

Antioxidant and Antibacterial Activities of *Juniperus phoenicea* Leaf and Berry Extracts

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Abstract

The leaves and berries of *Juniperus phoenicea* L., collected from eastern Algeria, were analyzed for their phenolic composition, antioxidant activity, and antibacterial effects against selected foodborne pathogens. The dried plant material was extracted with a methanol/water (80:20, v/v) solution and subsequently fractionated into chloroform, ethyl acetate, and n-butanol. The extracts and fractions were assessed for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity using DPPH, ABTS, CUPRAC, and galvinoxyl assays, and antibacterial activity through disk diffusion and minimum inhibitory concentration (MIC) determination. The leaves exhibited a higher phenolic content compared to the berries, with the ethyl acetate fraction of the leaves demonstrating the highest TPC ($459.78 \pm 9.60 \mu\text{g GAE/mg extract}$) and TFC ($47.34 \pm 2.45 \mu\text{g QE/mg extract}$). This fraction also exhibited the most potent activity in the ABTS ($\text{IC}_{50} = 4.39 \pm 0.12 \mu\text{g/mL}$), CUPRAC ($\text{A}_{0.5} = 8.17 \pm 0.62 \mu\text{g/mL}$), and galvinoxyl ($\text{IC}_{50} = 9.19 \pm 0.29 \mu\text{g/mL}$) assays. Antibacterial activity was predominantly associated with the methanolic fractions, particularly the berry methanolic extract against *Salmonella enteritidis* ($13.5 \pm 0.71 \text{ mm}$; $\text{MIC} = 0.15625 \text{ mg/mL}$). Overall, *J. phoenicea* exhibited distinct organ- and solvent-dependent profiles: the leaf ethyl acetate fraction was the most potent antioxidant, whereas the methanolic fractions were more significant for antibacterial screening. These findings warrant further investigation of *J. phoenicea* extracts as natural, multifunctional ingredients for food preservation.

Keywords: *Juniperus phoenicea*; phenolic compounds; antioxidant activity; antibacterial activity; foodborne pathogens

1. Introduction

Oxidative degradation and microbial spoilage negatively impact food quality, safety, and shelf life. As a result, plant-derived preservatives are increasingly being explored as potential alternatives or complements to synthetic additives in clean-label food systems (Silva et al., 2020; Efenberger-Szmechtyk et al., 2021; Oulahal & Degraeve, 2022).

Phenolic-rich extracts are of significant interest due to their ability to integrate radical-scavenging, reducing, and antimicrobial properties. Nonetheless, their effectiveness is contingent upon the botanical organ, solvent polarity, and extraction strategy employed (Gutierrez-del-Rio et al., 2018; Silva et al., 2020).

Juniperus phoenicea L., a species indigenous to the Mediterranean and North African regions, is traditionally utilized in medicinal practices. The majority of extant research has focused on essential oils and volatile terpenes, particularly fractions rich in alpha-pinene. In contrast,

there is a relative paucity of studies focusing on the non-volatile phenolic fractions derived from various plant organs (Ennajar et al., 2009; El Hamdaoui et al., 2018; Asbabou et al., 2024).

The principal research gap is the limited availability of comparative data on the non-volatile phenolic fractions extracted from *J. phoenicea* leaves and berries. These fractions are obtained through solvent partitioning and assessed using complementary antioxidant and antibacterial assays. Addressing this gap is essential, as fractionation can substantially influence phenolic enrichment and biological activity (Hayouni et al., 2007; Ince et al., 2020; Dervishi et al., 2023; Elsherif et al., 2024).

A multi-assay antioxidant approach was employed because DPPH, ABTS, CUPRAC, and galvinoxyl assays represent partially distinct reaction mechanisms and solvent environments (Apak et al., 2016; Yaseen et al., 2020; Danet, 2021).

This study sought to compare the leaves and berries of *J. phoenicea* collected in eastern Algeria, fractionate their hydroalcoholic extracts based on solvent polarity, and identify the fractions exhibiting the most significant antioxidant and antibacterial properties.

2. Materials and Methods

2.1 Plant material and sample preparation

Leaves and mature berries of *Juniperus phoenicea* L. were collected in early November 2022 from Djebel Doukkane (Tebessa region, eastern Algeria; altitude 960 m). After taxonomic identification, the material was cleaned, lyophilized, ground, and stored in airtight containers at room temperature away from light and humidity until extraction.



Figure 1. *Juniperus phoenicea* L. plant material used in the study.

2.2 Chemicals

DPPH, ABTS, BHA, BHT, potassium persulfate, ammonium acetate, gallic acid, copper(II) chloride, Folin–Ciocalteu reagent, galvinoxyl, sodium carbonate, neocuproine, aluminum nitrate nonahydrate, potassium acetate, and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany). Mueller–Hinton agar and Mueller–Hinton broth were used for antibacterial assays. All other solvents were of analytical grade.

2.3 Bacterial strains

The bacterial panel comprised two Gram-positive reference strains, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876, and two Gram-negative reference strains, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076.

2.4 Extraction and solvent partitioning

For each organ, 100 g of dried powder was macerated in 1 L of methanol/water (80:20, v/v) over three consecutive 24-hour periods at ambient temperature with periodic stirring. The combined filtrates were concentrated under reduced pressure at temperatures below 40 °C. The resulting residue was resuspended in water and sequentially partitioned with chloroform, ethyl acetate, and n-butanol to obtain fractions of increasing polarity. The extraction yield was expressed as the percentage of dry extract relative to the initial dry plant mass and was interpreted as mass recovery rather than direct phenolic enrichment.

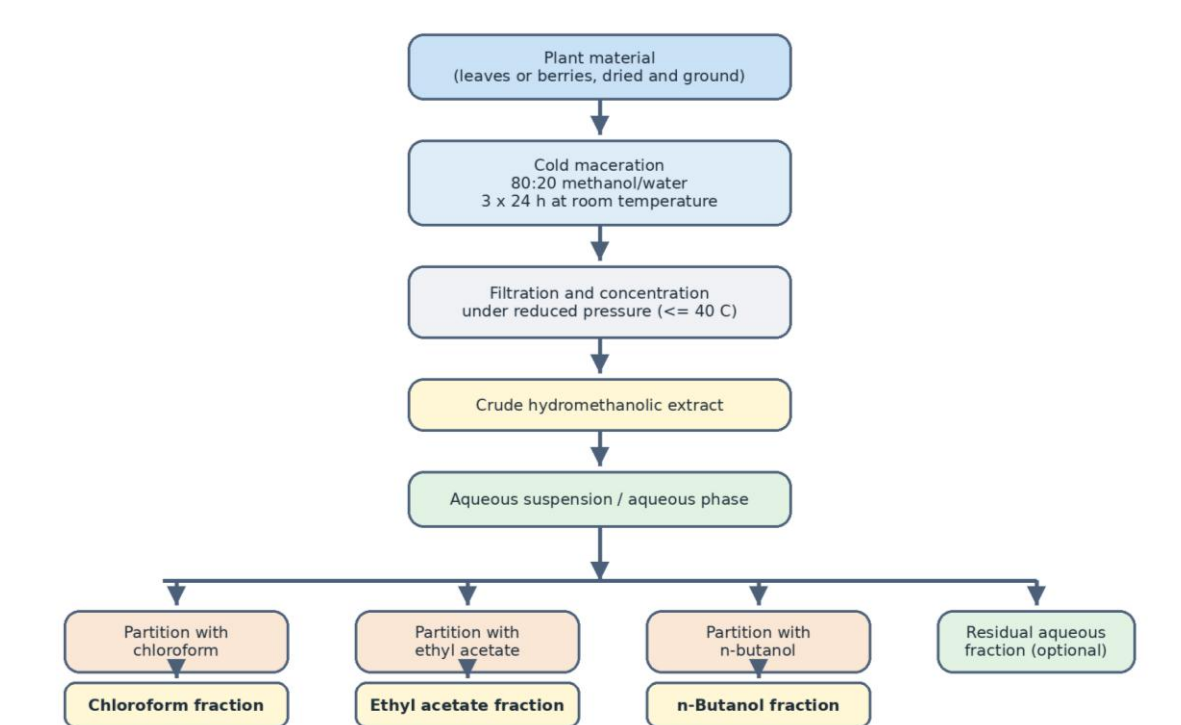


Figure 2. Redesigned extraction scheme for *Juniperus phoenicea* leaves and berries.

2.5 Determination of total phenolic content

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu microplate method, with absorbance measured at 750-765 nm. The results were expressed as micrograms of gallic acid equivalents per milligram of dry extract ($\mu\text{g GAE}/\text{mg extract}$). Gallic acid served as the calibration standard, and the samples were analyzed in replicate. Given that the Folin-Ciocalteu reagent also reacts with other reducing substances, the TPC values were interpreted as comparative indices of total reducing or phenolic richness (Singleton et al., 1999; Apak et al., 2016; Yaseen et al., 2020).

2.6 Determination of total flavonoid content

The total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric assay at a wavelength of 415 nm, with results expressed as micrograms of quercetin equivalents per milligram of dry extract ($\mu\text{g QE/mg extract}$). Quercetin served as the calibration standard, and the results are regarded as operational flavonoid-equivalent estimates due to the differential response of various flavonoid subclasses to AlCl_3 (Chang et al., 2002; Shraim et al., 2021).

2.7 DPPH radical scavenging assay

The DPPH scavenging activity was assessed at a wavelength of 517 nm following a 30-minute incubation period. The percentage inhibition was determined using the formula $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$. IC_{50} values were derived from concentration-response curves, with lower IC_{50} values indicating more potent radical-scavenging activity (Brand-Williams et al., 1995; Yaseen et al., 2020).

2.8 ABTS radical cation decolorization assay

The ABTS radical cation scavenging activity was assessed at a wavelength of 734 nm following a 10-minute reaction with preformed $\text{ABTS}^{\bullet+}$. The percentage inhibition and IC_{50} values were derived from concentration-response curves. Notably, lower IC_{50} values are indicative of stronger scavenging activity (Re et al., 1999; Dong et al., 2015; Yaseen et al., 2020).

2.9 CUPRAC reducing capacity assay

The reducing power was assessed using the CUPRAC assay at a wavelength of 450 nm, which involves the reduction of the Cu(II) -neocuproine complex. The results are reported as $A_{0.5}$, representing the concentration necessary to achieve an absorbance of 0.50; lower $A_{0.5}$ values denote a stronger reducing capacity (Apak et al., 2004; Apak et al., 2016).

2.10 Galvinoxyl radical scavenging assay

The galvinoxyl scavenging activity was assessed at a wavelength of 428 nm. The percentage inhibition and IC_{50} values were determined in a manner analogous to that used for DPPH, with lower IC_{50} values signifying greater activity. This assay was incorporated as a supplementary radical-scavenging test for systems of lower or intermediate polarity (Ochiai et al., 2003; Dervishi et al., 2023).

2.11 Antibacterial assays

Disk diffusion assays were conducted on Mueller-Hinton agar utilizing sterile 6-mm disks impregnated with 20 μL of each extract. Dimethyl sulfoxide (DMSO) at the corresponding concentration served as the solvent and negative control. Following incubation at 37 °C for 24 hours, inhibition zones were measured as the total diameter in millimeters, including the disk. Minimum inhibitory concentration (MIC) values were determined by broth microdilution from a 20 mg/mL DMSO stock using twofold serial dilutions, with the MIC

defined as the lowest concentration that prevents visible growth after 18-24 hours at 37 °C. Due to the absence of standard antibiotic comparators in the initial experimental design, the antibacterial results are interpreted as extract-screening findings rather than direct potency comparisons with reference antibiotics (Bauer et al., 1966; Balouiri et al., 2016; Wiegand et al., 2008).

3. Statistical Analysis

All measurements were conducted in triplicate or more and are reported as mean \pm standard deviation. IC₅₀ and A_{0.5} values were calculated from concentration-response regressions. Pearson correlation coefficients were utilized descriptively to compare antioxidant assay rankings, with interpretations made cautiously due to the limited number of fractions.

4. Results

4.1 Extraction yields

The methanol extraction yielded the highest yields for both plant organs, with 8.26% for leaves and 9.54% for berries, while the partition fractions resulted in lower yields. The methanolic crude extract from berries demonstrated the most substantial bulk recovery, whereas leaves produced higher yields of ethyl acetate and n-butanol fractions. These findings suggest that extraction yield is indicative of the total mass recovered rather than of an enrichment of phenolic compounds.

Table 1. Extraction yields of *Juniperus phoenicea* leaf and berry extracts.

Organ	Methanol (%)	Ethyl acetate (%)	Chloroform (%)	n-Butanol (%)
Leaves	8.26	0.53	0.11	2.60
Berries	9.54	0.22	1.40	0.28

4.2 Phenolic and flavonoid contents

In all examined solvent systems, the leaves exhibited a higher phenolic content compared to the berries. Notably, the ethyl acetate fraction of the leaves demonstrated the highest total phenolic content (TPC) at 459.78 ± 9.60 μg GAE/mg extract and total flavonoid content (TFC) at 47.34 ± 2.45 μg QE/mg extract, thereby confirming both an organ-specific effect and a solvent-selective enrichment effect. In contrast, the chloroform fraction of the berries exhibited only trace levels of phenolic and flavonoid compounds.

Table 2. Total phenolic and flavonoid contents of *Juniperus phoenicea* extracts. ND: not detected.

Organ	Fraction	TPC (μg GAE/mg extract)	TFC (μg QE/mg extract)
Leaves	Methanol	214.44 ± 16.01	9.47 ± 0.56
Leaves	Ethyl acetate	459.78 ± 9.60	47.34 ± 2.45
Leaves	Chloroform	94.39 ± 5.07	ND
Leaves	n-Butanol	263.02 ± 6.75	5.21 ± 0.21
Berries	Methanol	34.78 ± 4.01	2.80 ± 0.49
Berries	Ethyl acetate	199.49 ± 7.65	40.25 ± 5.84
Berries	Chloroform	5.27 ± 0.61	0.18 ± 1.17
Berries	n-Butanol	184.88 ± 10.49	8.69 ± 0.68

4.3 Antioxidant activity

The antioxidant activity demonstrated a hierarchy dependent on the method utilized. The methanol extract of the leaf exhibited superior efficacy in the DPPH assay, with an IC50 value of $20.38 \pm 1.33 \mu\text{g/mL}$, while the ethyl acetate extract of the berry was the most effective in the same assay, with an IC50 value of $22.19 \pm 1.37 \mu\text{g/mL}$. In the ABTS, CUPRAC, and galvinoxyl assays, the ethyl acetate fraction of the leaf consistently outperformed other fractions, with IC50 or A0.5 values of 4.39 ± 0.12 , 8.17 ± 0.62 , and $9.19 \pm 0.29 \mu\text{g/mL}$, respectively. This consistent performance across various assays substantiates its identification as the most potent antioxidant fraction. The principal trends are depicted graphically in Figure 3.

Table 3. Best-performing fractions in each antioxidant assay. Lower IC50 or A0.5 values indicate stronger activity.

Plant organ	Best DPPH fraction IC50 ($\mu\text{g/mL}$)	Best ABTS fraction IC50 ($\mu\text{g/mL}$)	Best CUPRAC fraction A0.5 ($\mu\text{g/mL}$)	Best galvinoxyl fraction IC50 ($\mu\text{g/mL}$)
Leaves	Methanol 20.38 ± 1.33	Ethyl acetate 4.39 ± 0.12	Ethyl acetate 8.17 ± 0.62	Ethyl acetate 9.19 ± 0.29
Berries	Ethyl acetate 22.19 ± 1.37	Ethyl acetate 11.75 ± 0.10	Ethyl acetate 29.74 ± 3.06	Ethyl acetate 18.27 ± 0.40

4.4 Correlation matrix of antioxidant assays

The Pearson correlation coefficients demonstrated a strong concordance between several assay pairs, notably between CUPRAC and galvinoxyl for berry extracts ($r = 0.998$). Conversely, the weaker associations observed with DPPH suggest that the ranking of antioxidants is partially dependent on the assay employed. Consequently, the matrix should be regarded as an exploratory representation of assay concordance rather than definitive evidence that one method can supplant another.

Table 4. Pearson correlation matrix for antioxidant assays. B = berries; L = leaves; Galv. = galvinoxyl. Coefficients are descriptive because only a small number of fractions were compared.

	ABTS-B	CUPRAC-B	DPPH-B	Galv.-B	ABTS-L	CUPRAC-L	DPPH-L	Galv.-L
ABTS-B	1	0.586	0.870	0.608	0.952	0.627	0.981	0.828
CUPRAC-B		1	0.279	0.998	0.612	0.952	0.455	0.881
DPPH-B			1	0.299	0.927	0.439	0.852	0.680
Galv.-B				1	0.625	0.941	0.477	0.895
ABTS-L					1	0.727	0.895	0.886
CUPRAC-L						1	0.489	0.912
DPPH-L							1	0.706

Galv.-L								1
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4.5 Antibacterial activity

The antibacterial efficacy was contingent upon both the organ and the solvent utilized. Methanolic fractions exhibited the most extensive effects, particularly against *S. aureus* and *S. enteritidis*. Notably, the methanolic extract from berries produced the largest inhibition zone against *S. enteritidis* (13.5 ± 0.71 mm), whereas the chloroform fraction from berries demonstrated the lowest minimum inhibitory concentrations (MICs) against *S. enteritidis* and relatively low MICs against *S. aureus* and *E. coli*. Among the tested organisms, *E. coli* was the least susceptible. These results suggest that the fractions exhibiting optimal antioxidant and antibacterial profiles were not identical.

Table 5. Disk diffusion inhibition zones. Values include the 6-mm paper disk; values ≤ 6 mm indicate no measurable inhibition.

Sample	Fraction	<i>S. aureus</i> (mm)	<i>E. coli</i> (mm)	<i>B. cereus</i> (mm)	<i>S. enteritidis</i> (mm)
Leaves	Methanol	10.0 ± 1.41	≤ 6	≤ 6	9.5 ± 0.71
Leaves	Ethyl acetate	≤ 6	≤ 6	≤ 6	9.5 ± 2.12
Leaves	Chloroform	≤ 6	≤ 6	≤ 6	≤ 6
Leaves	n-Butanol	≤ 6	≤ 6	≤ 6	9.5 ± 0.71
Berries	Methanol	11.5 ± 0.71	≤ 6	8.0 ± 1.41	13.5 ± 0.71
Berries	Ethyl acetate	7.5 ± 0.71	8.0 ± 1.41	≤ 6	8.5 ± 2.12
Berries	Chloroform	10.5 ± 0.71	6.5 ± 0.71	7.5 ± 0.71	12.0 ± 2.83
Berries	n-Butanol	≤ 6	≤ 6	≤ 6	≤ 6

Table 6. Minimum inhibitory concentrations (MICs). NT: not tested. Values of 100% indicate activity only at the highest tested concentration.

Sample	Fraction	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>S. enteritidis</i>
Leaves	Methanol	6.25% (1.25 mg/mL)	NT	NT	3.125% (0.625 mg/mL)
Leaves	Ethyl acetate	NT	NT	NT	100% (20 mg/mL)
Leaves	Chloroform	NT	NT	NT	NT
Leaves	n-Butanol	NT	NT	NT	100% (20 mg/mL)
Berries	Methanol	25% (5 mg/mL)	NT	1.5625% (0.3125 mg/mL)	0.78125% (0.15625 mg/mL)
Berries	Ethyl acetate	100% (20 mg/mL)	100% (20 mg/mL)	NT	100% (20 mg/mL)
Berries	Chloroform	3.125% (0.625 mg/mL)	3.125% (0.625 mg/mL)	1.5625% (0.3125 mg/mL)	0.78125% (0.15625 mg/mL)
Berries	n-Butanol	NT	NT	NT	NT

Key findings summary - Juniperus phoenicea leaf and berry extracts

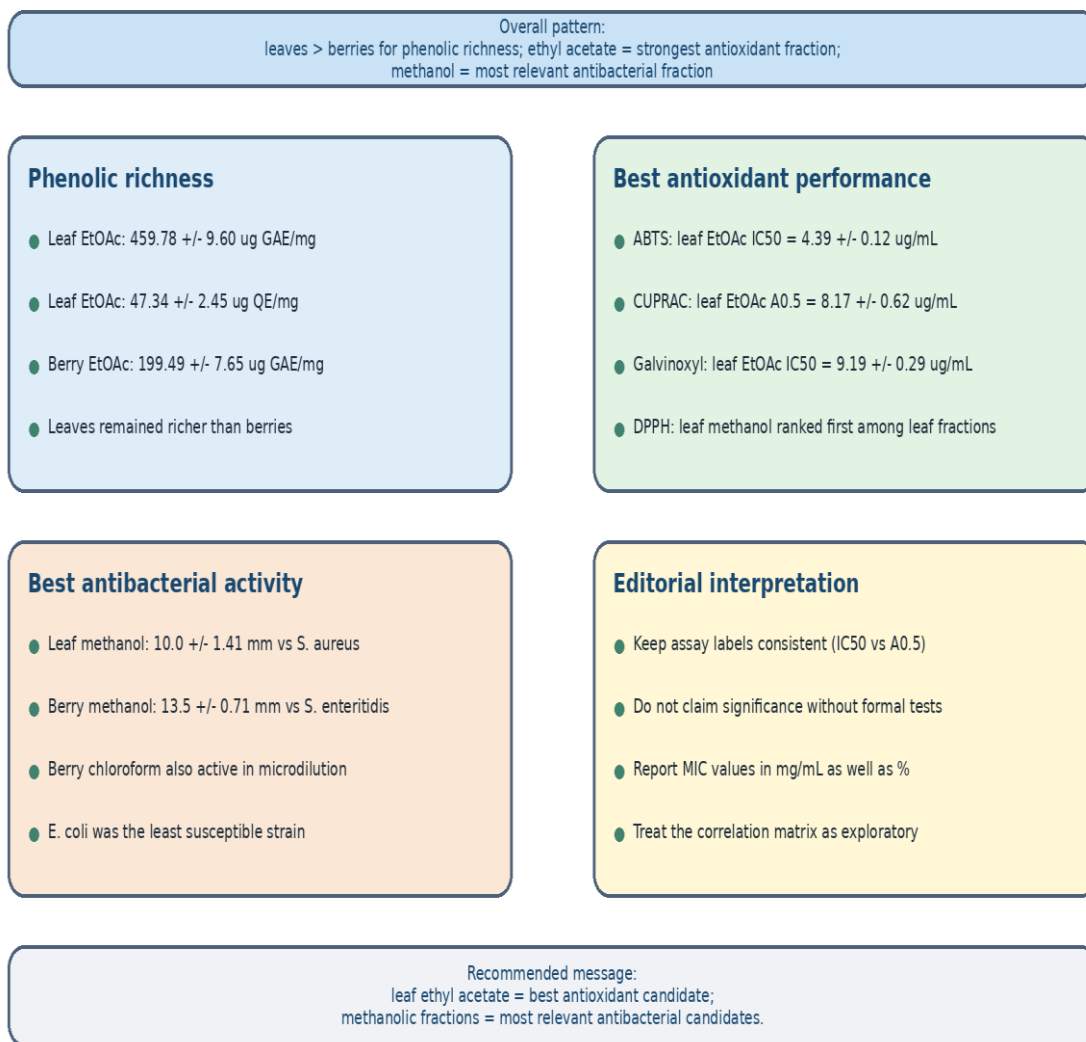


Figure 3. Graphical summary of the main antioxidant and antibacterial findings.

5. Discussion

The polarity of the solvent significantly affected both the recovery and composition of the extracts. Methanol was found to optimize the recovery of crude mass, while ethyl acetate selectively enriched antioxidant phenolic compounds, particularly in leaves. This observation is consistent with previous studies indicating that solvents of intermediate polarity are more effective at concentrating phenolic compounds than crude hydroalcoholic extracts (Hayouni et al., 2007; Ince et al., 2020; Dervishi et al., 2023).

The enhanced phenolic composition observed in leaves, as opposed to berries, is biologically plausible due to the direct exposure of leaves to light and oxidative stress. Recent research corroborates the bioactive potential of *J. phoenicea* preparations, including essential-oil, aqueous, and nano-based systems. However, these matrices differ from the non-volatile solvent fractions assessed in the present study (Asbabou et al., 2024; Elsherif et al., 2024; Zemmouli et al., 2025).

The antioxidant data substantiate the need to employ multiple assays. Although the leaf ethyl acetate fraction did not consistently achieve the highest ranking in the DPPH assay, it consistently ranked highest in the ABTS, CUPRAC, and galvinoxyl assays. This suggests a comprehensive antioxidant activity encompassing both radical-scavenging and reducing mechanisms, rather than an effect confined to a single assay system.

The correlation analysis substantiates this interpretation and confirms variations depending on the method employed. The robust relationship between CUPRAC and galvinoxyl indicates similar redox behavior in certain fractions. In contrast, the weaker relationships with DPPH highlight differences in radical type, solvent environment, reaction kinetics, and the accessibility of antioxidant constituents.

The antibacterial activity exhibited a distinct pattern compared to the antioxidant activity. Methanolic fractions, particularly the methanolic extract derived from berries, were more significant for antibacterial screening, while the chloroform fraction of the berries demonstrated favorable minimum inhibitory concentration (MIC) values. This indicates that the antibacterial effects may be contingent upon broader metabolite mixtures or lipophilic constituents rather than solely on phenolic concentration. The absence of a positive antibiotic comparator is recognized as a methodological limitation; thus, these findings should be regarded as preliminary data from extract screening.

From an applied perspective, the findings suggest two practical profiles: leaf ethyl acetate as a potential antioxidant and methanolic fractions as potential antibacterial agents. For applications in food preservation, future research should investigate these fractions within real food matrices, quantify marker compounds through chromatographic profiling, assess toxicity and sensory acceptability, and determine whether combined fractions can effectively provide both oxidative control and microbial inhibition.

Conclusion

The leaves and berries of *Juniperus phoenicea* from eastern Algeria have been identified as sources of fractions with distinct biological properties. Notably, the ethyl acetate fraction obtained from the leaves exhibited the highest concentration of phenolic antioxidants, whereas the methanolic fractions demonstrated significant antibacterial activity. These findings suggest the potential for further development of *J. phoenicea* extracts as natural multifunctional agents for food preservation, contingent upon the completion of chromatographic characterization, safety assessments, and validation within food matrices.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors report no conflicts of interest.

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AI Use Statement

We used artificial intelligence tools only for English language editing, translation, and figure preparation. The authors alone are responsible for all scientific interpretation, data analysis, and conclusions.

Authors' contributions: S.F. conceived the study, supervised the research, performed the experimental work, and drafted and revised the manuscript. M.A.M., H.L., and I.K. contributed to the experimental work, data interpretation, manuscript revision, and approval of the final version.

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