

ALGERIAN *Juniperus phoenicea* ESSENTIAL OIL: ASSESSEMENT OF HEAVY METAL LEVELS, PHYTOCHEMICAL SCREENING, ANTIOXYDANT, AND ANTIBACTERIAL ACTIVITIES

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Abstract

Juniperus phoenicea essential oil (JPEO) contains various biologically active constituents with remarkable biological activity. This study focuses on the chemical content, antimicrobial efficacy, heavy metal levels and antioxidant capacity of the essential oil extracted from the foliage of Algerian *Juniperus phoenicea*. The essential oil was obtained by hydrodistillation and then analysed by gas chromatography-mass spectrometry (GC-MS). To assess the heavy metal composition, energy dispersive X-ray fluorescence spectrometry (EDXRF) was applied to the extracted oil. The agar disk diffusion technique was employed to test antibacterial activity, and the MIC and MBC values were identified. Reducing power (RP) and DPPH assays were performed to evaluate the antioxidant capacity. The results showed significant antibacterial effects against Gram-positive bacteria, while Gram-negative bacteria were insensitive. The antioxidant activity was noteworthy compared to ascorbic acid, with IC₅₀ values of 1.10 ± 0.01 mg/mL (DPPH) and 1.55 ± 0.01 mg/mL (RP). The oil contained heavy metals at concentrations lower than those permitted by the World Health Organization. These results suggest that *Juniperus phoenicea* essential oil is an interesting new source of healthy natural compounds with antioxidant and antibacterial capacity, confirming its application in food biosafety as a preservative.

Key words: Djelfa region, essential oil, GCMS, Heavy metal, *Juniperus phoenicea*.

INTRODUCTION

Classified under the *Cupressaceae* family, *Juniperus phoenicea* is a shrub species native to the Mediterranean region, exhibiting a broad geographic range that stretches from Portugal in the west to Saudi Arabia in the east (Aouadi et al., 2022). *Juniperus phoenicea* holds a prominent role as a medicinal plant in Algeria's native vegetation, with its foliage and berries being widely employed in traditional therapeutic practices to treat diseases such as eczema, urinary tract diseases, rheumatism, sunstroke, diarrhea, acute gonococcal infections, dysmenorrhea, gastrointestinal disorders, and as a purifying disinfectant, as well as for gout and oedema (Harhour et al., 2018).

Juniperus phoenicea species essential oil (EO) was approved for its antimicrobial, antioxidant, antifungal, and cytotoxic effects (Abu-Darwish et al., 2014; Rahhal et al., 2019). This makes

Juniperus phoenicea a particularly noteworthy medicinal plant for the pharmaceutical and agro-food sectors. However, medicinal plants may contain toxic heavy metals from the air, soil, and water, which are likely to be contaminated by many industrial activities or during the use of agricultural aids, including fertilisers, organic pesticides, and contaminated irrigation water, and may pass into essential oils and threaten their safety and present a risk factor to human health (Abou-Arab et al., 1999; Ben Amor et al., 2021), making it necessary to carry out analytical monitoring of toxic metals in therapeutic plants (Zárate-Quiñones et al., 2021). Conversely, the essential oils' composition is influenced by variables such as their geographic origin, subsequently altering their biological properties (Rahhal et al., 2019), as well as, the level of various heavy metals, which can increase exponentially in particular ecosystems due to human activity (Samara et al.,

2023). With this in mind, we studied the chemical constitutions, antibacterial and antioxidant activity, along with heavy metal content (As, Mn, Hg, Fe, Cu, Pb, Zn, Cd, Cr) in the JPEO from Djelfa (Algeria).

MATERIALS AND METHODS

Sampling coordinates

The sample originated from the Djelfa region, centrally positioned in Algeria. It is part of the high plateau region. Situated at latitudes 33° to 35° North and longitudes 2° to 5° East, the region is categorised as part of the arid bioclimatic zone.

Plant materials

The leaves were harvested in October 2022 and subjected to air drying. An expert in plant taxonomy, affiliated with the University of Mascara's Department of Botany (Algeria), undertook the collected plant sample identification.

Dried *Juniperus phoenicea* leaves underwent a hydrodistillation procedure lasting two hours, with a Clevenger-type setup employed to obtain the essential oil (Clevenger, 1928). The steam charged with the oil droplets condensed as it passed through a cooler and was collected in a separating funnel where the oil and water separated owing to variation in density. The extracted oil preserved in a dark storage area at + 4°C (Rowshan et al., 2013). To quantify the essential oil yield, the formula outlined below was utilized (Teshale et al., 2022):

$$\text{Yield (\%)} = \frac{(w_1 \times 100)}{w_2} \quad (1)$$

W 1: defines the weight of EO.

W 2: defines the dry weight of the sample.

Heavy metal analysis

The essential oil was analysed using an EDXRF (Bruker S2 Puma type) equipped with 20 sample trays. Samples were analysed in sample cups supplied and sealed at one end with 6 µm polypropylene XRF film. 1.5 ml of sample was analyzed. Values were related as mg/kg.

Combined GC-MS analytical technique

A SHIMADZU GCMS-QP2020 system fitted with a fused Rxi®-5ms capillary column (30 m

× 0.25 mm, 0.25 µm film thickness; stationary phase: Crossbond® 5% diphenyl 95% dimethyl polysiloxane). This column is functionally equivalent to HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB-1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms, and aligns with USP phases G1, G2, and G38; it was utilised to analyse the essential oil. The analysis was conducted under the following conditions: A 0.5 µL sample was injected in split mode (1:50). The injector and detector temperatures were maintained at 250°C and 310°C, respectively. The oven temperature program began with an initial hold at 50°C for 2 minutes, followed by a ramp to 310°C at 3 °C/min, and a final hold at 310°C for 2 minutes. Ultra-pure helium (99.995%) was used as the carrier gas at a constant flow rate of 1 mL/min. The mass spectrometer operated with an ionisation energy of 70 eV, an ion source temperature of 200°C, and a mass scan range of 45-600 m/z.

Antioxydant activity

Following the method described by Brand-Williams et al. (1995), the DPPH free inhibition was evaluated. Five distinct concentrations of EO were prepared, and a freshly prepared 2 mL methanolic DPPH solution (4 mg/100 mL) was combined with 1 mL of each EO concentration. After incubating the mixtures under ambient temperature for 16 minutes, employing a spectrophotometer at 517 nm, absorbance was measured. The methanolic DPPH solution served as control, the extraction solvent served as the blank, and ascorbic acid acted as the positive control (Susanti et al., 2007). The appearance of yellow instead of purple indicates the activity of free radical scavenging (Dimitriu et al., 2021), quantified as a percentage with the following equation:

$$\text{DPPH inhibition activity (\%)} = \left[\frac{(A_1 - A_2)}{A_1} \right] \times 100 \quad (2)$$

A1: refers to the absorbance (DPPH) of the control lacking essential oil, while A2: denotes the absorbance following the addition of the EO. Percentage inhibition was plotted against the logarithm of the concentrations to create a graph. The IC50 was then estimated through linear regression analysis.

The RP was assessed following the method described by Oyaizu (1986). Briefly, for 20 minutes, tubes containing varying concentrations of essential oil (EO), 2.5 mL of potassium hexacyanoferrate (1%) and 2.5 mL of phosphate buffer (0.2 M, pH 6.6) were maintained in a water bath at 50°C. After adding 2.5 mL of 10% trichloroacetate, the mixture was centrifuged for 10 minutes at 3000 rpm. After centrifugation, 2.5 mL of the resulting supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% FeCl₃ solution. A blank solution was then used to test the absorbance at 700 nm, employing a blank solution that substituted the same volume of diluted EO with its corresponding vehicle. Various concentrations of ascorbic acid (62.5, 125, 250, 500, and 1000 mg/mL) were utilized as standards. Determining the 50% inhibitory concentration (EC50) was conducted graphically through linear regression.

Antibacterial activity

A total of six bacterial strains were evaluated using the pure EO, encompassing the Gram-positive bacteria *Streptococcus thermophilus*, *Staphylococcus aureus*, *Bacillus cereus* and *Lactobacillus lactis*, as well as the Gram-negative species *Pseudomonas aeruginosa* and *E. coli*. The selected bacterial strains were chosen due to their common association with food contamination and their pathogenic potential. These strains were obtained from meat samples. Standard biochemical tests and morphological assessments were used to verify the strains. After verification, they were cultured on nutrient agar, which supports their growth, for 24 hours at 37°C.

The colonies isolated from each bacterium were placed in tubes with sterile 0.9% sodium chloride solution, with the optical density adjusted to 0.08-0.1 absorbance at 625 nm, which corresponds to a McFarland standard of 0.5 and, thus contains approximately 10⁸ CFU/mL (Ambrosio et al., 2017).

The disk diffusion technique, as explained by Bauer et al. (1966) with some adjustments, was employed to assess the antibacterial property of JPEO. In summary, 100 µL of a suspension containing 10⁸ CFU/mL of bacterial cells was spread onto nutritional agar. Six-mm-diameter paper discs were soaked in 10 µL of EO and then

put on agar that had already been infected with the selected bacteria. The positive control was gentamicin (10 µg/plate), while the negative control was DMSO disks (10 µL). After being stored at 4°C for one hour, the Petri dishes were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter (in millimeters) of the inhibition zone, which included the 6 mm disk. The test was repeated three times, and the results were compared to the controls.

With some changes, the technique described by Aouadi et al. (2022) was followed in order to estimate MIC and MBC adopting the microdilution method, where 100 µL of an inoculum of 10⁸ CFU/mL of viable bacterial cells were introduced into tubes with 400 µL of Muller-Hinton broth. Subsequently, 500 µL of serially diluted essential oil prepared at concentrations ranging from 100 mg/mL to 0.781 mg/mL was added to each tube, achieving a final volume of 1 mL. These cultures followed by incubation at 37 ± 1°C for 24 hours. DMSO served as the negative control, while the positive controls comprised bacterial suspensions in Mueller-Hinton broth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no visible bacterial growth was observed. After the MIC was determined, a 10 µL sample from the limp tubes including the MIC and higher concentrations was inoculated using nutrient agar plates, which then underwent incubation overnight at 37°C. MBC represents the minimal concentration that kills 99.9% of the initial bacterial inoculum, showing no detectable bacterial growth.

Statistical analysis

The mean ± standard deviation (± SD) is used to display the data. The Student's t-test was used for statistical analyses, and p-values below 0.05 ($p < 0.05$) were deemed statistically significant.

RESULTS AND DISCUSSIONS

The extraction yield of *Juniperus phoenicea* leaf essential oil (0.64% of dry mass) shows a significant increase over values reported in various Algerian regions (0.28%, 0.32%, and 0.4%) and in Morocco (0.5%) (Cheraif et al., 2020; Oukadir et al., 2021; Houari et al., 2022).

Nonetheless, this yield does not reach the levels documented for Tunisian samples (1.69%) or for specimens collected in the Tiaret and Chelef regions of Algeria, which were reported at 0.8% and 0.73%, respectively (Boukhaloua et al., 2022; Mansour et al., 2023; Amokrane et al., 2024).

These discrepancies are likely due to a combination of intrinsic and extrinsic factors. Genotypic differences across *Juniperus phoenicea* populations may result in varied EO production levels, reflecting the influence of underlying genetic variability. Environmental parameters soil type, local climate, and specific microclimatic conditions such as light exposure and humidity also play crucial roles in EO yield by impacting metabolic pathways linked to secondary metabolite synthesis. The interplay of these variables suggests that each environmental condition may selectively influence specific biosynthetic routes in *Juniperus phoenicea*. Additionally, processing factors identified by Lalami et al. (2013), such as plant material freshness, drying methods, and the type of extraction apparatus, significantly affect EO yield, highlighting the need for standardized extraction protocols to ensure consistency in yield comparison across studies.

The EDXRF analysis of JPEO identified no detectable levels of hazardous metals, among them chromium (Cr), cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg), as mentioned in Table 1. This absence of toxic metals aligns with an environment that lacks significant anthropogenic activity, which often introduces metal contaminants (Rezaee et al., 2005). Factors such as soil composition, air quality, and climate significantly influence heavy metal uptake in plants (Šovljanski et al., 1990; Abu-Darwish et al., 2014). Therefore, the low or

undetectable levels in this sample imply a natural habitat with minimal exposure to pollutants.

In this analysis, the leaves were used as the source material, a choice that may account for the lack of detectable toxic metals. Previous studies have indicated that plant roots typically absorb and store higher concentrations of heavy metals like Pb, Cr, Cu, and Hg compared to aerial parts (Mussina et al., 2018). Additionally, metal uptake is influenced by both plant species and genetic factors; juniper leaves, in particular, are recognised for their ability to absorb pollutants in highly polluted environments, including those impacted by vehicle exhaust and industrial activities (Mussina et al., 2018; Radanovic et al., 2001). Consequently, the low contaminant levels in our sample suggest the leaves were sourced from an environment free from these influences.

In contrast, the essential micronutrients zinc (Zn), iron (Fe), and copper (Cu) were present in detectable quantities, while magnesium was absent (Table 1). These metals were ranked by concentration as $Cu > Zn > Fe$, all within WHO-recommended safety limits for medicinal plants (300 mg/kg for Cu, 100 mg/kg for Zn, 200 mg/kg for Fe and 10 mg/kg for Mn) (Ouedraogo et al., 2021). The regional geochemistry, particularly the limestone-rich soils, is likely responsible for the relatively low concentrations of several metals, as high bicarbonate levels decrease the solubility and bioavailability of trace elements (Kherfane, 2014; Madejon et al., 2017). The extraction process additionally minimizes the oil's level of heavy metal concentration, large metal molecules do not easily volatilize, which results in a purer oil suitable for medicinal use (Angelova & Ihtyarova, 2023).

Table 1. Heavy metal content in essential oil of *Juniperus phoenicea* leaves from Djelfa (mg/kg)

	Cd	As	Cr	Mn	Fe	Cu	Zn	Pb	Hg
JPEO	N/F	N/F	N/F	N/F	0.0272 ± 0.01	0.1159 ± 0.06	0.0687 ± 0.01	N/F	N/F

N/F: not found

The values recorded by GC/MS for JPEO leaves are presented in Table 2. A total of 101 components, representing 98.03% of the oil, were identified in *Juniperus phoenicea*. The monoterpene fractions were dominant (65.24%), with the latter being particularly rich

in monoterpene hydrocarbons (40.27%). The sesquiterpene fraction accounted for (32.04%), of which oxygenated sesquiterpenes were primarily composed (18.02%), followed by the diterpene fraction (0.55%).

The essential oil is characterized by the predominance of α -pinene, with a content of (26.92%), followed by Sabinene (5.41%), 4a (2 H)-Naphthalenol (3.22%), Linalool (3.15%), α -Terpineol (3.10%), cis-Calamenene (3%), Linalyl formate (2.92%) and α -Trimethyl acetate (2.42%). α -Pinene has been identified in previous research as a particular compound in *Juniperus phoenicea*, with concentrations that vary depending on the essential oil's geographic origin. Similar to the current study's results, previous research found α -pinene to be the main compound in JPEO, with concentrations of

60.21%, 44.2% and 12.63% respectively (Elmhalli et al., 2021; Oukadir et al., 2021; Mansour et al., 2023).

The main component of oil from the same species growing in Tiaret (Algeria) is β -pinene (35.7%) (Boukhaloua et al., 2022). This quantitative and qualitative variation in the composition of JPEO may be due to the nature of the soil, altitude, climatic conditions, sunshine, neighboring plant populations and genetic composition, which determine these different chemotypes (Lardry & Haberkorn, 2007).

Table 2. GC-MS analysis of *Juniperus phoenicea* essential oil constituents

n°	Compound	Ri	Rt	%
1	cis-3-Hexenol	853	6.087	0.04
2	Tricyclene	930	8.348	0.23
3	α - Pinene	948	9.088	26.92
4	1H-Pyrrole, 2-methyl-	799	9.350	0.13
5	Camphene	943	9.408	0.53
6	Verbene	925	9.603	0.22
7	β - Pinene	978	10.506	1.35
8	Myrcene	991	11.142	1.45
9	2-Carene	948	11.503	0.14
10	α - Phellandrene	1007	11.662	0.55
11	3-Carene	948	11.914	0.45
12	o-Cymene	1042	12.560	1.66
13	Sabinene	1013	12.828	5.41
14	γ -Terpinene	998	14.077	0.13
15	(3R)-1-methylidene-3-prop-1-en-2-ylcyclohexane	990	15.428	0.70
16	Linalool	1082	16.078	3.15
17	(2S,4S)-4-methyl-2-(2-methylprop-1-enyl)oxane	1114	16.485	0.15
18	trans-para-Menth-2-en-1-ol	1124	16.945	0.14
19	α - Campholenal	1155	17.148	0.47
20	Pinocarveol	1131	17.759	0.89
21	(1R)-cis-Verbenol	1136	17.875	0.16
22	Camphor	1121	17.977	0.41
23	trans-Verbenol	1136	18.067	0.90
24	3-Pinanone	1109	18.737	0.34
25	Pinocarvone	1114	18.832	0.17
26	2,6-Dimethyl-7-octenyl acetate	1243	19.001	0.21
27	α -Phellandren-8-ol	1125	19.075	0.59
28	Isopinocampone	1109	19.366	0.13
29	(-)-Terpinen-4-ol	1137	19.545	0.22
30	trans- Naphthalene, 1,2,3,4,4a,5,8,8a-octahydro-4a-methyl-	1157	19.920	0.40
31	α -Terpineol	1198	20.291	3.10
32	2-Pinen-10-ol	1191	20.478	0.38
33	Homomyrtenol	1290	20.795	0.21
34	Verbenone	1119	21.021	0.51
35	Carveol <trans->	1223	21.516	0.53
36	Oct-7-enol <3,7-dimethyl->	1179	22.019	1.27
37	Piperitone	1267	23.055	1.34
38	Linalyl formate	1212	23.258	2.92
39	Isopulegyl acetate	1273	24.101	1.03
40	(-)-Bornyl acetate	1277	24.545	0.20
41	Tetrahydro-2,2,6-trimethyl-6-vinyl-2H-pyran-3-yl acetate	1395	24.642	0.76
42	2,4-Decadien-1-ol	1274	25.835	0.64
43	1,4-Dimethyladamantane #	1027	26.238	0.27
44	Bicyclogermacrene	1497	26.837	0.24
45	α -Trimethyl acetate	1333	27.400	2.42
46	Copaene	1221	28.515	0.37

n°	Compound	Ri	Rt	%
47	Geranyl acetate	1380	28.820	0.15
48	(-)- β -Bourbonene	1339	28.892	0.17
49	Bicyclosesquiphellandrene	1435	29.115	0.12
50	Cyclohexane,1-Methyl-1-ethenyl-2,4-bis(1'-methylethenyl)	1398	29.209	0.39
51	Caryophyllene	1494	30.389	1.44
52	cis-Thujopsene	1416	30.827	0.10
53	γ -Elemene	1431	30.954	0.68
54	Naphthalene, 1,2,3,4,4a,5-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	1440	31.654	0.40
55	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	1579	31.790	0.78
56	β -Copaene	1216	32.390	0.10
57	Cadina-1(6),4-diene <10betaH->	1472	32.615	0.75
58	γ -cadinene	1435	32.746	0.56
59	(-)-Germacrene D	1515	32.926	0.79
60	β -Selinene	1469	33.129	0.51
61	(+)-epi-Bicyclosesquiphellandrene	1435	33.374	1.21
62	Sesquisabinene hydrate	1523	33.481	1.19
63	α -Muurolene	1440	33.708	0.68
64	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1. α ,2. α ,3. β ,6. α .)]-	1555	34.143	0.66
65	4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.0.1,5]decan-4-ol	1484	34.333	1.92
66	cis-Calamenene	1537	34.676	3.00
67	1,3-Dioxane-4,6-dione, 2,2-dimethyl-5-[1-[(1 methylethyl)amino]ethylidene]-	1899	34.745	0.44
68	Cubenene	1440	34.980	0.22
69	γ -Selinene	1502	35.097	0.11
70	2,6,10-Dodecatriene, 12-acetoxy-6-hydroxymethyl-2,10-dimethyl-, (E,E)-	2076	35.199	0.46
71	α -Maaliene	1403	35.366	0.37
72	α -Elemol	1546	35.663	1.40
73	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	1745	35.822	0.21
74	(E,E)-germacrene B	1603	35.974	1.03
75	cis-Nerolidol	1564	36.173	0.34
76	Citronellyl valerate	1624	36.641	0.36
77	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar- (1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	1536	36.761	0.30
78	Caryophyllene oxide	1507	36.977	1.36
79	Salvial-4(14)-en-1-one	1608	37.359	0.21
80	Humulene epoxide II	1592	37.967	0.76
81	Anthracene, 9-ethyl-9,10-dihydro-10-t-butyl-	2049	38.046	0.26
82	2-(1,4,4-Trimethyl-cyclohex-2-enyl)-ethanol	1313	38.283	0.52
83	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4S,4aS,8aR)-	1580	38.747	3.22
84	4,8,12,16-Octadecatetraen-1-ol, 4,9,13,17-tetramethyl-	2391	38.842	0.27
85	.tau.-Cadinol	1580	39.226	1.00
86	Cadinol	1580	39.365	0.27
87	β -Eudesmol	1656	39.517	0.72
88	γ -Eudesmol	1632	39.647	1.34
89	6-[(1E)-3-Hydroxy-1-butenyl]-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	1669	39.774	0.14
90	2-(4-Cyclopropylidenebutoxy)tetrahydro-2H-pyran	1467	39.987	0.12
91	(1R,4S)-4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol	1687	40.142	0.11
92	Spathulenol	1576	40.266	0.18
93	Ethanone, 1-(5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta[b]furan-5-yl)-121	1632	40.579	0.11
94	3-Phenylallyl isovalerate	1601	40.710	0.11
95	5-cyclodecen-1-ol, 4,10-bis(methylene)-7-(1-methylethyl)-, (1R,5E,7S)-	1699	40.831	0.36
96	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R- (1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	1555	41.013	0.78
97	((4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2- yl)methanol	1683	41.416	0.15
98	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	1680	41.954	0.45
99	β -Oplopenone	1540	43.725	0.16
100	Manoyl oxide	1978	51.155	0.16
101	(1R,4aR,4bR,10aR)-7-Isopropyl-1,4a-dimethyl-1,2,3,4,4a,4b,5,6,10,10a- decahydrophenanthrene-1-carbaldehyde	2097	60.02	0.13
Total identified				98.03%
Monoterpene hydrocarbons: 40.27% ; Oxygenated monoterpenes: 24.97% ; Sesquiterpene hydrocarbons: 14.02% ; Oxygenated sesquiterpenes: 18.02% ; Diterpene hydrocarbons: 0.26% ; Oxygenated diterpene: 0.29%				

Ri: Retention index; Rt: Retention time

The DPPH radical scavenging activity results demonstrate that the JPEO demonstrates notable antiradical activity, achieving results comparable to ascorbic acid (standard reference antioxidant) with statistical significance ($p < 0.05$) (Table 3). The pronounced scavenging activity observed may be owing to the combination effects of the various chemical components included in the EO, as highlighted by Selmi et al. (2023). When compared to other studies, the IC₅₀ value determined for our JPEO sample (1.10 ± 0.01 mg/mL) is substantially lower than values reported in Algerian studies by Boudiba et al. (2021) and Cheraif et al. (2020), which documented concentrations of 36.1 mg/mL and 15 mg/mL, respectively. Conversely, it is significantly higher than the values of 0.271 mg/mL and 0.093 mg/mL recently published by Ait-Mimoune et al. (2023) and Selmi et al. (2023), respectively. Such discrepancies in IC₅₀ values could be influenced by the essential oil's unique chemical composition as well as variations in the assay conditions, which include reaction temperature, the antioxidant to DPPH ratio, the solvent employed, pH, and the concentration of the sample (Noipa et al., 2011). The antioxidant

reducing power is a vital indicator of the antioxidant activity exhibited by substances. The results of the reducing power (RP) assay demonstrate that JPEO exhibits significant antioxidant activity, as reflected by its EC₅₀ value of 1.55 ± 0.01 mg/mL. While this value is slightly higher than that of ascorbic acid (1.12 ± 0.01 mg/mL), the difference between the two is statistically significant ($p < 0.05$), confirming JPEO's notable antioxidant potential (Table 3). These results are in agreement with other research indicating that the leaves of *Juniperus phoenicea* exhibit antioxidant capabilities with reported EC₅₀ values of 1.80 mg/mL, 14.5 mg/mL, and 46.85 μ g/mL, respectively (El Jemli et al., 2016; Aouadi et al., 2022; Mansour et al., 2023). This observed antioxidant activity is likely linked to the predominant component, α -pinene (26.92%), which has been documented for its antioxidant effects (Bajes et al., 2022). The reductive properties of α -pinene facilitate the transformation of Fe³⁺ or the ferricyanide complex into its ferrous form, thereby enhancing its efficacy as an antioxidant and contributing to the overall antioxidant capacity of the EO (Wang et al., 2019).

Table 3. IC₅₀ values for DPPH and RP (mg/mL)

IC ₅₀ (mg/ml) \pm SD	DPPH Reducing power	Ascorbic acid	<i>Juniperus phoenicea</i>
		1.73 ± 0.01	1.10 ± 0.01
		1.12 ± 0.01	1.55 ± 0.01

The results from the aromatogram analysis of JPEO regarding a number of bacterial species, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Streptococcus thermophilus*, *Bacillus cereus* and *Lactobacillus lactis* are provided in Table 4. Our findings demonstrate that JPEO exhibited the strongest antimicrobial effect against *Lactobacillus lactis*, yielding an inhibition zone of 24 ± 1.33 mm. Subsequent testing revealed progressively smaller zones of inhibition for *Streptococcus thermophilus* (19.33 ± 5.55 mm), *Bacillus cereus* (12 ± 2.66 mm), and *Staphylococcus aureus* (10.5 ± 0.5 mm), while *E. coli* and *Pseudomonas aeruginosa* showed greater resistance. The observed inhibition zones were smaller compared to those produced by conventional antibiotics.

Our analysis also indicated that Gram-positive bacteria, specifically *Streptococcus thermophilus*, *Lactobacillus lactis*, *Bacillus cereus*, and *Staphylococcus aureus*, were more vulnerable to JPEO than Gram-negative bacteria, such as *Pseudomonas aeruginosa* and *E. coli* ($p < 0.05$). This observed sensitivity can be attributed to the existence of a complex outer membrane in Gram-negative bacteria, characterised by an abundance of lipopolysaccharides that hinder the penetration of hydrophobic substances. Conversely, Gram-positive bacteria loses this external membrane and possess a thinner peptidoglycan wall, which facilitates access to the cell membrane, enhanced by lipophilic lipoteichoic acid that promotes the uptake of hydrophobic substances (Rahhal et al., 2019). Responses to JPEO varied among microorganisms previously studied,

aligning with the results of Ait-Mimoune et al. (2023), who documented similar effects against *Pseudomonas aeruginosa* and *Escherichia coli*. Other studies, such as those by Boukhaloua et al. (2022), Benjemaa et al. (2022), and Rahhal et al. (2019), documented significant antimicrobial effects against *Pseudomonas aeruginosa*, with inhibition zone diameters of 28 mm, 10.67 mm,

and 17.20 mm, respectively, and against *Escherichia coli* with diameters of 20 mm, 6 mm, and 11.32 mm. The variations in bacterial sensitivity to JPEO are likely influenced not only by the high levels of α -pinene but also by the essential oil's overall chemical profile, which may exhibit synergistic effects (Mercier et al., 2009).

Table 4. Diameters of inhibition zones of *Juniperus phoenicea* essential oil

	<i>Pseudomonas aeruginosa</i>	<i>Lactobacillus lactis</i>	<i>E. coli</i>	<i>Sataph. aureus</i>	<i>Streptococcus thermophilus</i>	<i>Bacillus cereus</i>
JPEO inhibition zone (mm)	--	24 ± 1.33	--	10.5 ± 0.5	19.33 ± 5.55	12 ± 2.66
ATB (genta)	26 ± 0.5	31 ± 0.66	30.33 ± 1.11	28 ± 0.66	24.66 ± 1.11	30 ± 0.66

(--): Resistant strain

Table 5 summarises the findings from MIC and MBC assessments, reflecting the bacteriostatic and bactericidal properties of JPEO. The MBC values ranged from 6.25 to 25 μ L/mL, with MIC value of 3.125 μ L/mL for the examined bacterial strains. Additionally, JPEO demonstrated significant bactericidal activity against *Staphylococcus aureus*, *Streptococcus thermophilus*, and *Bacillus cereus*, while exhibiting bacteriostatic effects against *Lactobacillus lactis*, as evidenced by an MBC/MIC ratio below 4, comparable to reference values (Himed et al., 2016). Notably, MIC and MBC values could not be established for *E. coli* and *Pseudomonas aeruginosa*, both of which demonstrated pronounced resistance.

A wide range of results has been reported concerning variability in the antibacterial efficacy of JPEO when tested against the identical microbial strains analysed in this research. Aouadi et al. (2022) found that the essential oil exhibited higher MIC and MBC values against *Staphylococcus aureus* and *Bacillus cereus* compared to our findings. Similarly, Samara et al. (2023) observed lower MIC and MBC values for *Staphylococcus aureus* than those recorded in the current study. The discrepancies in microbial sensitivity to EO may be correlate with the origin of the microbial strains and their varying susceptibilities to these natural compounds (Kalemba & Kunicka, 2003).

Table 5. Values of the MIC and MBC of the essential oil of *Juniperus phoenicea* for the examined bacteria

samples	<i>Staphylococcus aureus</i>		<i>Lactobacillus lactis</i>		<i>Streptococcus thermophilus</i>		<i>Bacillus cereus</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
JPEO (μ L/mL)	3.125	6.25	3.125	25	3.125	12.5	3.125	6.25

CONCLUSIONS

This study underscores the critical role of α -pinene as the principal component of *Juniperus phoenicea* essential oil (JPEO), which exhibits remarkable antibacterial properties against Gram-positive bacteria. The observed MIC and MBC highlight the strong efficacy of JPEO, contributing valuable insights into its potential applications in combating bacterial contamination. In contrast, the resistance

observed in Gram-negative bacteria, particularly *E. coli* and *Pseudomonas aeruginosa*, emphasizes the need for targeted approaches when utilizing JPEO as an antimicrobial agent. Additionally, the antioxidant capacity of JPEO, demonstrated through DPPH and reducing power (RP) assays, positions it as a promising source of ferric ion-reducing antioxidants and effective free radical scavengers, thereby enhancing the understanding of the health benefits associated with *Juniperus phoenicea*.

The analysis of heavy metal concentrations revealed that essential oil contains iron (Fe), zinc (Zn), and copper (Cu) at levels well below the World Health Organization (WHO) limits, while manganese (Mn), cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr) and arsenic (As) were not detectable. This finding further supports the safety of JPEO for human consumption. In summary, this research highlights the significant antibacterial and antioxidant properties of JPEO from the Djelfa region, affirming its potential as a safe and effective additive in food products. The contributions of this study provide a foundation for further exploration of *Juniperus phoenicea* in various applications, enabling novel advancements in health and nutrition.

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