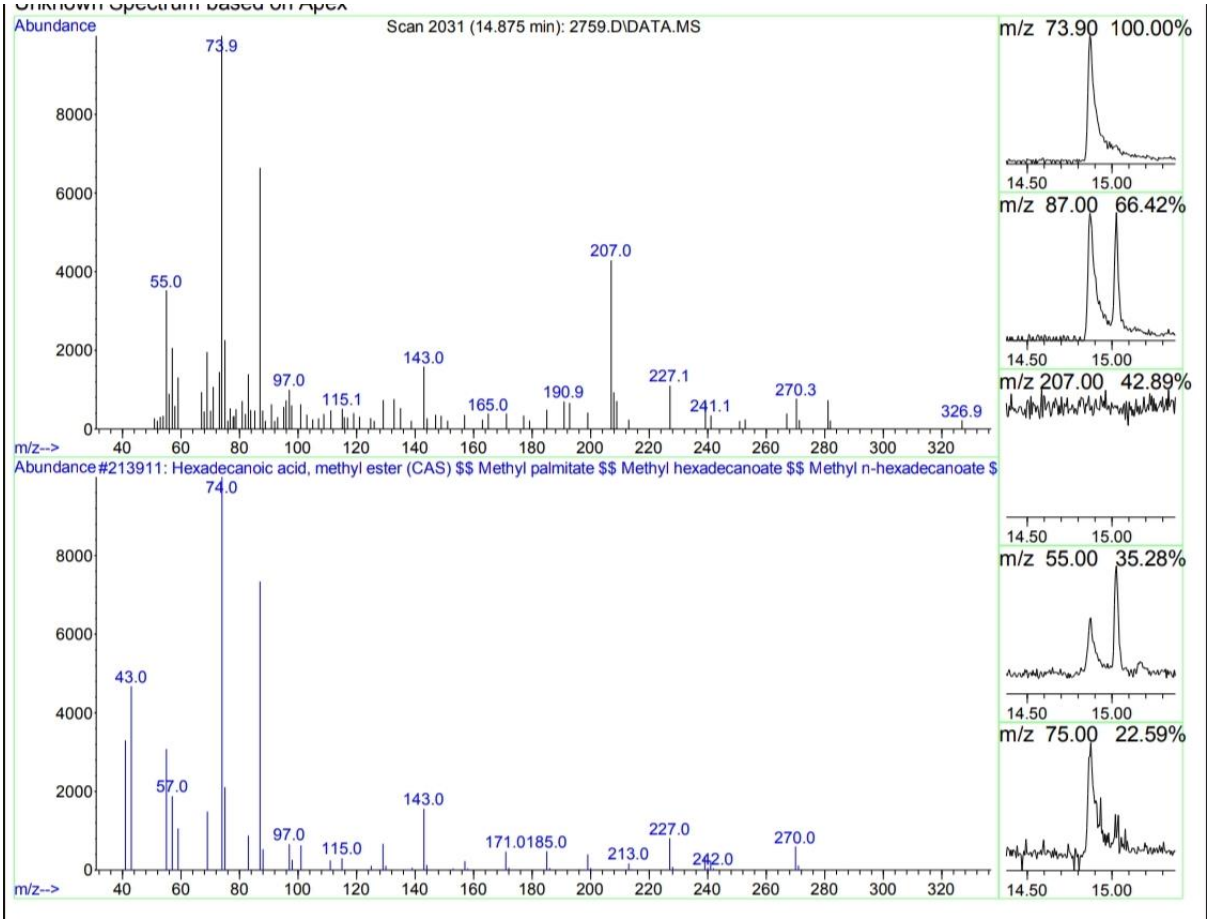

Figure 1. Total ion chromatogram (TIC) of the hexane extract of *R. heudelotii* seeds analyzed by GC-MS. Major peaks are identified as methyl palmitate (retention time = 14.879 min) and methyl stearate (retention time = 16.751 min). The inset shows the representative mass spectrum of methyl palmitate with molecular ion [M^+] at m/z 270 and characteristic fragment ions.

Total Phenolic Content (TPC)

The total phenolic content of the *R. heudelotii* seed ethanol extract (Fraction F2) was 29.65 ± 1.18 mg GAE/g extract (n = 3).

DPPH Radical Scavenging Activity

The ethanol extract (Fraction F2) demonstrated concentration-dependent DPPH radical scavenging activity, as shown in table 4 and figure 3. The percentage of inhibition increased progressively with rising extract concentration, exhibiting a sigmoidal relationship when plotted against the logarithm of concentration (R^2 = 0.9995). Non-linear regression analysis (GraphPad Prism 8.0.2) yielded an IC_{50} value of 64.51 µg/mL (95% CI: 44.28 – 119.0 µg/mL) for the extract (R^2 = 0.7197), while the reference standard ascorbic acid showed an IC_{50} of 14.70 µg/mL (95% CI: 10.41 – 19.83 µg/mL, R^2 = 0.8150).

Table 4: DPPH radical scavenging activity and IC_{50} values of *R. heudelotii* seed ethanol extract compared to ascorbic acid standard

Conc. (µg/mL)	Inhibition (%) - Extract	Inhibition (%) - Ascorbic acid
6.25	12.45 ± 7.45	28.91 ± 10.91
12.5	19.87 ± 9.87	45.23 ± 13.23
25	30.56 ± 12.56	62.45 ± 14.45
50	45.23 ± 15.23	78.90 ± 13.90
100	58.91 ± 16.91	95.34 ± 13.34

Values are presented as mean ± SD (n = 3).

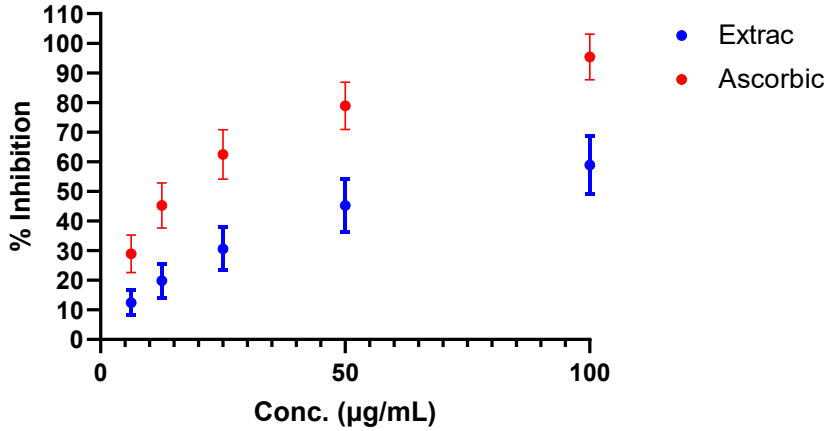

Figure 2: DPPH radical scavenging activity of *R. heudelotii* seed extract and ascorbic acid. Values are mean ± SD (n = 3).

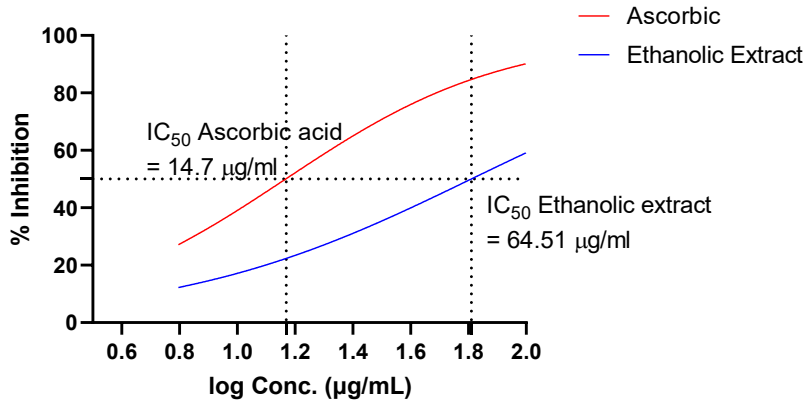

Figure 3: Dose-response curves for DPPH radical scavenging activity. IC_{50} values were determined by non-linear regression (four-parameter logistic model).

Total Antioxidant Capacity (T-AOC)

Total antioxidant capacity was determined using a ferric reduction-based colorimetric kit (Elabscience, E-BC-K136-S) following the manufacturer's protocol. The R. *heudelotii* seed ethanol extract (Fraction F2) exhibited a T-AOC of 23.91 ± 0.37 U/mg protein (n = 3), while the positive control (ascorbic acid, 0.1 mM) gave 45.62 ± 2.14 U/mg protein (n = 3). An unpaired t-test revealed a statistically significant difference between the extract and ascorbic acid (mean difference = 21.71 U/mg protein, 95% CI: 18.23 to 25.19, $p < 0.05$). The high R^2 value (0.9868) indicates that 98.7% of the variance is explained by the treatment. These results demonstrate that the ascorbic acid standard possessed superior total antioxidant capacity compared to the seed extract (Figure 4)

Table 5: Total Antioxidant Capacity (T-AOC) of *R. heudelotii* seed ethanol extract (Fraction F2) and ascorbic acid standard

Sample	T-AOC (U/mg protein), n=3
R. heudelotii extract (F2)	23.91 ± 0.37
Ascorbic acid (0.1 mM)	45.62 ± 2.14 *

Values are presented as mean ± SD. Unpaired t-test: $p < 0.05$.

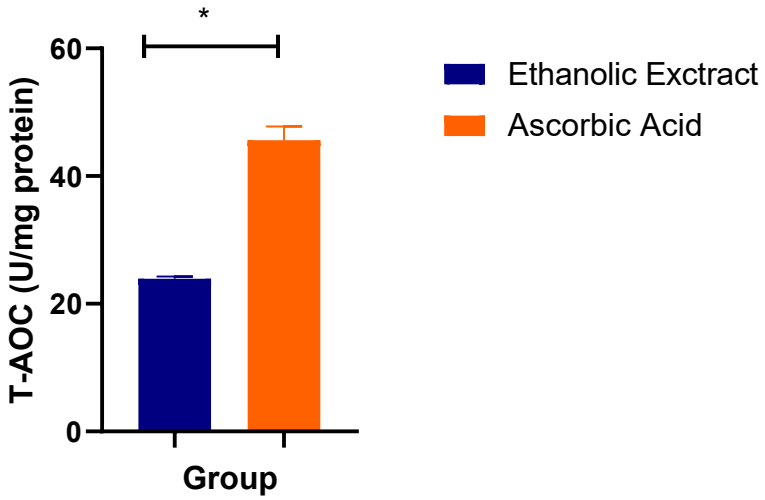

Figure 4: Total Antioxidant Capacity (T-AOC) of *R. heudelotii* seed ethanol extract (Fraction F2) and ascorbic acid (0.1 mM). Values are expressed as mean ± SD (n = 3). Unpaired t-test: *$p < 0.05$.

Correlation Analysis

Pearson correlation analysis revealed a very strong, statistically significant positive correlation between DPPH radical scavenging activity and total antioxidant capacity for ascorbic acid ($r = 0.9998$, $r^2 = 0.9995$, $p = 0.0141$), confirming the internal consistency of the two colorimetric assays. For the *R. heudelotii* seed extract, a moderate positive correlation was observed that did not reach statistical significance ($r = 0.500$, $r^2 = 0.250$, $p = 0.667$).

Table 6: Correlation between DPPH radical scavenging activity and total antioxidant capacity (T-AOC).

Sample	n	r	r^2	P value
R. heudelotii extract	3	0.500	0.250	0.667
Ascorbic acid	3	0.9998	0.9995	0.0141*

*Significant at $p < 0.05$.

4. DISCUSSION

The qualitative phytochemical screening confirmed that *R. heudelotii* seeds contain flavonoids, tannins, saponins, alkaloids, terpenoids, and volatile oils. These findings are consistent with previous reports on the leaves and bark of this species (1,6). Flavonoids and tannins are well-established antioxidants that scavenge free radicals primarily through hydrogen atom donation (16,17). The presence of these compound classes in the seed extract likely explains the observed concentration-dependent radical scavenging activity.

Gas chromatography-mass spectrometry (GC-MS) analysis of the hexane extract identified methyl palmitate (9.71% peak area) and methyl stearate (11.17% peak area) as the major non-polar constituents. Methyl palmitate has been shown to exhibit anti-inflammatory effects by inhibiting nuclear factor kappa B (NF-κB) activation and reducing pro-inflammatory cytokine production (18,19). Methyl stearate possesses antimicrobial and antioxidant properties (20). The limited number of identified compounds reflects the selectivity of hexane for non-polar constituents; polar bioactive compounds, such as gallic acid derivatives and the ellagitannin corilagin, remain preferentially partitioned into the ethanol fraction (6).

The total phenolic content (TPC) of the *R. heudelotii* seed ethanol extract was 29.65 ± 1.18 mg GAE/g. This value is comparable to other medicinal seeds with established antioxidant properties (17). The DPPH radical scavenging assay revealed concentration-dependent activity for both the extract and ascorbic acid standard. Non-linear regression analysis yielded an IC_{50} value of 64.51 μg/mL (95% CI: 44.28 – 119.0 μg/mL) for the extract, while ascorbic acid showed an IC_{50} of 14.70 μg/mL (95% CI: 10.41 – 19.83 μg/mL). The higher IC_{50} value of the extract indicates that the seed extract possesses moderate antioxidant activity, though it is less potent than the pure standard. This difference in potency is expected given that crude plant extracts contain complex mixtures of bioactive and non-bioactive compounds, whereas ascorbic acid is a purified reference standard (16).

The total antioxidant capacity (T-AOC) assay further supported the antioxidant potential of the seeds. The extract exhibited a T-AOC of 23.91 ± 0.37 U/mg protein, while ascorbic acid (0.1 mM) showed significantly higher activity (45.62 ± 2.14 U/mg protein). An unpaired t-test revealed a statistically significant difference between the extract and ascorbic acid (mean difference = 21.71 U/mg protein, 95% CI: 18.23 – 25.19, $p < 0.05$), confirming the superior reducing capacity of the pure standard.

Pearson correlation analysis revealed a very strong, statistically significant positive correlation between DPPH radical scavenging activity and T-AOC for ascorbic acid ($r = 0.9998$, $r^2 = 0.9995$, $p = 0.0141$, $n = 3$). This near-perfect correlation validates the internal consistency of the two colorimetric assays and confirms that the experimental conditions were appropriate for detecting linear dose-response relationships. For the *R. heudelotii* seed extract, a moderate positive correlation was observed between T-AOC and DPPH activity that did not reach statistical significance ($r = 0.500$, $r^2 = 0.250$, $p = 0.667$, $n = 3$). Similarly, the correlation between TPC and DPPH IC_{50} showed a strong negative trend ($r = -0.97$) that was not statistically significant ($p = 0.15$, $n = 3$), primarily due to the limited sample size.

The modest and non-significant correlations observed for the seed extract, in contrast to the near-perfect correlation of ascorbic acid, can be explained by several factors. First, the total phenolic content of the extract (29.65 mg GAE/g) is relatively moderate compared to phenolic-rich medicinal plants. Lower phenolic concentrations produce weaker assay signals and reduce the signal-to-noise ratio, making it more difficult to detect statistically significant linear relationships, particularly with a small sample size ($n = 3$). Second, plant seeds are complex matrices containing substantial amounts of fats (approximately 24.6%) and proteins (approximately 30.6%) that can interfere with colorimetric assays and introduce biological variability (3,4). These non-phenolic constituents do not contribute to radical scavenging but may absorb light or react with assay reagents, thereby obscuring the true contribution of phenolic compounds. Third, the small sample size ($n = 3$) inherently limits statistical power; with only three data points, a correlation coefficient greater than 0.997 is required to achieve $p < 0.05$. The moderate r-value of 0.500 observed for the extract falls well below this threshold, reflecting the biological variability inherent in crude plant extracts rather than a true absence of relationship.

The antioxidant activity observed in this study is consistent with previous reports on other medicinal plants containing similar phytochemical constituents. The presence of phenolic acids, including caffeic, chlorogenic, and ferulic acids, has been associated with potent radical scavenging activity in various plant species (22). Additionally, flavonoids, including silibinin and related compounds, have been shown to exert antioxidant effects through the inhibition of cyclic AMP-phosphodiesterase, leading to reduced oxidative stress (23,24). The phytochemical profile

of *R. heudelotii* seeds, which includes alkaloids, flavonoids, saponins, tannins, and terpenes, aligns with the general understanding that such compounds collectively contribute to the overall antioxidant capacity of medicinal plants.

The T-AOC value (23.91 U/mg protein) further supports the antioxidant potential of the seeds. Oxidative stress is implicated in the pathogenesis of chronic diseases, including cardiovascular disorders, diabetes, and cancer (25,26). The antioxidant properties of *R. heudelotii* seeds documented in this study provide mechanistic support for their traditional use in managing inflammatory and oxidative stress-related conditions (2,7).

5. Limitations

This study had several limitations. First, the phytochemical screening was qualitative, providing information on the presence or absence of compound classes but not individual quantification. Second, the GC-MS analysis focused on non-polar constituents (hexane extract), while polar bioactive compounds such as phenolic acids and flavonoids remain in the ethanol fraction. Third, the small sample size (n = 3) for correlation analysis limited statistical power. Future studies should include quantitative analysis of individual phenolic compounds using HPLC, isolation of pure compounds, and larger sample sizes for robust correlation analysis.

6. CONCLUSION

This study demonstrated that R. heudelotii (Akpi) seeds are rich in bioactive phytochemicals including flavonoids, tannins, saponins, alkaloids, terpenoids, and volatile oils. GC-MS analysis identified methyl palmitate (9.71%) and methyl stearate (11.17%) as major non-polar constituents. The seeds exhibited antioxidant activity with a DPPH IC_{50} of 64.51 μg/mL (95% CI: 44.28 – 119.0 μg/mL), T-AOC of 23.91 ± 0.37 U/mg protein, and TPC of 29.65 ± 1.18 mg GAE/g. These findings support the traditional use of Akpi as an antioxidant agent and highlight its potential for nutraceutical applications

7. Declaration

Ethics Statement: No ethical approval was required for this study as no human or animal subjects were involved.
Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.
Conflicts of Interest: The authors declare no conflicts of interest.
Author Contributions: Conceptualization, A.M.H. and H.M.M.; methodology, investigation, and data curation, A.M.H.; software, validation, formal analysis, resources, supervision, and writing - review and editing, H.M.M.; writing - original draft preparation and visualization, A.M.H.; project administration, A.M.H. All authors have read and agreed to the published version of the manuscript.
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