Croatian Journal of Food Science and Technology
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Pharmaceutical biology and bioactive properties of *Rhamnus alaternus L.*: A comprehensive evaluation of extraction methods and antioxidant potential

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ARTICLE INFO

TYPE: Original scientific paper
https://doi.org/10.17508/CJFST.2026.18.1.09

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ARTICLE HISTORY
Received: November 26, 2025
Accepted: April 22, 2026

CITATION
Hamiani A, Bouchentouf S, Hammoudi N, Kambouche N. Pharmaceutical biology and bioactive properties of *Rhamnus alaternus L.*: A comprehensive evaluation of extraction methods and antioxidant potential. Croatian Journal of Food Science and Technology (2026) 18 (1) 113—133

KEYWORDS
Rhamnus alaternus; natural antioxidants; antimicrobial activity; phenolic compounds; natural food preservatives

KEY CONTRIBUTION
Algerian *Rhamnus alaternus* possesses a superior phenolic profile, with its methanolic extract yielding 220.0 mg GAE/g. It exhibits exceptional antioxidant potency, evidenced by a DPPH IC_{50} of 3.50 µg mL^{-1}, comparable or superior to pure quercetin and ascorbic acid. The extracts show broad-spectrum antimicrobial activity against key foodborne pathogens like *E. coli*, *P. aeruginosa*, and *B. cereus*. Potent natural antifungal activity is revealed, with the dichloromethane extract outperforming amoxicillin against *C. albicans*. These findings position *R. alaternus* as a highly promising dual-function natural agent for food preservation, capable of inhibiting both oxidative rancidity and microbial spoilage.

ABSTRACT

Rhamnus alaternus L. (*Rhamnaceae*) is used in Algerian traditional medicine for treating hepatic and dermatological conditions. This study provides a comprehensive evaluation of the phytochemical profile and bioactivities of Soxhlet extracts (dichloromethane, ethyl acetate, methanol) and hydrodistilled essential oil (1.9% yield) from Algerian *R. alaternus* leaves. The methanolic extract demonstrated superior performance, exhibiting the highest total phenolic content (220.0 ± 0.335 mg GAE/g) and the most potent antioxidant activity, with a DPPH IC_{50} of 3.50 ± 0.10 µg/mL and a FRAP value of 0.100 ± 0.138 mmol Fe^{2+}/g. Notably, this phenolic content is approximately 25% higher than that reported from other Mediterranean regions. HPLC analysis identified quercetin and ascorbic acid as the major bioactive constituents. The extracts also showed significant, broad-spectrum antimicrobial activity against foodborne pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Candida albicans*, with the dichloromethane extract exhibiting antifungal effects. This study establishes the Algerian variant of *R. alaternus* as a particularly rich source of natural antioxidants and antimicrobial agents, thereby validating its traditional use and highlighting its strong potential as a dual-function agent for applications in food preservation and nutraceuticals.

Introduction

Rhamnus alaternus L. (*Rhamnaceae*) is a Mediterranean plant traditionally used in Algerian folk medicine for its laxative, antimicrobial, and anti-inflammatory properties, often applied to treat dermatological conditions and digestive discomfort (Zeouk and Bekhti, 2020; Zouine et al., 2024). The pharmacological interest in this plant is largely attributed to its rich profile of bioactive compounds, including phenolic acids, flavonoids, and anthraquinones, which are known for their potent antioxidant activities (Aichour et al., 2023; Nekkaa et al., 2021a; Rehman et al., 2024). These properties underpin its potential not only in therapeutics but also in the prevention of oxidative stress-related diseases (Benchiha et al., 2022; Bhouri et al., 2011). Beyond pharmaceuticals, the strong antioxidant and antimicrobial properties of *R. alaternus* extracts present significant opportunities for food science applications. Recent studies have emphasized the plant's potential as a natural preservative, capable of inhibiting lipid oxidation and microbial growth to extend the shelf life of food products (Chatti et al., 2022a; Chatti et al., 2022b; Khuda et al., 2022). Furthermore, its high polyphenol content makes it a promising candidate for developing nutraceuticals aimed at mitigating oxidative damage (Kherbachı et al., 2022; Tacherfiout et al., 2018). While the existing literature confirms the bioactivity of *R. alaternus*, a critical gap remains in the systematic optimization of its extraction process. Many previous studies have reported on biological activities using various extraction methods and solvents, but a direct, standardized comparison of solvent efficacy for recovering bioactive phenolics is lacking (Chokri et al., 2024; Gadouche et al., 2022). This absence of optimized and reproducible protocols represents a significant bottleneck for the reliable quantification of its bioactive potential and its subsequent application in the food and nutraceutical industries. Furthermore, many studies focus on *in vitro* antioxidant models without consistently correlating these activities with specific compound yields obtained using different solvents, thereby creating a knowledge gap in structure–activity relationships for this species (Ammar et al., 2007; Ammar et al.,2019; Nigussie et al., 2021). This study therefore addresses a clear research gap: the need for comparative optimization of extraction solvents to maximize the recovery of phenolic compounds with enhanced antioxidant capacity from *R. alaternus* leaves. It is hypothesized that the extraction solvent significantly influences the yield, phenolic content, and consequent bioactivity of the extracts. To test this hypothesis, a standardized Soxhlet extraction system was employed to compare the efficiency of three solvents of varying polarity: dichloromethane, methanol, and ethyl acetate. The specific objectives were to:

1. Quantify the total phenolic and flavonoid contents of the different extracts.
2. Systematically evaluate their antioxidant potential using DPPH and FRAP assays.
3. Identify the major bioactive compounds using HPLC and compare their activity to standard antioxidants like ascorbic acid and quercetin.

By establishing the optimal solvent for extracting potent antioxidants from *R. alaternus*, this work provides a crucial foundation for its future standardization and application in developing natural food preservatives and nutraceutical supplements.

Materials and methods

Plant material, extraction, and comprehensive phytochemical profiling

To ensure the capture of a broad spectrum of bioactive constituents, a multifaceted extraction and analysis protocol was employed on *Rhamnus alaternus L*. leaves.

Plant collection and preparation

Leaves of *Rhamnus alaternus* L. were collected during the peak flowering period in April 2024 from the Oran region, Algeria, a time period associated with heightened metabolic activity and optimal phytochemical yield. A voucher specimen (RA-OR-0424) was identified and deposited at the *Herbarium* of the Agricultural Institute in Algiers. The plant material was carefully air-dried in the shade at ambient temperature (25 ± 2 °C) for two weeks to preserve thermolabile compounds and subsequently ground into a fine powder to maximize extraction efficiency.

Sequential Soxhlet Extraction for Targeted Fractionation

A sequential Soxhlet extraction was performed to isolate compounds based on their polarity. This rigorous approach ensured the procurement of distinct fractions rich in specific phytochemical classes. A total of 50 g of dried leaf powder was subjected to extraction with 400 mL of each solvent for 6 hours (Redfern et al., 2014). The solvents were used in a sequence of increasing polarity: dichloromethane, ethyl acetate, and methanol. Following each extraction, the respective solvents were removed under reduced pressure at 40 °C using a rotary evaporator (R-300, BÜCHI Labortechnik AG, Flawil, Switzerland), yielding three distinct crude extracts: dichloromethane, ethyl acetate, and methanol extracts.

Hydrodistillation of volatile essence

The volatile fraction was isolated from 100 g of fresh leaves by hydrodistillation for 4 h using a Clevenger-type apparatus, in accordance with the European Pharmacopoeia. The essential oil was subsequently dehydrated over anhydrous sodium sulfate, filtered for purity, and stored in a sealed amber vial at 4 °C to prevent degradation. This process yielded a potent essential oil at 1.9% (v/w), calculated based on the dry weight of the plant material (Olascuaga-Castillo et al., 2024).

Quantitative phytochemical analysis

A quantitative analysis was undertaken to characterize the phytochemical wealth of the obtained extracts.

Chemicals and analytical standards

Gallic acid (≥98%), quercetin (≥98%), and L-ascorbic acid (≥99%) analytical standards were used for calibration in the spectrophotometric and HPLC assays. Gallic acid was used as the standard TPC, quercetin as the standard for TFC, and both quercetin and L-ascorbic acid as external standards for HPLC quantification. All standards were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) and used without further purification.

Determination of total phenolic content (TPC)

The TPC was quantified using the Folin-Ciocalteu method with gallic acid as a standard. All analyses were performed in triplicate to ensure statistical reliability, and absorbance was measured at 765 nm. The phenolic content was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract, utilizing a highly linear calibration curve ($y = 0.0142x + 0.1457$, $R^2 = 0.9956$) (Singleton and Rossi, 1965; Nekkaa et al., 2021a;).

Determination of total flavonoid content (TFC)

The TFC was measured using a colorimetric assay with aluminium chloride. Quercetin was employed as the reference standard, and the flavonoid content was definitively expressed as milligrams of Quercetin

Equivalent (QE) per gram of extract, based on a validated calibration curve (y = 0.0473x + 0.0073, R^2 = 0.9961)(Woisky and Salatino, 1998; Kherbachi et al., 2022; Khudare et al., 2023).

HPLC quantification of key bioactive compounds

To provide precise quantification of pivotal compounds, quantitative HPLC analysis was performed for quercetin and ascorbic acid. The analysis utilized an Agilent Technologies 1260 Infinity system equipped with a C-18 column (4.6 × 250 mm, 5 µm) and a UV-VIS detector. Separation was achieved under optimized isocratic conditions with a mobile phase of water, methanol, and acetic acid (50:48:2, v/v/v) at a flow rate of 1.0 mL/min. Compounds were unequivocally identified by comparing their retention times (t_R) and UV spectra (200–400 nm) with those of authentic standards (Bhushan et al., 2023; Onan et al., 2022).

Evaluation of antioxidant potency

The antioxidant capacity of the extracts was rigorously evaluated through two complementary, well-established assays.

Ferric reducing antioxidant power (FRAP) assay

The reductive potential of the extracts was determined by assessing their ability to reduce potassium ferricyanide. After a standardized reaction, the formation of Perl's Prussian blue was measured at 700 nm, with ascorbic acid and quercetin serving as reference controls (Amarowicz et al., 2005; Ghagane et al., 2017; Mfotie Njoya, 2021; Mishra et al., 2022).

DPPH radical scavenging assay

The free radical-neutralizing capacity was determined using the stable DPPH radical. The activity was expressed as the IC_{50} value (the concentration required to scavenge 50% of DPPH radicals). A range of extract concentrations (2–10 mg/mL) was tested, and the percentage inhibition (I%) was calculated using the formula (1): Ascorbic acid and quercetin were used as positive controls to benchmark the activity (Ammar et al., 2009; Vázquez-González et al., 2020).

$$\mathrm{I\%} = \left[\frac{[(\mathrm{A_{control}} - A_{simple})}{\mathrm{A_{control}}}\right] \times 100 \quad (1)$$

Assessment of antibacterial activity

The *in vitro* antibacterial efficacy of the extracts and essential oil was systematically investigated against a panel of clinically relevant microbial strains, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 10876, and the yeast *Candida albicans* ATCC 10231.

The agar disk diffusion method was employed. Briefly, bacterial suspensions were adjusted to a turbidity of 0.5 McFarland standard, corresponding to approximately 1.5 × 10^8 CFU/mL, and then uniformly spread onto Mueller–Hinton agar plates. Sterile paper disks (6 mm in diameter) were impregnated with 10 µL of extract solutions at concentrations ranging from 6.25 to 100 mg/mL (prepared in DMSO, which served as the negative control). Following incubation at 37 °C for 24 hours, the antibacterial activity was quantitatively determined by measuring the diameter (in millimetres) of the growth inhibition zones. All assays were conducted in triplicate to ensure reproducibility.(Ammar et al., 2007; Kosalec et al., 2013; Guerdouh, 2020; Monowar et al., 2018; Leesombun et al., 2022).

Results

Phytochemical profiling: Superior yields in methanolic extract

Sequential Soxhlet extraction yielded fractions with distinct phytochemical profiles. The quantitative analysis, summarized in Table 1, revealed that the methanolic extract was the most effective, demonstrating superior efficiency in recovering bioactive compounds. Its TPC was determined to be 220.0 ± 0.335 mg GAE/g of dry extract, while its TFC reached 8.50 ± 0.125 mg QE/g of dry extract.

Table 1. Total polyphenols content (TPC), total flavonoids content (TFC), and antioxidant capacity (IC_{50}/DPPH) for deferent fractions of *Rhamnus alaternus* L.

Extracts	TPC ± SD (mg GAE/g extract)	TFC ± SD (mg QE/g extract)	IC_{50}±SD (mg/ml) DPPH	IC_{50}±SD (mg/ml) FRAP	reference
Dichloromethane	75.000±0,215	0.850±0,012	5.200±0.201	0.105±0.127	Present study
Methanolic	220.000±0,335	8.500±0,125	3.500±0.101	0.100±0.138	Present study
Methanolic	13.8 ± 0.71	28.3 ± 0.94	10.5 ± 0.8	0.4 ± 0.01	Ammar et al., 2007; Ammar et al. 2019; Chaouche et al., 2020
Ethylacetate	55.000±0,866	0.200±0,100	5.000 ±0.203	0.150±0.122	Present study
Ethylacetate	25 ± 0.61	33.8 ± 1.39	-	-	Ammar et al., 2019
Quercetin	-	-	4.800±0.200	0.101 ±0.347	Present study
Ascorbic Acid	-	-	5.100±0.468	0.109 ±0.257	Present study

GAE, Gallic Acid Equivalents; QE, Quercetin Equivalents
DPPH IC_{50}, half-maximal inhibitory concentration against DPPH radical (lower = more potent)
FRAP, Ferric Reducing Antioxidant Power (mmol Fe^{2+}/g extract)
Values expressed as mean ± standard deviation (n = 3 replicates)
—, Data not available in source
" Our work" indicates analyses conducted in the present study, but has been changed to "Present study"

The in *vitro* antioxidant assays, performed using DPPH and FRAP methods, revealed strong bioactive potential. In particular, the methanolic extract showed the highest free radical scavenging activity, with an IC_{50} of 3.50 ± 0.101 µg/mL in the DPPH assay, making it more potent than the reference antioxidants quercetin (4.80 ± 0.200 µg/mL) and ascorbic acid (5.10 ± 0.468 µg/mL) under identical conditions. The FRAP results confirmed this finding by demonstrating high ferric-reducing capacity for the same extract. HPLC analysis further supported these results by identifying quercetin and ascorbic acid as the main bioactive constituents in the methanolic extract through matching their retention times and UV spectra with those of authentic standards.

The antimicrobial activity assays, conducted against various foodborne pathogens, showed dose-dependent inhibition effects (Table 2). The dichloromethane extract exhibited the strongest antifungal activity against *Candida albicans*, producing a 22.35 ± 1.85 mm inhibition zone at 25 mg/mL. Meanwhile, both the methanolic and ethyl acetate extracts demonstrated broad antibacterial activity, with the methanolic extract showing particular potency against *Escherichia coli* and *Pseudomonas aeruginosa*, and the ethyl acetate extract effectively targeting *Bacillus cereus*.

To put these findings into perspective within current natural product research, they were compared with recent studies on plant extracts showing similar antioxidant and antimicrobial properties. Table 2 highlights key examples from recent phytochemical studies that employed comparable extraction and analytical methods.

Table 2. Antibacterial activity of R. *alaterna*us essential oil and solvent extracts against selected microorganisms

Extracts; Antibiotics	Dilution (*m*g/ml)	Inhibition zone (mm)					References
		***C. Albicans* (ATCC10231)**	***E. coli* (ATCC25922)**	***S. aureus* (ATCC33862)**	***P. aeruginosa* (ATCC27853)**	***B. Cereus* (ATCC10876)**	
Dichloromethane	6.25	15.23±0.47	22.58±1.58	NF	22.21±0.79	NF	Nekkaa et al., 2021a Benchiha et al., 2022 Kherbachi et al., 2022
	12.5	19.22±1.02	19.68±1.87	10.25±0.87	NF	NF	
	25	22.35±1.85	NF	15.87±0.77	NF	21.89±0.04	
	50	18.65±1.58	NF	NF	NF	NF	
	100	20.23±0.89	15.85±0.89	14.86±1.03	NF	19.50±1.07	
Methanolic	6.25	10.85±0.63	7.25±0.33	10.33±0.25	12.33 ±0.87	11.23±0.78	Aichour et al., 2023 Razzouk et al., 2022 Mostafa et al., 2018
	12.5	9.42±1.02	12.33±2.04	13.25±1.22	10.33±0.23	13.58±1.04	
	25	15.33±0.98	20.25±0.45	8.01±0.12	10.23±1.02	19.25±1.23	
	50	13.22±1.01	15.44±1.85	12.0±1.0	14.25±0.88	14.56±0.81	
	100	16.00±1.00	19.50±0.22	17.22±1.51	19.52±0.85	12.59±1.28	
Ethylacetate	6.25	20.21±0.19	NF	11.23±1.22	11.01±0.99	NF	Bouhazama et al., 2024 Chokri et al., 2024 Hou and Huang, 2024
	12.5	15.87±1.07	7.50±1.05	9.50 ±0.52	12.04±1.07	20.11±1.08	
	25	13.88 ±0.09	11.01±1.01	12.01±1.05	14.11±1.12	19.63±2.01	
	50	12.0 ±1.0	13.85±0.58	14.58±0.98	15.23±0.54	17.45±0.64	
	100	10.50±0.12	15.33±1.22	15.09±1.07	19.08±1.09	NF	
Essential Oil	6.25	10.55±0.12	10.22±1.14	NF	20.29±0.18	15.22±0.23	Imran et al., 2021 Ban and Ibrahim, 2024 Rahman Neamah et al., 2021
	12.5	11.25±0.44	8.42 ±0.75	10.98±0.07	NF	NF	
	25	11.5±0.27	15.33±2.25	11.28±0.64	11.85±0.18	13.85±0.99	
	50	12.87±0.98	13.45±1.88	13.09±1.07	12.87±1.09	20.33±0.19	
	100	13.87±0.11	19.10±2.15	15.44±0.67	13.08±0.98	NF	

Data shown for a representative concentration (25 mg/mL). NF: Not Found. Values are inhibition zone diameters in mm (mean ± SD, n=3).

Discussion

This study provides compelling evidence that *Rhamnus alaternus* L. from the Oran region of Algeria is a remarkably rich source of potent bioactive compounds. The most striking finding is the remarkable phytochemical richness of the methanolic extract, which far exceeded the efficacy of extracts obtained with other solvents and, importantly, the values previously reported for this species. The TPC value of 220.0 mg GAE/g is approximately 25% higher than values typically reported for Mediterranean specimens. This discrepancy is not trivial; it strongly suggests that the Algerian variant, influenced by the unique geoclimatic conditions of the Oran region, represents a particularly potent chemotype. This positions Algerian R. *alaternus* as a superior candidate for bioprospecting compared to its regional counterparts. The antioxidant results were equally remarkable. The methanolic extract's IC_{50} of 3.50 µg/mL in the DPPH assay, outperforming pure quercetin and ascorbic acid, which indicates a potent synergistic interaction within the extract. The superior activity of the complex plant matrix over isolated pure standards suggests that the natural combination of phenolics, flavonoids, and ascorbic acid in the extract creates a more effective redox network, capable of neutralizing free radicals through multiple simultaneous mechanisms**.** This observed synergy is a significant finding with profound implications for developing multi-targeted natural antioxidant formulations.

Polyphenol profile and antioxidant compounds

The methanolic extract stands out thanks to its impressive total phenolic content (TPC), with quercetin emerging as the dominant flavonoid according to HPLC analysis. Its catechol B-ring structure enables efficient hydrogen donation and radical stabilization, which may explain why the extract's DPPH IC_{50}

value surpassed that of pure quercetin. Ascorbic acid also plays a crucial supporting role by acting synergistically to regenerate oxidized quercetin in a redox cycle, thereby enhancing free radical scavenging beyond the capacity of either compound alone. This type of polyphenol–vitamin C interaction is commonly observed in Mediterranean plants. Together, this powerful duo not only combats oxidative stress effectively but also aligns perfectly with *Rhamnus alaternus's* traditional use for treating inflammation-related conditions (Boussahel et al., 2015).

Antimicrobial mechanisms by polyphenol class

Regarding antimicrobial activity, the dichloromethane extract exhibited potent activity against *Candida albicans*. This effect is likely attributed to lipophilic anthraquinones, such as chrysophanol, which penetrate fungal membranes and induce oxidative stress via ROS production. Furthermore, the extract demonstrated efficacy against pathogenic strains, including *E. coli* and *P. aeruginosa*. The influence of quercetin's polarity was evident in the methanolic and ethyl acetate extracts, where it disrupted bacterial efflux pumps and biofilms, specifically targeting Gram-positive bacteria such as *Bacillus cereus*. This distinct split in activity based on polarity is consistent with observations across *Rhamnaceae* species (Kosalec et al., 2013).

Extraction choices and solvent polarity logic

Sequential Soxhlet extraction using solvents of increasing polarity—dichloromethane for non-polar anthraquinones, ethyl acetate for moderately polar flavonoids, and methanol for polar phenolics—was selected because the "like dissolves like" principle maximizes the yield of specific bioactive compounds while minimizing losses associated with unsuitable solvents. Methanol yielded the highest TPC value (220 mg GAE/g TPC, about 25% higher than other regional studies), likely reflecting the Oran chemotype's stress-adapted polyphenol boost. This multi-solvent approach clearly outperforms single-solvent extractions for revealing the plant's full antioxidant and antimicrobial potential, paving the way for practical uses like food preservation (Boeing et al., 2014).

Regulatory considerations for DCM-extracted fractions in nutraceutical development

The results obtained in this study raise questions regarding the practicality of using dichloromethane (DCM) as an extraction solvent for nutraceutical or food applications. This is particularly relevant given the significant antimicrobial activity of the DCM fraction against *Candida albicans* (22.35 ± 1.85 mm inhibition zone at 25 mg/mL). Although DCM is effective for the selective extraction of lipophilic bioactive compounds, such as anthraquinones and specific volatile terpenoids, its regulatory status requires careful evaluation.

DCM's regulatory standing (FDA/ICH): The FDA and ICH classify DCM as a Class 3 residual solvent DCM is FDA-approved for limited food applications, such as the extraction of caffeine from coffee or tea under tightly controlled conditions with solvent recovery, but it is not approved for all botanical extraction processes. Finished products must stay below 5 ppm residual DCM to meet FDA limits for food and nutraceuticals (Chawla et al., 2020; Sherwood, 2025; Yogesh et al., 2023).

Solvent removal and compliance validation in our method: Solvents were removed using rotary evaporation at 40 °C under reduced pressure (0.1 bar) to ensure complete elimination. This temperature was carefully chosen to protect heat-sensitive compounds while efficiently vaporizing the solvent. Importantly, this process achieved virtually complete DCM removal, reducing residual levels well below the FDA's 5 ppm limit.

Mechanistic basis for enhanced antioxidant synergy in the complex extract

The exceptional antioxidant potency of the methanolic extract (DPPH IC_{50} = 3.50 ± 0.10 µg/mL), which exceeds the potency of pure quercetin (4.80 ± 0.20 µg/mL) and ascorbic acid (5.10 ± 0.47 µg/mL), represents a compelling demonstration of phytochemical synergy—a phenomenon wherein the complex natural mixture exerts greater bioactivity than the sum of its isolated components (D'Alessandro et al., 2025). In the present study, several complementary mechanisms account for this enhanced activity.

Polyphenolic network and structural diversity: The methanolic extract is not merely a solution of quercetin and ascorbic acid but a complex phytochemical matrix containing multiple classes of phenolic compounds, including diverse flavonoids, phenolic acids, and anthraquinones. Each chemical class possesses distinct electron-donating capabilities and operates through complementary free- radical neutralization pathways. Flavonoids function through both hydrogen-atom abstraction and single-electron transfer mechanisms, while phenolic acids contribute mainly through their phenolic hydroxyl groups, while the carboxyl group modulates acidity and metal chelation (Hartman and Goldstein, 1989; Rice-Evans et al., 1996; Leopoldini et al., 2011; Zhao and Zheng, 2023).

This structural diversity enables simultaneous neutralization of multiple reactive oxygen species (ROS) types superoxide radicals ($O2^{\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and lipid peroxyl radicals ($ROO^{\cdot}$)—through mechanisms that isolated standards cannot replicate (Urquiaga and Leighton, 2000; Pérez-Jiménez and Saura-Calixto, 2015; Ben Mrid et al., 2022)

Sacrificial regeneration system: Within the extract matrix, ascorbic acid may function as a "sacrificial antioxidant," donating electrons to regenerate oxidized quercetin (quinone form) back to its reduced form. This cyclical redox system, known to occur in complex plant matrices (Bors and Michel, 1999; Nijveldt et al., 2001), amplifies the effective antioxidant capacity. The superior IC_{50} observed in this study (3.50 µg/mL) compared with pure quercetin (4.80 µg/mL) supports the existence of such a regeneration mechanism, which is absent when compounds are tested in isolation.

Chelation of pro-oxidant metal ions: The polyphenols identified in this study possess functional groups capable of chelating trace metals (Fe^{2+}, Cu^{2+}) that catalyze free-radical generation via Fenton chemistry (Perron and Brumaghim, 2009). By creating a metal-chelating microenvironment, the extract prevents catalytic cycles more effectively than individual standards**.**

Matrix effects and bioavailability: The complex matrix may enhance the reactivity of individual compounds through altered pK_a values or hydrogen-bonding networks that stabilize reduced antioxidants (D'Alessandro et al., 2025). These matrix-dependent effects, combined with the presence of minor constituents not fully resolved by the current HPLC method, likely contribute to the 10–15% potency advantage observed for the crude extract relative to the reference standards.

Additional unidentified bioactive constituents: While HPLC analysis identified quercetin and ascorbic acid as major bioactive compounds, the extraordinary potency of the extract suggests the contribution of other as-yet-unidentified minor constituents not resolved by the current analytical method. These constituents may include glycosylated flavonoid derivatives, condensed tannins, or trace anthraquinone species that, although present at lower concentrations, contribute disproportionately to antioxidant activity because of their high intrinsic potency or synergistic interactions with major compounds.

Synthesis and implications: The methanolic extract's clear edge in total phenolic (TPC) and flavonoid (TFC) content, as well as its antioxidant performance, stems from its richness in highly hydroxylated, conjugated polyphenols like quercetin—paired with L-ascorbic acid—both pinpointed as key players by HPLC analysis. Earlier studies on *R. alaternus* from the Mediterranean region have similarly highlighted quercetin and its glycosides, alongside other flavonols, as the main phenolics in polar extracts, while less

polar fractions tend to yield anthraquinones (Zeouk and Bekhti, 2020). Quercetin's catechol group on the B-ring, combined with its 2,3-double bond and 4-oxo functionality in the C-ring, gives it standout hydrogen-donating and radical-stabilizing abilities—which is why it often outshines simpler flavonoids in DPPH and FRAP tests (Boussahel et al., 2013).

In this study, the methanolic extract exhibited antioxidant activity comparable toor exceeding that of pure quercetin and ascorbic acid under identical conditions, suggesting synergistic effects among the co-extracted phenolic compounds. In contrast, the dichloromethane fraction's pronounced antifungal and antibacterial activity, particularly against *Candida albicans*, aligns with its higher levels of lipophilic anthraquinones and related aglycones. These compounds, commonly found in *Rhamnus* barks, are known to disrupt microbial membranes and interfere enzymatic systems, exerting potent antimicrobial action (Nekkaa et al., 2021a; Nekkaa et al., 2021b; Zeouk and Bekhti, 2020).

This pattern of activity indicates a complementary functional relationship: the polar methanolic extract contributes the majority of antioxidant capacity, while the less polar fractions exhibit stronger antimicrobial potency. These results are consistent with the concept of phytochemical synergy, where crude plant extracts often outperform isolated compounds due to complementary interactions among their diverse constituents. Consequently, standardized crude extracts or minimally processed fractions represent promising candidates for therapeutic applications (Ait Atmane et al., 2025).

In the realm of antimicrobial activity, the results extend the potential applications of this plant beyond antioxidants. The notable efficacy of the dichloromethane fraction against *C. albicans* and the broad-spectrum activity of the polar extracts against challenging Gram-negative pathogens such as *E. coli* and *P. aeruginosa* are highly relevant. The significant inhibition of key food spoilage organisms, including spore-forming *B. cereus*, directly highlights the extract's potential as a natural, plant-based preservative to enhance food safety and shelf-life. However, the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values is a crucial next step to quantitatively benchmark this activity and facilitate the development of applied formulations.

Collectively, these findings do more than just validate the traditional use of R. *alaternus*; they elevate its status to that of a promising, multifunctional natural resource. The convergence of exceptional antioxidant power linked to a unique phytochemical profile with significant antimicrobial activity opens dual avenues for its application in nutraceuticals aimed at oxidative stress mitigation and in natural food preservation strategies.

To better position these findings within the current landscape of phytochemical research, it is essential to compare the Algerian R. *alaternus* chemotype with other recently reported bioactive plant extracts (Nekkaa et al., 2021a; Devarajan et al., 2026; Toul and Djendar, 2023)

Comparative analysis: Rhamnus alaternus in the context of recent phytochemical research

To contextualize the exceptional bioactivity of our findings within the contemporary landscape of natural product research, we compiled a comprehensive comparison of recently published studies investigating plant extracts with similar antioxidant and antimicrobial activities. The following Table 3 presents a curated selection of state-of-the-art phytochemical studies employing comparable extraction and assessment methodologies.

Table 3 demonstrates that the methanolic extract from Algerian *R. alaternus* ranks among the top performers in recent studies, exhibiting significant antioxidant capacity (DPPH IC_{50} = 3.50 µg/mL) and high phenolic content (220.0 mg GAE/g).

While *Punica granatum* peel extract shows comparable antioxidant power (IC_{50} = 3.80 µg/mL), the *R. alaternus* extract possesses superior phenolic levels. This profile—obtained through sequential Soxhlet

extraction with solvents of varying polarity—supports the hypothesis that strategic solvent selection can elucidate latent bioactivity in Mediterranean flora. Combined with its broad antimicrobial effects (see Table 2), Algerian *R. alaternus* demonstrates high potential for application in natural preservatives and the nutraceutical market.

Table 3. Comparative phytochemical analysis of plant extracts: DPPH antioxidant potency and phenolic content

Plant Species	Extract Type	TPC (mg GAE/g)	DPPH IC_{50} (μg/mL)	Primary Application	Ref.
***Rhamnus alaternus* L.**	**Methanolic**	**220.0±0.34**	**3.50 ± 0.10**	**Antioxidant/ Antimicrobial**	**Present study**
Rhamnus alaternus L.	Methanolic	13.8 ± 0.71	10.5 ± 0.8	Food preservation	(Ammar et al., 2009; Moussi et al., 2015; Chaouche et al., 2020; Nekkaa et al., 2021b; Chatti et al., 2022b; Kherbachı et al., 2022; Aichour et al., 2023; Bouhazama et al., 2024)
Rhamnus purpurea	Methanolic	95.3 ± 2.1	7.2 ± 0.5	Antimicrobial/ Antioxidant	(Khuda et al., 2022)
Crataegus aronia	Ethanolic	180.5 ± 3.2	4.1 ± 0.2	Cardiovascular protection	(Ljubuncic et al., 2005; Bahri-Sahloul et al., 2014; Omairi et al., 2020; Al Mobideen et al., 2022)
Origanum vulgare L.	Hydroethanolic	156.2 ± 2.8	5.8 ± 0.3	Food additive/ Nutraceutical	(Zhang et al., 2014; Koldaş et al., 2015; Oniga et al., 2018; Moghrovyan et al., 2019; Pandey et al., 2019; Lombrea et al., 2020; Simirgiotis et al., 2020; Parra et al., 2021; Bora et al., 2022; Khorsand et al., 2022; Benkaddour et al., 2025; Mrabet et al., 2025; Nurzyńska-Wierdak and Walasek-Janusz, 2025; Wahab et al., 2025; Zakarya et al., 2025)
Sambucus nigra L.	Methanolic	142.0 ± 1.9	6.5 ± 0.4	Antioxidant/ Immunomodulation	(Viapiana and Wesolowski, 2017; Domínguez et al., 2021; Ferreira-Santos et al., 2021; Ferreira et al., 2022; Liu et al., 2022; Haş et al., 2023; Milkova-Tomova et al., 2023; Floares (Oarga) et al., 2025; Vardapetyan et al., 2025)
Rosmarinus officinalis L.	Ethanolic	128.7 ± 2.1	8.3 ± 0.6	Food preservation/ Antioxidant	(Kasparavičienė et al., 2013; Nieto et al., 2018; Amaral et al., 2019; de Oliveira et al., 2019; de Macedo et al., 2020; Yu et al., 2021; Bejenaru et al., 2024; Boubker et al., 2025)
Hypericum perforatum L.	Aqueous	89.5 ± 1.5	9.4 ± 0.7	Anti-inflammatory/ Antioxidant	(Silva et al., 2005; Öztürk et al., 2009; Barnes et al., 2019; Makarova et al., 2021; Nazari et al., 2022; Brankiewicz et al., 2023; Kakouri et al., 2023)
Punica granatum L.	Methanolic	198.3 ± 3.1	3.8 ± 0.2	Antioxidant/ Polyphenol source	(Bekir et al., 2013a; Bekir et al., 2013b; Belkacem et al., 2014; Peršurić et al., 2020; Yassin et al., 2021; Yu et al., 2021; Leesombun et al., 2022; Maphetu et al., 2022; Sweidan et al., 2023; Zhao et al., 2024; Bourroubey et al., 2025; Duysak et al., 2025)
Thymus serpyllum L.	Ethanolic	112.4 ± 1.8	6.9 ± 0.5	Antimicrobial/Food additive	(Mihailovic-Stanojevic et al., 2013; Jarić et al., 2015; Vergun et al., 2022; Jalil et al., 2024; Al-Mijalli et al., 2025)

TPC: expressed as mg Gallic Acid Equivalents per gram dry extract, DPPH IC_{50}, half-maximal inhibitory concentration against DPPH radical (lower values indicate greater antioxidant potency), Values presented as mean ± standard deviation where available, Extraction methods and assay conditions standardized to allow meaningful comparison, Bold highlighting indicates the present study's findings, demonstrating superior performance relative to contemporary alternatives.

The methanolic extract's remarkable antioxidant strength (IC_{50} = 3.50 µg/mL), which exceeds that of pure quercetin (4.80 µg/mL) and ascorbic acid (5.10 µg/mL), is attributed to synergistic interactions among its phytochemicals. Quercetin and ascorbic acid are the major contributors, but the mixture also includes various phenolic acids, other flavonoids, and anthraquinones that neutralize free radicals through different mechanisms such as hydrogen transfer, electron donation, and metal chelation, providing broader and more effective protection than any single compound alone. In addition, ascorbic acid likely regenerates oxidized quercetin in a recycling loop that is absent in pure standards. This kind of synergistic redox system is a big reason why whole plant extracts often outperform isolated ingredients, opening doors for powerful natural antioxidant products.

Conclusion

The results of this study demonstrate that *Rhamnus alaternus* L. from the Oran region of Algeria contains significant bioactive compounds. The methanolic extract exhibits potent antioxidant effects, primarily attributed to polyphenols such as quercetin and ascorbic acid. In contrast, the dichloromethane extract demonstrates high antimicrobial efficacy, particularly against *Candida albicans* and key foodborne pathogens. These results support the plant's longstanding role in Algerian traditional medicine and point to real-world potential for food preservation and natural supplements. They also demonstrate how selecting the appropriate solvent optimizes the extraction of compounds from polar versus non-polar fractions. Future research will focus on the isolation of the primary bioactive constituents through bioactivity-guided fractionation, alongside the precise determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. Furthermore, the efficacy of these extracts will be evaluated in complex food matrices, such as oils or meat products. These investigations are essential to establish the feasibility of practical applications within the food industry.

Author Contributions: AH: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation. SB: Validation, Data Curation, Writing – Review & Editing, Visualization. NH: Investigation, Methodology, Formal Analysis. NK: Conceptualization, Resources, Supervision, Project Administration, Writing – Review & Editing.

Funding: None.

Acknowledgements: The authors would like to thank the Ministry of Higher Education and Scientific Research of Algeria for support.

***Conflicts of Interest*:** The authors declare no conflict of interest.

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