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Composition, antioxidant and antibacterial properties of *Rosmarinus officinalis L.* essential oils from Mostaganem, Algeria: Regional valorisation perspective

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Abstract

The objective of this study is to characterize the chemical profile and evaluate the antibacterial and antioxidant properties of *Rosmarinus officinalis L.* essential oil (REO) from the Mostaganem region, Algeria. The EO was extracted from leaves and flowers by hydro distillation, yielding 0.62%. Physicochemical analyses revealed an acid index of 2.24 and an ester index of 325.10, confirming compliance with AFNOR quality standards.GC-MS allowed the identification of 15 major constituents (92.56% of total oil), notably 1,8-cineole, camphor, and α-pinene. Antibacterial activity was assessed against Staphylococcus strains using agar-diffusion assays, with inhibition zones reaching 21 mm. Antioxidant capacity, measured by the DPPH radical-scavenging test, demonstrated moderate activity (64%). Special emphasis is placed on the regional agro-economic relevance of REO, illustrating its potential to diversify agricultural production under challenging climates. The results further address practical applications in agri-food sectors including its use as a natural preservative, aromatic ingredient, and functional additive. Standardized protocols ensure methodological reproducibility and facilitate transferability to other agricultural contexts.

Keywords: *Rosmarinus officinalis L*, Essential oils, Antibacterial activity, Antioxidant activity, Algerian, Sustainable agriculture

Introduction

Ever since ancient times, plants have served as foundational resources for remedy development, providing essential bioactive compounds that underpin pharmaceutical innovation and traditional therapeutic practices worldwide. The World Health Organization **(WHO 2023)**, estimates that a substantial proportion of the global population relies on traditional medicine, with over 35,000 plant species utilized for medicinal purposes, and 70% of modern pharmaceutical raw materials derived from botanical sources **(Abada et al.2025, Vaishnavi et al.2025).**

Algeria hosts remarkable botanical diversity due to its varying climates, soils, and geographic features, with wild medicinal flora comprising over 3,000 species, about 15% of which are endemic **(Karouche et al.2021)**. Despite this abundance, Algerian medicinal plants remain underexplored phytochemically and pharmacologically **(Lamera et al. 2025).** The Mostaganem region exemplifies this richness, notably with rosemary (*Rosmarinus officinalis L.*), which is traditionally employed for mild analgesic and anti-inflammatory applications (**Bekhechi et al. 2024).**

Recent pharmacological research confirms that rosemary exhibits a broad spectrum of bioactivities, antimicrobial, anti-inflammatory, antioxidant, antitumorigenic, antinociceptive, and neuroprotective, benefiting mood, cognition, pain, and anxiety **(Alaboudi et al.2025).** Its essential oil, rich in monoterpenes and major compounds like 1,8-cineole, α-pinene, camphor, and trans-caryophyllene, demonstrates potent antibacterial action against pathogenic strains **(Soliman et al.2024, Kumar et al. 2025).**

Plant extracts, especially methanolic, ethanolic, and aqueous, offer remarkable chemical diversity and are valued for their antioxidant, antimicrobial, and anti-inflammatory effects **(Singh et al.2024)**. The biological efficacy of essential oils is closely associated with their chemical composition, specifically the presence of major functional groups (phenols, alcohols, aldehydes, terpenes, ketones) and the overall phytochemical content **(Ben Miri et al. 2025).** Variations in chemical profiles and bioactivity are influenced by geographical origin, harvest timing, and extraction protocols (**Zejli et al.2023)**.

Essential oils, volatile secondary metabolites synthesized by aromatic plants, are recognized for their characteristic scents and wide-ranging biological activities **(Achagar et al.2024).** Their natural origin and demonstrated health benefits have led to broad utilization in pharmaceuticals, food preservation, and the cosmetics industry **(Rodilla et al.2024).** Notably, essential oils are increasingly valued for their antimicrobial properties, with proven efficacy against multidrug-resistant bacteria **(Visan et al.2024, Sharma et al.2024).**

Plant-derived polyphenols are acknowledged for their multifunctional pharmacological attributes, including antioxidant, antibacterial, anticancer, antihypertensive, and anti-inflammatory effects **(Gago et al.2025).** These compounds help counteract oxidative stress and mitigate age-related conditions such as cancer, cardiovascular, and neurodegenerative diseases **(Shankar et al.2024).**

Recent years have seen growing interest in the cultivation and utilization of REO, due to its high economic value, diverse pharmaceutical applications, and natural preservative capacity. The methods in this study are directly applicable for value enhancement of rosemary cultivated under northern conditions. The findings provide transferable insights on stress adaptation, chemical profiles under climatic constraints, and sustainable exploitation of medicinal plants in high-latitude environments.

The present study provides the first comprehensive analysis of the phytochemical composition and bioactive properties of REO from Mostaganem, Algeria, a region with distinctive environmental conditions. By linking chemical and biological characteristics to local climatic and soil factors, this research clarifies the impact of geographic origin on rosemary's therapeutic potential. These findings deliver actionable insights for the sustainable valorisation of Mediterranean flora and support the development of resilient crops for agricultural systems facing climate-related constraints.

Materials and methods

Study Area and Plant Material

The plant material was collected in March 2024 from the Mostaganem region (35°56′ N, 0°05′ E), a Mediterranean coastal area in northwestern Algeria.The sampling location was chosen to assess the effect of regional climatic and edaphic factors on the biological activity of the studied essential oil.

Only healthy aerial parts were selected and carefully air-dried at 25°C (Fig. 1). Once dried, the leaves were manually separated and ground into a fine powder and stored in paper bags under controlled conditions until analysis.

Figure 1. Fresh leaves of *Rosmarinus officinalis L.*

Hydrodistillation using a Clevenger apparatus

Essential oils were extracted by hydrodistillation of 100 g dry plant material using a Clevenger apparatus for 3 hours, in accordance with the optimised protocol described by **Fadil et al. (2015)**. The essential oil was carefully collected and dried over anhydrous sodium sulphate to remove residual moisture. The purified oil was stored in sealed amber vials at 4°C to preserve its chemical integrity until further analysis. Each experiment was performed in duplicate to ensure reproducibility.

Yield of Essential Oils

The yield of the REO was calculated as the mass of the extracted oil (g) relative to 100 g of dry plant material in accordance with the following equation **(Ban et al. 2016)**:

$$\text{Yield (\%)} = \frac{\text{Dry plant material mass (g)}}{\text{Amount of extracted oil (g)}} \times 100$$

Phytochemical characterisation of rosemary essential Oil

Chemical composition was determined by GC-MS using dual-capillary columns with helium as carrier gas. The temperature profile was set from 50°C to 200°C. Identification and quantification of essential oil constituents were based on retention indices and mass spectra. Phenolic and flavonoid contents were quantified by HPLC-MS, using a C18 column and gradient elution, targeting key metabolites (carnosic acid, carnosol, rosmarinic acid, luteolin). Structural confirmation relied on MS/MS data.

Analyses of antimicrobial activity

Antibacterial efficacy was assessed by disc-diffusion on Mueller–Hinton agar against reference strains (Staphylococcus aureus, Pseudomonas aeruginosa),in accordance with the protocol described by **Gajic et al. (2022).**Paper discs were impregnated with 10 µL of essential oil dissolved in 10% (v/v) dimethyl sulfoxide (DMSO). Inhibition zones were measured after 24 h incubation at 37°C. All assays were performed in duplicate. Results were expressed as the mean diameter of inhibition zones and categorized according to strain sensitivity as outlined in Table 1. Positive and negative controls (standard antibiotics and DMSO alone, respectively) were included to validate the assay.

Table1. Sensitivity of microbial strains according to inhibition zone diameters (Boutabia et al. 2016)

Sensitivity	Inhibition Zone Diameter
Not sensitive or resistant (-)	diametre < 8mm
Sensitive (+)	diameter between 9 and 14 mm
Very sensitive (++)	diameter between 15 à 19 mm
Extremely sensitive (+++)	diametre > 20 mm

Analysis of antioxidant activity

The antioxidant activity was measured using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH°), in accordance with the protocol described by **El Maimouni et al. (2024)**. Serial dilutions of the essential oil and ascorbic acid standard were tested. Absorbance at 517 nm was measured after 30 minutes in the dark. The free radical-scavenging activity was expressed as the percentage of DPPH° reduction. The percentage of reduction (PR%) was calculated using the following formula: $PR = (AC - AE) / AC \times 100$

where

AE: Absorbance of the DPPH° solution in the presence of the essential oil or ascorbic acid

AC: Absorbance of the DPPH° solution in the absence of the essential oil and ascorbic acid

The variation in the reduction power as a function of the concentration of the essential oil and ascorbic acid also allowed the calculation of the EC_{50} parameter, were determined by linear regression from three separate trials.

Data Analysis

All experiments were performed in triplicate. Data, expressed as mean ± were analyzed using ANOVA ($p < 0.05$) with post hoc Tukey HSD for group comparisons.

Results and discussion

Yield Extraction of Rosemary essential oil

The essential oil yield from the aerial parts of Rosemary using the Clevenger hydrodistillation method in this study was 0.62%, consistent with the findings of previous reports. **Elyemni et al. (2022)** reported a similar yield of 0.54% in rosemary samples collected in Morocco, while **Mwithiga 2022** observed yields ranging from 0.45% to 0.59%. Additionally, **Teshale et al. (2022)** documented a yield of 0.54%, reinforcing the consistency of these extraction values across different locations and conditions.

Variations in the essential oil yield from rosemary can be attributed to numerous extrinsic and intrinsic factors. According to our study, geographical origin, drying methods, extraction techniques, and distillation duration have shown significant influence on REO yield and chemical profile, as noted by do **Nascimento et al. (2020)**, Chen **et al. (2021)** and **Hendelet al. (2024)**. Genetic traits, climatic variability, and plant variety further contribute to the observed variabilities in yields.

Chemical composition of Rosemary essential oil

The chemical composition of REO by hydrodistillation using the Clevenger apparatus and analysed by GC/MS is summarised in Table 2 and Figure 2. Fifteen compounds accounting for 99.80% of the total oil were identified. The predominant constituent was 1,8-cineole (31.20%), followed by camphor (16.54%) and α-pinene (15.82%).

Table 2. List of volatile compounds in the REO. Their % content is presented along with the experimental and literature retention indices. Results are presented as mean ± standard deviation from triplicate analysis.

No	Compounds	Kovat's index **Adams et al. (2017)**	CH (%)
	Monoterpene hydrocarbons		37.19 ± 0.65
1	α-Pinene	939	15.82± 0.49
2	Camphene	954	9.77± 0.12
3	β-Pinene	979	3.56± 0.00
4	α-Terpinene	1017	2.44 ± 0.03
5	para-Cymene	1025	4.79 ± 0.03
6	Limonene	1028	0.81± 0.00
	Oxygenated monoterpenes		61.76± 0.63
7	Cineole	1030	31.2± 0.65
8	β -myrcene	1048	3.75± 0.05
9	Linalool	1097	1.49± 0.05
10	Camphor	1146	16.54 ± 0.49
11	Borneol	1169	1.47± 0.02
12	α-Terpineol	1199	7.16 ± 0.19
13	Verbenone	1205	0.15± 0.00
	Sesquiterpene hydrocarbons		0.11± 0.00
14	β-Caryophyllene	1419	0.08± 0.00
15	α-Caryophyllene	1423	0.03 ± 0.00
	Total oxygenated compounds		62.5± 2.50
	Total nonoxygenated compounds		37.3± 0.65

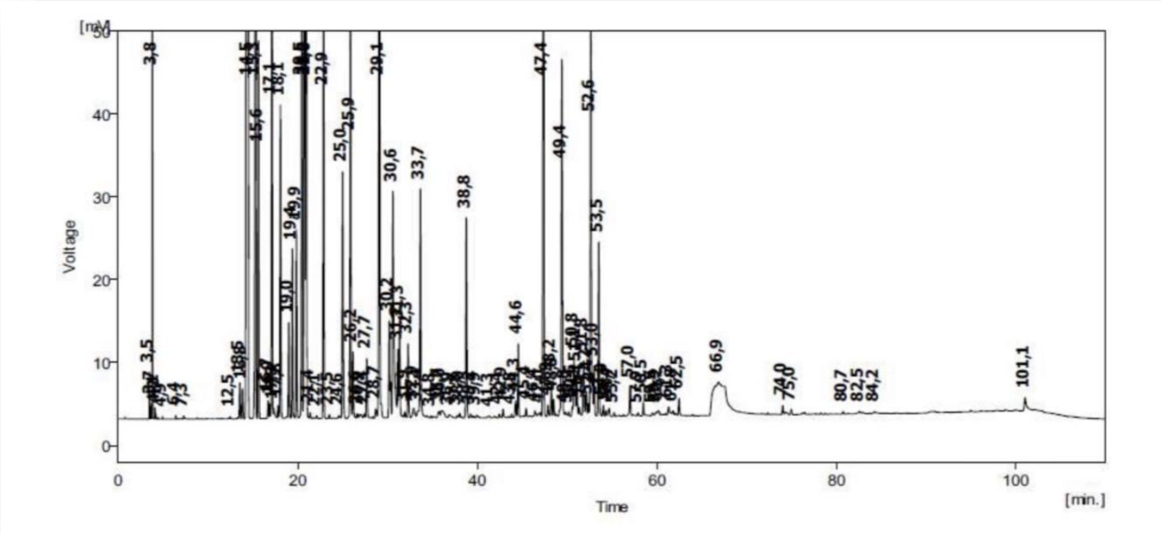

Figure 2. GC-MS of of Rosemary Essential Oil.

A detailed analysis revealed a significantly higher proportion of oxygenated monoterpenes relative to monoterpene hydrocarbons. This predominance of oxygenated compounds is likely attributable to the low water content of the extraction system and the rapid heating rate during hydrodistillation, factors that reduced thermal and hydrolytic degradation **(Athanasiadis et al. 2024, Mahboub et al. 2025)**. Oxygenated compounds, which possess higher dipole moments, are more readily extracted in comparison with monoterpene hydrocarbons with weaker dipole moments.

The chemical profile observed in this study is consistent with previously reported findings emphasising geographic variability in REO composition. For example, REO harvested in the Bordj-Bou-Arreridj region of Algeria exhibited a higher concentration of 1,8-cineole (52.4%) and camphor (12.6%) **(Annemer et al. 2022)**. Thus, the geographic and environmental influences identified in our study align with the findings of prior reports highlighting the influence of genetic and bioclimatic factors on the phytochemical makeup of R. officinalis essential oils **(Al-Maharik et al. 2022, Barut et al. 2022, Liu et al. 2024)**.

Results for Antibacterial Activity

REO collected in the Mostaganem region exhibited potent antibacterial activity against both Staphylococcus aureus and Pseudomonas aeruginosa (Table 3). The pure oil produced inhibition zones of 27 mm for S. aureus and 15 mm for P. aeruginosa, indicating substantially greater inhibition of the Gram-positive strain. This pattern of differential susceptibility aligns with previous findings demonstrating that REO tend to elicit stronger activity toward Gram-positive bacteria than Gram-negative species **(Mouas et al. 2017)**. In their work, Mouas et al.

observed inhibition zones of 22.75-23.75 mm (Blida) and 14.25-16.75 mm (Djelfa) for S. aureus and P. aeruginosa, respectively.

Table 3. Antibacterial effect of REO against S. aureus and P. aeruginosa.

(/)

	Diameter of inhibition zones (mm)	
Essential oil concentration (%)	S. aureuse	P .aeruginosa
100	27	15
75	22	9
50	15	/
25	9	/

Absence of inhibition zone

Further supporting these observations, **Fenghour et al. (2023)** reported that Algerian rosemary oil yielded inhibition zones ranging from 8.5 to 12.33 mm for S. aureus and up to 14.66 mm for Klebsiella pneumoniae, with the inhibition diameters for oils obtained from eastern Algeria rarely exceeding 20 mm even for the undiluted oil. **Saleh et al. (2022)** documented inhibition zones of 15-19 mm against Bacillus species and 7-14 mm against other strains, confirming the relatively greater sensitivity of Gram-positive bacteria. **Husein et al. (2025)** also noted inhibition zones from 10 mm (for E. faecalis) to 16 mm (for E. coli), emphasising inter-strain variability and the moderate antibacterial potency of rosemary extracts.

Phytochemical characterisation of Rosemary essential oil

The phytochemical profile of REO was characterised by HPLC–MS analysis, which enabled the identification and quantification of key phenolic acids and flavonoids. Carnosic acid was the predominant compound, eluting at 18.2 min with a deprotonated molecular ion $[M-H]^-$ at m/z 331.2, and its concentration was 25.2 ± 2.3 mg mL^{-1}. It was followed by carnosol, which was detected at 19.5 min (m/z 329.2) with concentration of 6.2 ± 0.7 mg mL^{-1}, and rosmarinic acid, which eluted at 16.4 min (m/z 359.1) with concentration of 3.5 ± 0.5 mg mL^{-1} (Fig. 3).

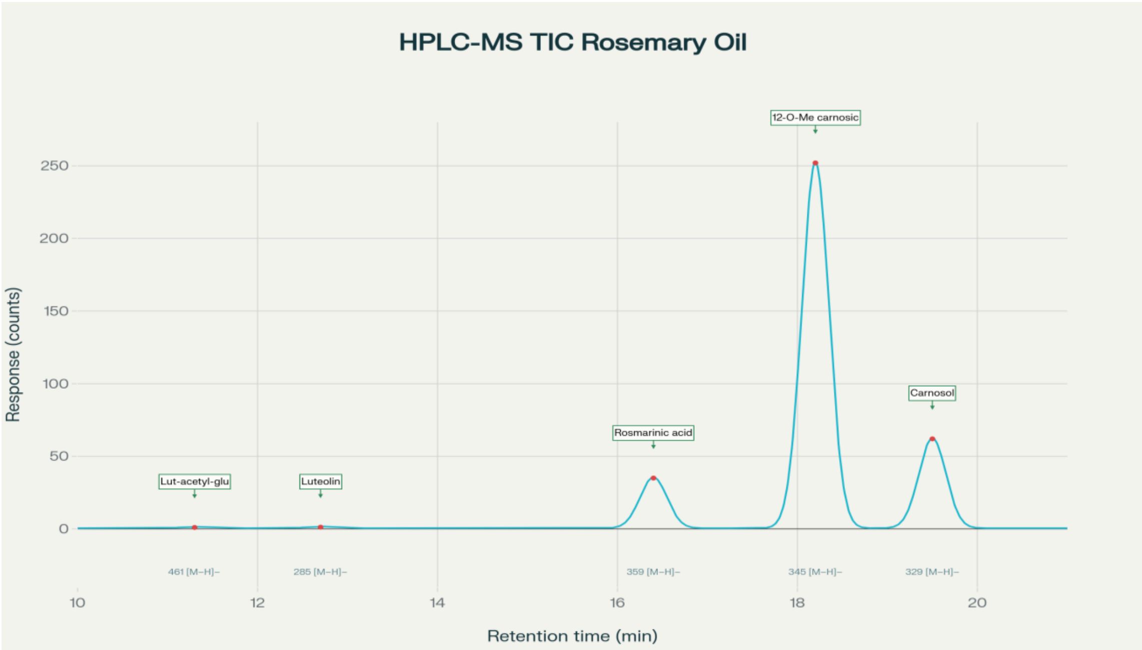

Figure 3. Total ion chromatogram for HPLC–MS analysis in negative ionization mode of REO

Flavonoids were present at the lower levels: luteolin showed a retention time of 12.7 min with m/z 285.0 and concentration of 0.12 ± 0.03 mg mL^{-1}, while luteolin-7-O-glucoside showed a retention time of 11.3 min with m/z 447.1 and concentration of 0.10 ± 0.01 mg mL^{-1} (Fig. 4 ; Table 4). The total phenolic content was 35.66 mg mL^{-1}, and the total flavonoid content was 1.15 mg mL^{-1}. Flavonoids, including luteolin and its glycosides, were identified as minor components.

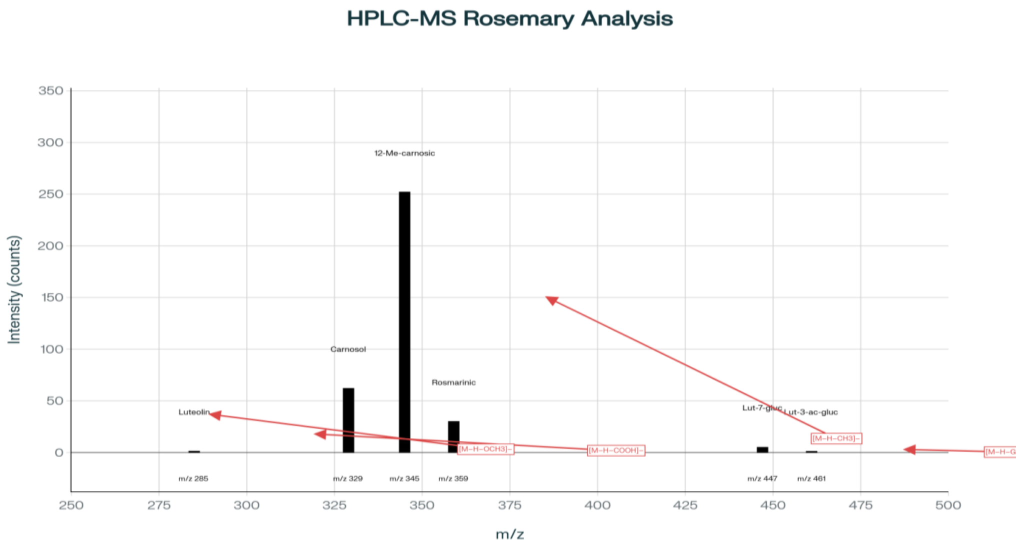

Figure 4. MS/MS Fragmentation Spectra of Key Phenolic Compounds

Table4. HPLC–MS identification and quantification of metabolites of the REO. Concentration is presented as mean ± standard deviation derived from triplicate analysis.

Compound	Retention Time (min)	Negative Ionization (m/z)	Positive Ionization (m/z)	Concentration (mg/mL)	Literature range (mg/mL) (Mena et al.2016)
Luteolin -3-O-acetyl-O-glucuronide	11.3	461 $[M - H]^-$ 923 $[2M - H]^-$ 285 $[\text{luteolin} - H]^-$	463 $[M + H]^+$	0.10 ± 0.01	0.01 – 0.15
Luteolin	12.7	285 $[M - H]^-$ 571 $[2M - H]^-$	287 $[M + H]^+$	0.12 ± 0.03	0.05 – 0.35
Rosmarinic acid st	16.4	359 $[M - H]^-$ 719 $[2M - H]^-$	383 $[M + Na]^+$ 743 $[2M + Na]^+$	3.5 ± 0.5	1.2 – 8.4
12-O-Methyl carnosic acid	18.2	345 [M − H]−	369 $[M + Na]^+$ 715 $[2M + Na]^+$	25.2 ± 2.31	9.5 – 32.6
Carnosol	19.5	329 $[M - H]^-$ 375 $[M + FA - H]^-$	331 $[M + H]^+$ 353 $[M + Na]^+$ 683 $[2M + Na]^+$	6.2± 0.7	1.8 – 9.0

The predominance of carnosic acid, carnosol, and rosmarinic acid observed in this study is in agreement with previously reported phenolic profiles of Rosmarinus officinalis essential oil analysed by advanced mass spectrometric techniques **(Ayyildiz et al.2024,Udaypal et al.2024)**.Comparative metabolomic studies similarly identified these diterpenoids as dominant constituents, with minor flavonoids such as luteolin and its glycosides contributing to phytochemical diversity and bioactivity **(Aamer et al.2023, Bencharif-Betina et al.2023)**. Solvent selection has been shown to substantially influence phenolic and flavonoid extraction efficiencies and antioxidant activities, highlighting the importance of methodological considerations in quantitative assessments **(De-Montijo-Prieto et al., 2021, Pogorzelska-Nowicka et al. 2024)**. The variability in phenolic and flavonoid levels among rosemary samples is consistent with the influences of genotype, environmental conditions, seasonality, and extraction protocols reported in the literature **(Mohammed et al.2024,Bakrim et**

al.2025). The high levels of carnosic acid and rosmarinic acid measured here align with the findings of previous reports connecting these compounds to antioxidant capacity and potential nutraceutical benefits of REO.

Results of Antioxidant activity analyses

The antioxidant potential of REO has been extensively investigated; however, the existing studies have yielded results with considerable variability. These variations are primarily attributed to differences in the geographical origin of plant material, extraction methods, and the analytical assays employed to evaluate antioxidant capacity.

In the present study, the antioxidant activity of REO demonstrated a strong concentration-dependent effect. Increasing the concentration of the essential oil samples proportionally enhanced the free radical-scavenging activity, resulting in higher overall antioxidant activity, as depicted in Figure 6. Specifically, the DPPH radical-scavenging assay revealed that at the lowest tested concentration (5 mg mL^{-1}), REO achieved approximately 42% inhibition, emphasising significant radical-neutralising capacity even at low doses.

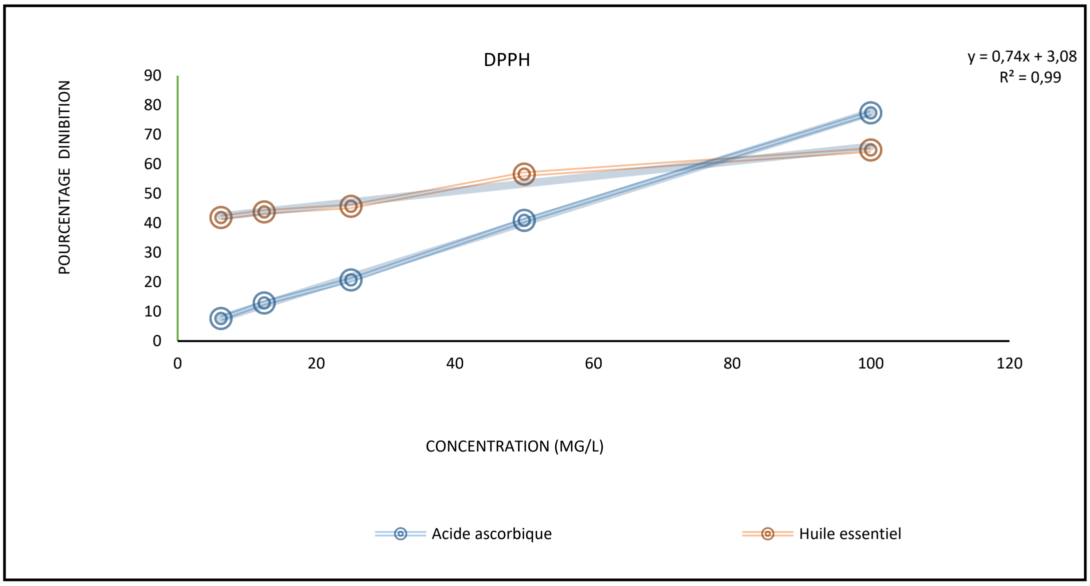

Figure 5. DPPH radical scavenging activity of REO

This significant antioxidant efficacy is largely attributable to the phenolic constituents within REO. Structure-activity relationship studies have established that antioxidant capacity correlates with both the quantity and positional arrangement of hydroxyl groups on the flavonoid nucleus, which facilitate free radical stabilisation. **Shen et al. (2022)** highlighted this relationship, and **Al-jaafreh 2024** further corroborated that the robust antioxidant activity of REO derives from its rich polyphenolic profile.

Optimisation of extraction protocols for polyphenols, flavonoids, and other antioxidant molecules is critically dependent on the solvent system utilised and can be further influenced by the specific plant organs from which the extracts are derived. Variabilities in radical-scavenging activity among different REO samples has also been linked to quantitative differences in their principal chemical constituents **(Rafya et al.2024).** Among monoterpene components, 1,8-cineole has been identified to be particularly more effective than other monoterpenes in reducing reactive oxygen species **(Afonso et al.2021, Kabubii et al.2024)**. Moreover, the terpenes in REO exhibit antioxidant capabilities comparable to phenolic mixtures **(Belbachir et al.2025)**. In summary, the antioxidant properties of REO could be attributed to synergistic interactions among its diverse chemical constituents, including both major and minor compounds. This complex chemical interplay fundamentally governs the oil's overall efficacy as a potent natural antioxidant.

Conclusions

The valorization of Rosmarinus officinalis essential oil holds notable potential not only for Mediterranean but also for regional agricultural systems. Our results demonstrate the high quality and pronounced bioactivity of the essential oil sourced from the Mostaganem region, highlighting its suitability as a candidate for crop diversification in marginal, or climate-challenged environments. By introducing R. officinalis into agroecosystems, producers could benefit from an expanded range of value-added crops, thereby reinforcing local economic resilience and adapting to evolving climate limitations. Furthermore, the integration of this bioresource into agri-food chains as a natural preservative or functional ingredient aligns with market trends and sustainability goals. The standardized methodology adopted here enables reliable transfer and reproducibility of results in different geographic contexts. Future research should address field validation in northern agricultural settings and evaluate the broader socioeconomic impact of such diversification strategies.

Declaration of competing interests

The authors declare that they have no conflicts of interest in relation to the current study.

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