Phytochemical Profiling, Biological activities, and in Silico Molecular Docking of Mentha pulegium L. Essential Oil from Northwestern Algeria

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Abstract

Mentha pulegium L., an aromatic plant widely distributed in North Africa, was investigated for its essential oil composition, antioxidant and antimicrobial activities, and molecular interactions. The essential oil, extracted by hydrodistillation from aerial parts collected in Northwestern Algeria, yielded 0.72% (w/w) and was analyzed by GC-MS (Gas Chromatography-Mass Spectrometry). Pulegone (55.03%) and isomenthone (16.85%) were identified as the major constituents, along with limonene (3.13%) and menthol (2.11%). The oil exhibited potent antioxidant activity with an IC_{50} of 22.17 µg/mL in the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, comparable to ascorbic acid. Antibacterial evaluation against six pathogenic strains showed the highest susceptibility for Bacillus subtilis (14.33 ± 0.58 mm), Bacillus cereus (13.67 ± 1.16 mm), Staphylococcus aureus (10.00 ± 0.00 mm) and Escherichia coli (10.33 ± 1.53 mm); while Pseudomonas aeruginosa and Salmonella typhi were resistant. MIC values ranged from 0.62 to 1.25 µL/mL, with bactericidal activity (MBC/MIC=1) observed against Staphylococcus aureus and Escherichia coli. Molecular docking against bacterial DNA gyrase revealed that cis- and trans-isopulegone exhibited the strongest binding affinity (ΔG = -6.2 kcal/mol), supporting the experimental antimicrobial data. These findings highlight M. pulegium essential oil from northwestern Algeria as a promising natural source of bioactive compounds for food preservation and pharmaceutical applications, with molecular docking providing mechanistic insights into its antibacterial potential.

Keywords: Mentha pulegium L., essential oil, GC-MS, biological activities, molecular docking.

1. Introduction

Aromatic herbs have long been used in food for their organoleptic properties and their ability to enhance flavor. Beyond their culinary applications, these plants have attracted increasing scientific interest because of their potential health benefits and their richness in bioactive compounds. They can be used fresh, dried, powdered, or in the form of extracts, essential oils, and purified metabolites (Grigore-Gurgu et al., 2025; Latif and Nawaz, 2026).

Medicinal and aromatic plants are important sources of secondary metabolites, including polyphenols, flavonoids, and terpenoids, which exhibit various biological activities. These compounds contribute to the antioxidant and antimicrobial properties of plant extracts and essential oils, increasing their potential applications in the pharmaceutical, biomedical, and food sectors. Consequently, growing attention has been directed toward plant-derived products as potential natural alternatives to synthetic chemicals and conventional preservatives (Boubker et al., 2025).

Mentha pulegium L., commonly known as pennyroyal, is an aromatic species belonging to the Lamiaceae family and the genus Mentha. It is widely distributed in Europe, North Africa, Asia Minor, and the Middle East (Mohammadi et al., 2024). Its essential oil is characterized by the presence of bioactive compounds, particularly pulegone, menthone, and isomenthone, which are associated with antioxidant, antimicrobial, and other biological activities (Lopez et al., 2005; Mahboubi and Haghi, 2008). Traditionally used in herbal medicine, M. pulegium has also gained increasing interest as a potential natural preservative and antimicrobial agent for pharmaceutical and food applications (Bouazza et al., 2022; El Omari et al., 2025; Amara et al., 2025; Souiy et al., 2026).

In Algeria, several studies have investigated M. pulegium in different regions; however, information regarding its distribution

and biological properties in western Algeria remains limited. Therefore, the present study aims to catalogue Mentha species in the daïra of Achaacha (Mostaganem, Algeria) and to investigate the biological potential of M. pulegium. Specifically, the study focuses on characterizing the chemical composition of the essential oil obtained from M. pulegium collected in the Mostaganem region and evaluating its antioxidant and antimicrobial activities.

The significance of this work lies in the valorization of a local aromatic plant rich in bioactive compounds and in the exploration of its potential as a natural alternative to synthetic additives. Such an approach may contribute to improving the oxidative stability and microbiological quality of foods while supporting the sustainable use of plant-based resources. More specifically, the novelty of this study lies in the integrated evaluation of the essential oil obtained from local Mentha pulegium L. populations from Mostaganem, combining its chemical and biological characterization, molecular docking analysis to investigate potential interactions between its major bioactive compounds and relevant molecular targets, and the assessment of its potential application as a natural preservative in a food matrix. This comprehensive approach provides new insights beyond previous studies mainly focused on the plant's chemical composition and in vitro biological activities.

2. Materials and Methods

2. 1 Plant Material

The aerial parts of Mentha pulegium L. were collected in March 2025 from the Achaâcha region, located in the eastern part of Mostaganem Province, Northwestern Algeria (Fig. 1). The study area is situated at approximately 36°14′47″ N and 0°38′03″ E. The botanical identification of the sample was performed by Dr. Mostari Abassia, botanist and professor at Abdelhamid Ibn Badis University of Mostaganem, Algeria.

Fig. 1. Map of the Mostaganem region.

Location where the plant material was collected (the Achaâcha region in Northwestern Algeria, Mostaganem province).

2.2 Preparation of the plant

After harvesting, the plant material was washed with distilled water, then dried in the shade at room temperature in a well-ventilated area for one week (Fig.2). The dried samples were then stored in airtight containers, protected from light and heat, to preserve their physicochemical and biological properties.

Fig. 2. Fresh and dried aerial parts of the plant Mentha pulegium L. (Original photo).

2.3 Extraction of Essential Oil

2.3.1 Steam Distillation

According to Boukhatem et al. (2019), the essential oil of Mentha pulegium L. was extracted by steam distillation (Fig. 3). This method involves passing steam at approximately 100 °C through 2 kg of plant material, using 2.5 L of distilled water. The steam carries the volatile compounds, which are subsequently condensed, yielding an aqueous phase (hydrosol) and an organic phase corresponding to the essential oil. The absence of direct contact between the liquid water and plant material helps minimize the hydrolysis of volatile compounds, thereby preserving the chemical quality of the essential oil. After 2 h of distillation, the hydrosol and essential oil were collected and separated.

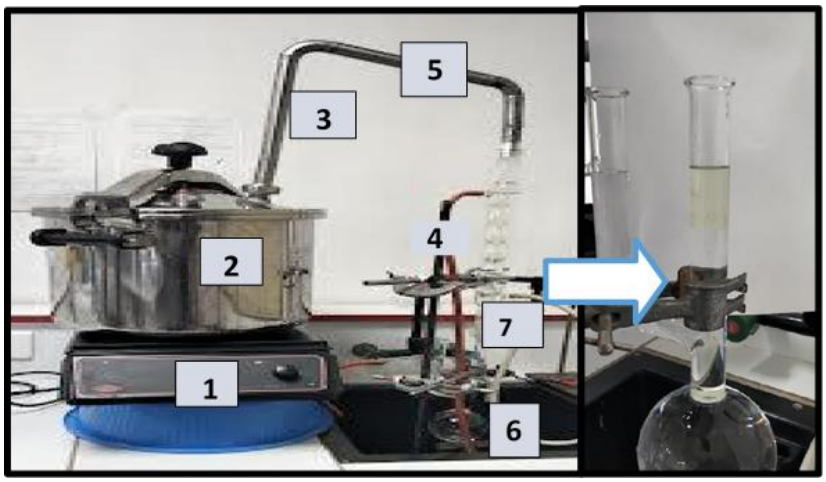


Fig. 3. Water-vapor entrainment distillation apparatus (Original photo).

1: Heating plate 2: Pressure cooker 3: Condenser 4: Water outlet 5: Coolant 6: Water inlet 7: Graduated tube

2.4 Determination of extraction yield

According to Souiy et al. (2023), the essential oil yield is the ratio of the mass of oil extracted to the weight of the plant material used. It is generally expressed as:

$$yield\ (\%) = \frac{M0}{Mp} \times 100$$

M0: masse of essential oil (g).
Mp: masse of plant material (g).

2.4.1 Organoleptic Analysis

The organoleptic properties of the essential oil were evaluated based on three criteria: appearance, color, and odor, in accordance with the method described by Afrokh et al. (2024).

2.4.2 Physicochemical analysis

2.4.2.1 Density

The relative density of the essential oil was determined at 20 °C using a 5 mL pycnometer, according to NM ISO 279:2008 and the method described by Inić et al. (2026). It was calculated as the ratio of the mass of the essential oil to that of an equal volume of distilled water.

$$D = M / M'.$$

D: the relative density.
M: the mass of the oil (g).
M': the mass of distilled water (g).

2.4.2.2 Hydrogen ion concentration (pH)

A defined quantity of essential oil was transferred into a clean, dry beaker. The pH of the sample was then measured using a pH meter previously calibrated with standard buffer solutions. This method allows for the determination of the acidity or alkalinity of the essential oil, according to the protocol described by Badache et al. (2026).

2.4.2.3 Solubility test

The miscibility of the essential oil was evaluated in ethanol and methanol at 20 °C, in accordance with the method described by Rached et al. (2025). Miscibility was determined by the formation of a clear solution following the gradual addition of the solvents to the essential oil.

2.5 Chemical Characterization and identification of Mentha pulegium L. essential oil by GC-MS

The essential oil was analyzed using a Shimadzu GC-2010 gas chromatograph connected to an MS-QP2010 SE mass spectrometer at the Organic and Macromolecular Chemistry Research Unit (URCOM), University of Le Havre, France.

The essential oils were first diluted 1:100 (v/v) in 96° ethanol. The compounds were separated using a ZB-5MS capillary column (5% phenyl, 95% dimethyl siloxane, 30 m × 0.25 mm, 0.25 µm film thickness). The carrier gas used was helium (He) at a flow rate of 1 mL/min. The injection volume was 1 µL of the ethanol-based essential oil, injected in split mode (split ratio 1/50). The column was initially set to 50 °C for 3 min, then gradually heated to 270 °C at a ramp rate of 5 °C/min, and subsequently held at that temperature for 15 min.

The mass spectrometer operated in electron impact mode with an ionization energy of 70 eV; analysis was performed within the 35–300 m/z scan range. The oil components were identified by co-injection with standards (where possible) and confirmed using the National Institute of Standards and Technology (NIST) V.2.0 GC-MS Library. The relative concentration of each compound in the essential oil was expressed as a percentage by normalizing the peak area (Babushok et al., 2011).

2.6 Antioxidant Activity

2.6.1 DPPH free radical scavenging activity

Antioxidant activity was assessed using the DPPH assay according to the method described by Brand-Williams et al. (1995). A volume of 750 µL of DPPH methanol solution was mixed with 250 µl of the sample at various concentrations, then incubated for 30 minutes in the dark at room temperature. Absorbance was measured at 517 nm. Methanol served as the negative control, while ascorbic acid was used as the positive control. The results were expressed as the percentage of inhibition (%).

$$I\% = (Negative\ control\ A - Sample\ A\ /\ Negative\ control\ A) \times 100$$

I%: percentage of antioxidant activity
A: Absorbance

The IC_{50} value, corresponding to the concentration required to inhibit 50% of DPPH radicals, was determined by linear regression between the sample concentration and the percentage of inhibition, according to Bencheikh (2017). All analyses were performed in triplicate.

2.7 Antibacterial activity

2.7.1 Microbiological materials

The antibacterial activity of the essential oil of Mentha pulegium L. was evaluated against six reference bacterial strains, including three Gram-positive bacteria (Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 10876, and Bacillus subtilis ATCC 11778) and three Gram-negative bacteria (Escherichia coli ATCC 25922, Salmonella typhi ATCC 14028, and Pseudomonas aeruginosa ATCC 27853). The strains, sourced from the American Type Culture Collection (ATCC), were provided and identified by the microbiology laboratory of the Faculty of Natural and Life Sciences at Abdelhamid Ibn Badis University in Mostaganem (Algeria).

2.7.2 Disk diffusion method (aromatogram)

The antibacterial activity of the essential oil was determined using the disk diffusion method (aromatogram), according to the protocol described by Vanegas et al. (2021). Bacterial inoculum was prepared from fresh cultures (18–24 h) by suspending an isolated colony in sterile saline (0.85% NaCl). The suspension was homogenized using a vortex mixer and then adjusted to a turbidity equivalent to the 0.5 McFarland standard (≈ 1×10^8 CFU/mL), corresponding to an optical density of 0.08–0.10 at 600 nm (Balouiri et al., 2016).

The bacterial suspensions were inoculated onto Mueller–Hinton agar plates. Sterile Whatman No. 1 paper discs (6 mm in diameter), The discs were impregnated with 5 µL of pure essential oil, were aseptically placed on the surface of the inoculated agar. A ciprofloxacin disc (5 µg/disc) was used as a positive control to compare the antibacterial activity of the essential oil with that of a reference antibiotic. After a 30-minute diffusion period at room temperature, the plates were incubated at 37 °C for 24 hours (Touré, 2015).

Antibacterial activity was assessed by measuring the diameter of the inhibition zones (DIZ), expressed in millimeters (mm). The susceptibility of the bacterial strains was interpreted according to the criteria of Djendi et al. (2023): resistant (IBD < 8 mm), susceptible (9–14 mm), highly susceptible (14–19 mm), and extremely susceptible (≥ 20 mm).

2.7.3 Determination of the (MIC)

The minimum inhibitory concentration (MIC) of the essential oil of Mentha pulegium L. was determined using the broth microdilution method on 96-well microplates, according to Vanegas et al. (2021). Successive dilutions of the essential oil, prepared from a 10% (v/v) stock solution in DMSO, were made in Mueller-Hinton broth and then inoculated with a standardized bacterial suspension (1.5×10^8 CFU/mL). After incubation at 37 °C for 24 h, bacterial growth was detected using 2,3,5-triphenyltetrazolium chloride (TTC) at a concentration of 0.2% (w/v) as a colorimetric indicator of microbial viability. After

18 hours of incubation at 37°C, the MIC was defined as the lowest concentration at which no visible growth was observed.

2.7.4 Determination of the minimum bactericidal concentration (MBC)

According to Wang et al. (2020), the minimum bactericidal concentration (MBC) was determined by streaking 10 µL from wells showing no visible growth onto Mueller-Hinton agar, followed by incubation at 37 °C for 24 h. The MBC corresponds to the lowest concentration at which no bacterial regrowth occurs. The antibacterial effect of the essential oil was then interpreted based on the MBC/MIC ratio, where a ratio ≤ 2 indicates bactericidal activity and a ratio ≥ 4 indicates bacteriostatic activity (El Amri et al., 2014).

2.8 Data analysis

All experiments were conducted in triplicate for each treatment. The data were analyzed using Microsoft Excel, and the results are expressed as mean ± standard deviation.

2.8.1 Molecular Docking study

The three-dimensional structure of Escherichia coli DNA gyrase subunit B (PDB ID: 4PRX) was obtained from the RCSB Protein Data Bank (Burley et al., 2019). Prior to docking, the protein file was processed using BIOVIA Discovery Studio Client 2025 (Dassault Systèmes, France) to eliminate crystallographic water molecules and the native ligand (ADP). UCSF Chimera (v1.19) was then employed to add polar hydrogens and assign Gasteiger partial charges (Pettersen et al., 2004). The test ligands corresponded to the main phytoconstituents detected in the M. pulegium essential oil via GC-MS analysis. Their 3D conformations were sourced from PubChem and converted into PDB format using Open Babel (v3.1.1) (O'Boyle et al., 2011). Docking simulations were carried out in PyRx (v0.8) using the AutoDock Vina algorithm (Dallakyan and Olson, 2015).

The search parameters included an exhaustiveness of 32, an energy range of 3 kcal/mol, and a maximum output of eight poses per ligand. Binding energies were expressed as the lowest predicted free energy change (ΔG, kcal/mol). Visualization of the best-scoring complexes was performed with BIOVIA Discovery Studio Client 2025 to characterize intermolecular interactions, such as hydrogen bonds, hydrophobic contacts, and van der Waals forces.

The reliability of the docking protocol was assessed through a self-docking validation. The co-crystallized ligand (ADP) was re-docked into the binding site of 4PRX under identical conditions. The root-mean-square deviation (RMSD) between the re-docked and original crystallographic poses was computed for each target. An RMSD threshold of ≤ 2.0 Å was set as the criterion for validating the docking accuracy and reproducibility.

3. Results and Discussion

3.1 Yield

The essential oil yield of Mentha pulegium L. obtained by hydrodistillation was 0,72 ± 0,04 %. This value is higher than that reported by Amara et al. (2025) (0.5%). This value was lower than those reported by Bouyahya et al. (2017) and Rached et al. (2025), who obtained yields of 5.4% and 2.31%, respectively. Differences in yield can be attributed to various factors, including geographic origin, harvest period, growing conditions, the condition of the plant material, and extraction parameters, as well as the morphological and biochemical variability of the species (Saba and Anwar, 2018; Omer et al., 2022).

3.2 Organoleptic Analysis

Steam distillation of the dried aerial parts of Mentha pulegium L. yielded a liquid essential oil that was yellow to pale yellow in color and had a characteristic aromatic odor (Table 1). These organoleptic characteristics are consistent with those reported by Zekri et al. (2023), attesting to the quality of the essential oil obtained.

Table 1. Organoleptic characteristics of the essential oil of Mentha pulegium L.

	Organoleptic characteristics		
	Color	**Odor**	**Appearance**
AFNOR (2000)	Nearly color less to pale yellow	Characteristic fresh odor, with a camphor-like note depending on the source	Free-flowing, clear liquid
Essential Oil of *M. pulegium* L.	Pale yellow	Strong and fresh	Liquid

3.3 Physicochemical analysis of the essential oil Density

The measured density of Mentha pulegium essential oil is 0.8936 ± 0,0025, a value slightly higher than that reported by Bouhaddouda (2016), which was 0.878.

3.4 pH

The essential oil of Mentha pulegium L. had a pH of 5.22 ± 0,10, close to the values reported by Lahrech (2010) (5.44), El Ghorab (2006) (≈ 5.30) and Teixeira et al. (2012) (≈ 5.15). This value is consistent with the pH range generally described for essential oils of the genus Mentha (5.1–5.5).

3.5 Solubility Test

The essential oil of Mentha pulegium L. was found to be soluble in methanol and ethanol but insoluble in water, reflecting its hydrophobic nature. These results are consistent with the observations of Dhifi et al. (2016) and Rached et al. (2025), who attribute this property to the lipophilic nature of essential oils.

3.6 Chemical composition of essential oil

Chromatographic analysis of the essential oils of Mentha pulegium L. led to the identification of 26 compounds accounting for 89.42% of the total composition. The main compounds identified (Table 2) are: pulegone (55.03 %), isomenthone (16.85 %), limonene (3.13 %), menthol (2.11 %), and 1,8-cineole (1.25 %). The other compounds were present at concentrations below 1 %. These results are consistent with those reported in several recent studies. Indeed, Rached et al. (2025) identified pulegone (80.04 %) as the major compound, along with menthone (8.14 %), isopulegone (1.37 %), limonene (0.49 %), and menthol (0.27 %) in a Moroccan essential oil of M. pulegium. Similarly, El Omari et al. (2025) reported an essential oil dominated by pulegone (41.0 %) and menthone (21.2 %). These quantitative variations among the major constituents are generally attributed to differences in geographic origin, climatic conditions, phenological stage, harvest period, and genetic factors influencing the biosynthesis of secondary metabolites (Mollaei et al., 2020; Nebié, 2023).

Table 2. Chemical composition of the essential oil isolated from the aerial parts of M. pulegium L collected in the Mostaganem region.

No.	Compounds	Chemical formula	RT (min)	Area	RI	%
1	α-Pinene	$C_{10}H_{16}$	9.092	400230	933	1.05
2	Sabinene	$C_{10}H_{16}$	10.557	78150	972	0.2
3	β-Pinene	$C_{10}H_{16}$	10.716	242960	977	0.64
4	3-Octanone	$C_8H_{16}O$	11.031	211428	985	0.55
5	Myrcene	$C_{10}H_{16}$	11.19	83357	989	0.22
6	3-Octanol	$C_8H_{18}O$	11.457	217522	997	0.57
7	Limonene	$C_{10}H_{16}$	12.571	1194779	1029	3.13
8	1,8-Cineole	$C_{10}H_{18}O$	12.672	476663	1032	1.25
9	(E)-β-ocimene	$C_{10}H_{16}$	13.168	74692	1046	0.2
10	Linalool	$C_{10}H_{18}O$	15	138535	1099	0.36
11	Fenchol	$C_{10}H_{18}O$	15.652	66003	1119	0.17
12	Delta-terpineol	$C_{10}H_{18}O$	16.627	414815	1150	1.09
13	Isomenthone	$C_{10}H_{18}O$	16.817	6430786	1156	16.85
14	trans-Isopulegone	$C_{10}H_{16}O$	17.1	215541	1164	0.56
15	Menthol	$C_{10}H_{20}O$	17.262	805011	1169	2.11
16	cis-Isopulegone	$C_{10}H_{16}O$	17.428	340085	1175	0.89
17	Terpinene-4-ol	$C_{10}H_{18}O$	17.587	90424	1,180	0.24
18	Pulegone	$C_{10}H_{16}O$	19.337	21005292	1238	55.03
19	Piperitone	$C_{10}H_{16}O$	19.803	74625	1254	0.2
20	Menthyl acetate	$C_{12}H_{22}O_2$	20.353	236435	1272	0.62
21	Piperitenone	$C_{10}H_{14}O$	22.194	254546	1,338	0.67
22	Methyleugenol	$C_{11}H_{14}O_2$	23.844	70695	1398	0.19
23	Beta-caryophyllene	$C_{15}H_{14}$	24.462	185499	1,422	0.49
24	α-Humulene	$C_{15}H_{24}$	25.39	326950	1458	0.86
25	Germacrene D	$C_{15}H_{24}$	26.04	100934	1483	0.26
26	Benzyl acetate	$C_9H_{10}O_2$	31.425	85652	1704	0.22
	Total identified compounds (%)					89.42
	Monoterpene hydrocarbons					5.44
	Oxygenated monoterpenes					80.84
	Sesquiterpene hydrocarbons					1.61
	Other compounds					1.53

RI: Retention Index calculated, **RT:** Retention Time.

3.7 Antioxidant activity

3.7.1 DPPH free radical scavenging

The antioxidant activity of the essential oil of Mentha pulegium L., as assessed by the DPPH assay, revealed a strong ability to scavenge free radicals, with an IC_{50} value of 22.17 µg/mL indicating high antioxidant activity. These findings are consistent with those reported by Messaoudi et al. (2022), who obtained an IC_{50} value of 25.68 µg/mL for the essential oil of Mentha pulegium L. This relatively low IC_{50} value further supports the strong antioxidant potential of M. pulegium essential oil. Differences in IC_{50} values may be related to environmental conditions, extraction methods, and variations in the chemical composition of the essential oil. Although pulegone is a major bioactive constituent, the antioxidant activity of Mentha pulegium L. essential oil may also result from additive or synergistic interactions among other major compounds, such as isomenthone, limonene, and menthol, as well as minor constituents. These findings highlight the potential of M. pulegium L.

essential oil as a natural source of antioxidants for potential applications in the agri-food sector.

Figures 4 and 5 show an increase in the percentage inhibition of the DPPH radical as a function of the concentration of the essential oil of M. pulegium L. and ascorbic acid, highlighting a dose-dependent antiradical activity. The essential oil exhibited an IC_{50} value of 22.17 µg/mL, compared with 21.10 µg/mL for ascorbic acid, used as the reference antioxidant. Although the IC_{50} values were numerically similar, no statistical comparison was performed to determine whether the observed difference was statistically significant.

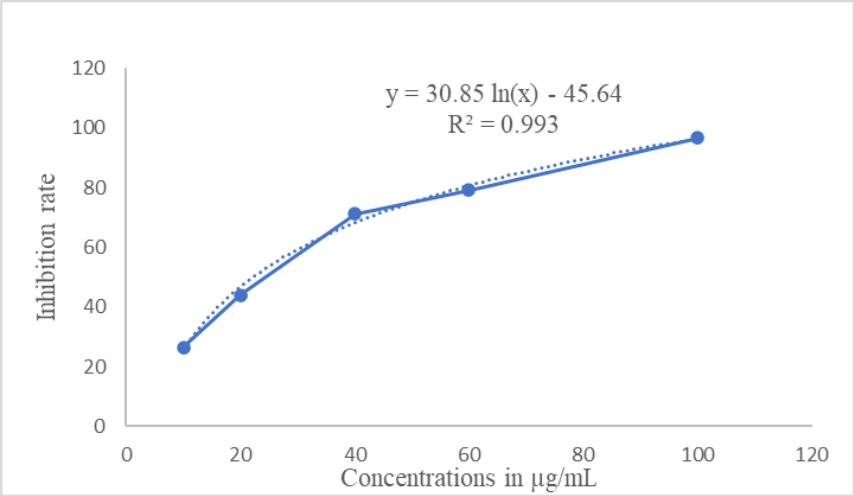


Fig. 4. Free radical scavenging activity of Mentha pulegium L. essential oil.

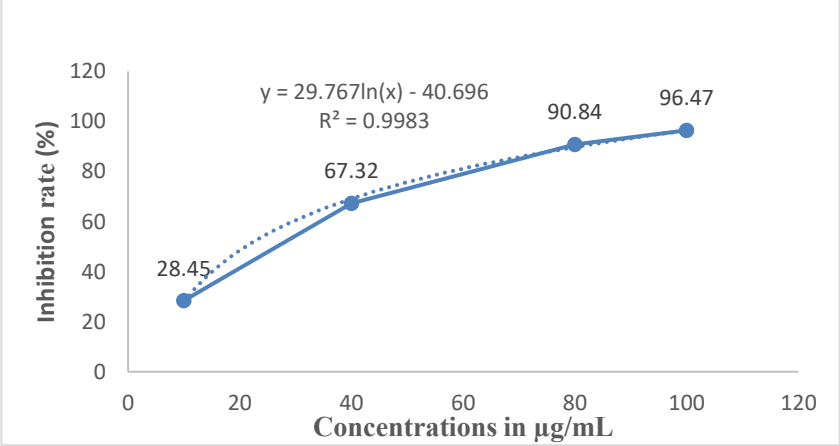


Fig. 5. Antiradical activity of the standard, ascorbic acid.

3.8 Antibacterial Activity

The essential oil of Mentha pulegium L. exhibited antibacterial activity against Gram-positive strains (Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus) as well as against the Gram-negative strain Escherichia coli. The highest sensitivity was observed in B. subtilis, with a mean inhibition zone diameter of 14.33 ± 0.58 mm (Table 3).

Table 3. Inhibition zone diameters (in mm) of pathogenic microorganisms by Mentha pulegium essential oil and the antibiotic CIP (ciprofloxacin)

Microorganisms Pathogenic	Mentha pulegium L. essential oil		Antibiotics (CIP 5 µg)	
	Inhibition Zones (mm)	Sensitivity	Inhibition Zones (mm)	Sensitivity
Staphylococcus aureus	10.00 ± 0.00	+	28.67 ± 0.58	+++
Bacillus cereus	13.67 ± 1.16	+	31.00 ± 1.00	+++
Bacillus subtilis	14.33 ± 0.58	++	37.67 ± 1.53	+++
Escherichia coli	10.33 ± 1.53	+	28.33 ± 1.53	+++
Salmonella typhi	7.67 ± 1.53	-	27.00 ± 2.0	+++
Pseudomonas aeruginosa	/	/	30 ± 1.00	+++

Not susceptible (–); Susceptible (+); Highly susceptible (++); Extremely susceptible (+++); /: No inhibition (Djendi et al., 2023).

The results confirm the antibacterial activity of Mentha pulegium L. essential oil against the strains studied. Bacillus subtilis was the most susceptible (14.33 ± 0.58 mm), followed by Escherichia coli (10.33 ± 1.53 mm), consistent with the observations of Allali et al. (2021). Moderate inhibitory activity was observed against Staphylococcus aureus (10.00 ± 0.00 mm and Bacillus cereus (13.67 ± 1.16 mm). In contrast, Salmonella typhi showed low susceptibility (7.67 ± 1.53 mm), while Pseudomonas aeruginosa proved to be resistant, with no inhibition zone, which is consistent with the results of Messaoudi et al. (2022). These results confirm that the antibacterial efficacy of the essential oil of M. pulegium L. varies depending on the bacterial strain. Overall, Gram-positive bacteria are more susceptible than Gram-negative bacteria, particularly Pseudomonas aeruginosa, which is notable for its high resistance.

3.9 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil of Mentha pulegium L. were determined for the bacterial strains found to be susceptible during the aromatogram test. The results are presented in Table 4.

Table 4. MBC/MIC ratios of M. pulegium essential oil against the tested bacterial strains.

Strains	MIC (µL/mL)	MBC (µL/mL)	MBC/MIC	Nature of the activity
Staphylococcus aureus	1.25 ± 0,13	1.25 ± 0,13	1	Bactericidal
Escherichia coli	0.62 ± 0,06	0.62 ± 0,06	1	Bactericidal
Bacillus subtilis	1.25 ± 0,13	10 ± 1,00	8	Bacteriostatic
Bacillus cereus	1.25 ± 0,13	10 ± 1,00	8	Bacteriostatic

The minimum inhibitory concentrations (MICs) of the essential oil of Mentha pulegium L. ranged from 0.62 µL/mL for Escherichia coli to 1.25 µL/mL for Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus. These low MIC values indicate significant inhibitory activity against the tested bacterial strains. The minimum bactericidal concentrations (MBC) of the essential oil of Mentha pulegium L. varied depending on the bacterial strain. The MBC values were identical to the MICs for Staphylococcus aureus (1.25 µL/mL) and Escherichia coli (0.62 µL/mL), indicating bactericidal activity. In contrast, higher MBCs were observed for Bacillus subtilis and Bacillus cereus (10 µL/mL), reflecting primarily bacteriostatic activity.

The MBC/MIC ratio revealed bactericidal activity of the essential oil of M. pulegium L. against Staphylococcus aureus and Escherichia coli (MBC/MIC = 1), whereas bacteriostatic activity was observed against Bacillus subtilis and Bacillus cereus (MBC/MIC = 8). These results are generally consistent with recent data in the literature. Rached et al. (2025) also reported bactericidal activity of the essential oil against S. aureus (MIC = 2 µL/mL; MBC = 4 µL/mL) and E. coli (MIC = MBC = 10 µL/mL), while El Abdali et al. (2024) confirmed its bactericidal effect against these two strains. The variations observed between the MIC and MBC values can be attributed to differences in the chemical composition of the essential oil, particularly the pulegone content, as well as to the geographic origin of the plant material, cultivation conditions, extraction methods, and experimental protocols. Furthermore, our results corroborate those of Bouyahya et al. (2017), who also demonstrated bactericidal activity against S. aureus and E. coli. However, those authors reported bactericidal activity against all the strains studied, including B. subtilis, unlike our results, in which bacteriostatic activity was observed for B. subtilis and B. cereus.

3.10 Molecular Docking Results

The molecular docking of the five Mentha pulegium essential oil compounds against the E. coli DNA gyrase subunit B active site (PDB: 4PRX) (Table 5), revealed binding affinities ranging from –6.2 to –5.7 kcal/mol. Cis-isopulegone and trans-isopulegone exhibited the highest values at –6.2 kcal/mol, followed closely by pulegone at –6.1 kcal/mol, while menthol and isomenthone showed slightly lower affinities at –5.8 and –5.7 kcal/mol, respectively. These negative values indicate favorable and spontaneous binding interactions between all tested compounds and the gyrase B active site.

Regarding the interaction profile, Cis-isopulegone formed a conventional hydrogen bond with PHE A:213 (2.64 Å), alongside hydrophobic interactions including a Pi-alkyl bond with LEU A:226 and an alkyl bond with TYR A:318. Trans-isopulegone displayed a similar interaction pattern, with a conventional hydrogen bond at PHE A:213 (2.65 Å), a Pi-alkyl interaction with TYR A:318, and alkyl bonds with LEU A:226, as well as an additional Pi-alkyl interaction with TYR A:318, suggesting slightly more extensive hydrophobic contacts compared to its cis-isomer. Pulegone engaged in a carbon-hydrogen bond with ALA A:225 (3.57 Å) and established multiple alkyl interactions with MET A:311, LEU A:315, VAL A:224, and LEU A:226, along with a Pi-alkyl bond with PHE A:213. This compound exhibited the most diverse hydrophobic interaction network. Menthol showed the simplest interaction profile, with only a single Pi-alkyl bond involving TYR A:318 at 5.22 Å, which may account for its relatively lower binding affinity. Isomenthone formed a conventional hydrogen bond with LEU A:226 (2.36 Å) and exhibited the most extensive set of hydrophobic interactions, including multiple alkyl bonds with MET A:311, VAL A:224, LEU A:315, and LEU A:226, as well as Pi-alkyl interactions with PHE A:213 and TYR A:318.

Across all ligands, the most frequently involved residues across all ligands were PHE A:213, LEU A:226, and TYR A:318, all of which are located within the ATP-binding pocket of gyrase B. The consistent involvement of these hydrophobic residues suggests that hydrophobic interactions play a predominant role in ligand binding, while hydrogen bonds (conventional or

carbon-hydrogen) contribute to the stability and specificity of the ligand-enzyme complexes. The superior binding affinities of cis- and trans-isopulegone (–6.2 kcal/mol) may be attributed to the presence of a hydrogen bond with PHE A:213 combined with effective hydrophobic packing within the active site. In contrast, menthol's reduced affinity (–5.8 kcal/mol) correlates with its limited interaction network, relying solely on a single Pi-alkyl contact.

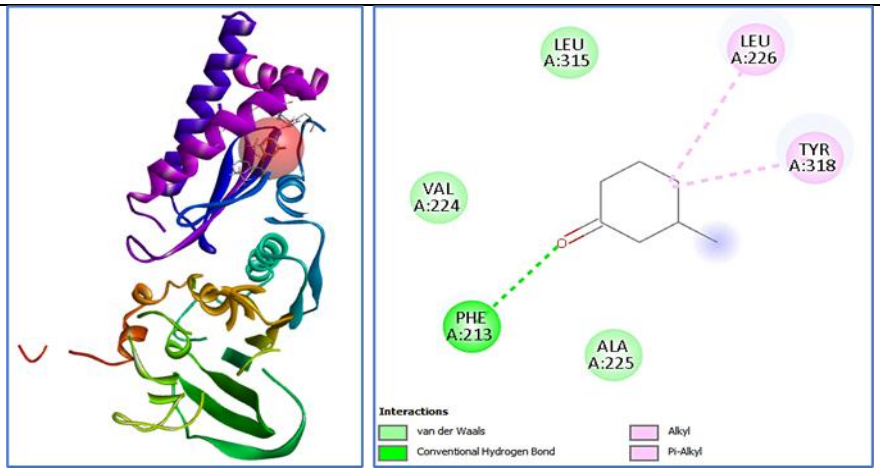
The 2D and 3D interaction maps (Fig. 6-9) visually confirmed the binding modes described above. Cis- and trans-isopulegone (Fig. 6) showed overlapping binding poses with similar contact patterns, while pulegone (Fig. 7), menthol (Fig. 8), and isomenthone (Fig. 9) each adopted distinct orientations within the active site, reflecting their specific interaction profiles. The 3D representations further illustrated the proper accommodation of all ligands within the gyrase B ATP-binding pocket, supporting the feasibility of these compounds as potential inhibitors.

The docking protocol was validated by redocking the co-crystallized ligand into the same binding site. The resulting root-mean-square deviation (RMSD) values were below the accepted 2.0 Å threshold, confirming the reliability of the scoring function and grid box parameters. This validation step ensured that the docking procedure was reproducible and that the binding poses generated for the tested compounds accurately reflected their predicted interactions within the gyrase B active site.

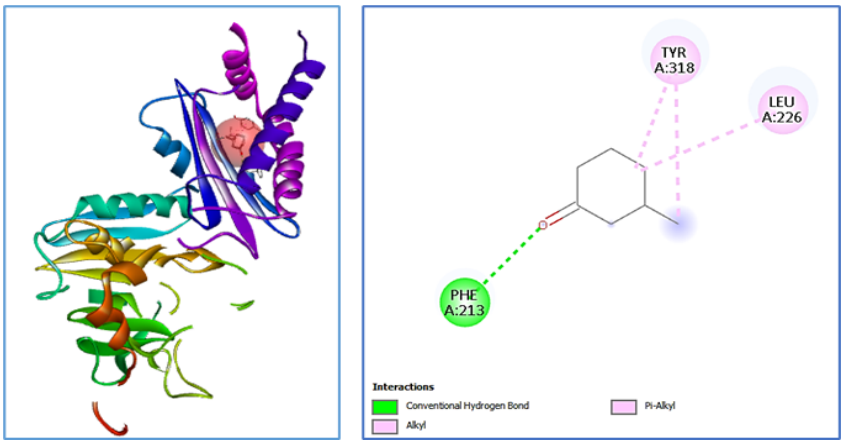
Overall, the docking results demonstrate that all five M. pulegium compounds possess favorable binding affinities for E. coli gyrase B, with cis- and trans-isopulegone showing the most promising inhibitory potential. The predominance of hydrophobic contacts, supplemented by hydrogen bonds, appears to be the primary driving force for ligand recognition. These findings suggest that Mentha pulegium essential oil and its major constituents could serve as potential leads for the development of novel antibacterial agents targeting DNA gyrase.

Table 5. Binding affinities and key residues of M. pulegium compounds at the gyrase B active site (PDB: 4PRX).

Ligand	Binding affinity (kcal/mol)	Amino acids and interaction types	Distance (A°)
Cis-isopulegone	–6.2	PHE A: 213: Conventional hydrogen bond LEU A: 226: Pi-Alkyl TYR A: 318: Alkyl	2.64 3.90 4.47
Trans-isopulegone	–6.2	PHE A: 213: Conventional hydrogen bond TYR A: 318: Pi-Alkyl LEU A: 226: Alkyl TYR A: 318: Pi-Alkyl	2.65 3.77 4.48 4.66
Pulegone	–6.1	ALA A: 225: Carbon hydrogen bond MET A: 311: Alkyl LEU A: 315: Alkyl Val A: 224: Alkyl LEU A: 226: Alkyl PHE A: 213: Pi-Alkyl	3.57 3.72 3.93 4.58 4.61 5.39
Menthol	–5.8	TYR A: 318: Pi-Alkyl	5.22
Isomenthone	–5.7	LEU A: 226: Conventional hydrogen bond MET A: 311: Alkyl VAL A: 224: Alkyl LEU A: 315: Alkyl LEU A: 315: Alkyl PHE A: 213: Pi-Alkyl LEU A: 226: Alkyl LEU A: 226: Alkyl TYR A: 318: Pi-Alkyl	2.36 3.55 4.05 4.25 4.46 4.85 4.90 5.24 5.30



(a) Cis-isopulegone



(b) Trans-isopulegone

Fig. 6. Molecular docking of cis- and trans-isopulegone into E. coli gyrase B active site (PDB: 4PRX): 2D/3D interaction maps.

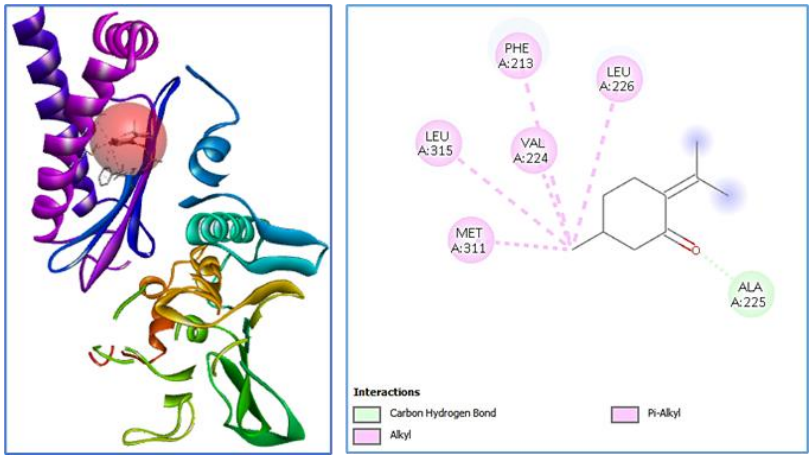


Fig. 7. Molecular docking of Pulegone into E. coli gyrase B active site (PDB: 4PRX): 2D/3D interaction maps.

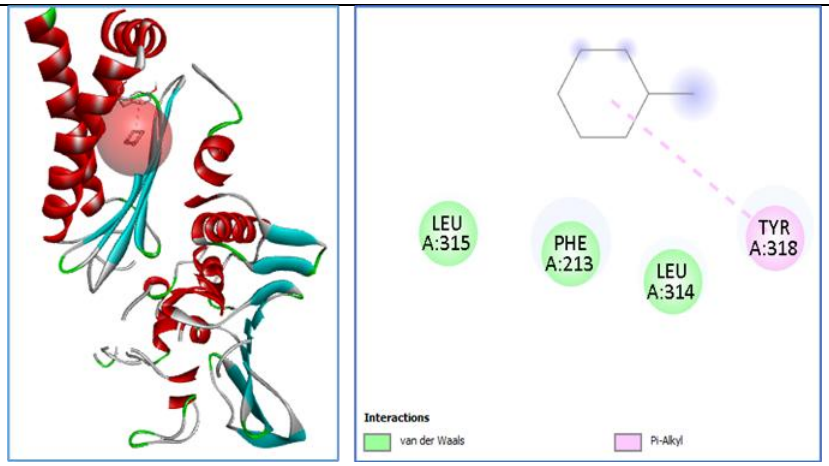


Fig. 8. Molecular docking of Menthol into E. coli gyrase B active site (PDB: 4PRX): 2D/3D interaction maps.

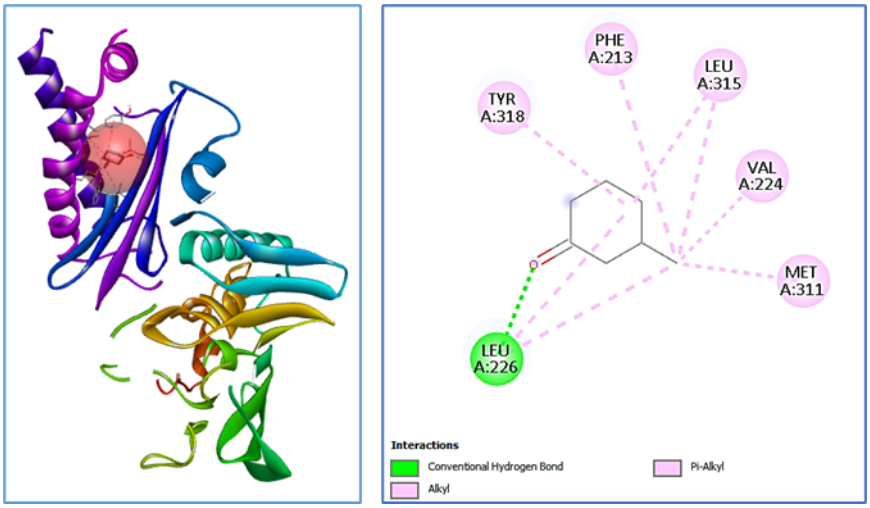


Fig. 9. Molecular docking of isomenthone into E. coli gyrase B active site (PDB: 4PRX): 2D/3D interaction maps.

4. Conclusion

This study demonstrates the significant biological potential of the essential oil derived from Mentha pulegium L. harvested in the Mostaganem region of Algeria, thereby contributing to the valorization of a local botanical resource with substantial economic and biotechnological relevance. Gas chromatography-mass spectrometry (GC/MS) analysis revealed a diverse profile of bioactive compounds, with pulegone identified as the primary constituent. This compound is the principal contributor to the observed biological activities, including a high antioxidant capacity (IC_{50} = 22.17 µg/mL), which indicates an efficient free radical scavenging potential.

Regarding antimicrobial properties, the essential oil exhibited significant inhibitory effects against several foodborne pathogens, with Bacillus subtilis demonstrating susceptibility (14.33 ± 0.58 mm). Determinations of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) confirmed bactericidal activity against Staphylococcus aureus and Escherichia coli, alongside bacteriostatic effects against Bacillus subtilis and Bacillus cereus. These findings underscore the efficacy of the essential oil against microorganisms associated with food spoilage and contamination.

The molecular docking results indicate that the major constituents of Mentha pulegium L. exhibit favorable interactions with the B subunit of Escherichia coli DNA gyrase, with cis-isopulegone and trans-isopulegone showing the most promising binding profiles. These findings support the potential of M. pulegium essential oil as a source of antibacterial compounds targeting DNA gyrase.

These results establish the essential oil of M. pulegium L from Western Algeria as a promising source of bioactive compounds for applications as natural antioxidant and antimicrobial agents. Furthermore, the findings highlight the value of this endemic species within the Algerian floristic heritage for the development of innovative solutions in the food industry, particularly in the context of "clean label" product formulation and natural preservation. Current research trends support the utilization of essential oils as sustainable alternatives to synthetic preservatives, contingent upon rigorous characterization of their chemical composition, safety profiles, and functional efficacy within complex food matrices.

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Authors' Contribution

IS, AA and KB contributed to the conception and design of the study. SI and KB performed the analysis. All authors read and approved the submitted version.

Conflict of Interest

The authors declare that they have no conflict of interests.

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