Trop J Nat Prod Res, November 2021; 5(11):1966-1972 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Chemical Composition, Antioxidant and Antibacterial Efficiency of Essential Oils from Algerian *Juniperus phoenicea* L. Against Some Pathogenic Bacteria

Kamela Mehira[1], Abdelkader Douaoui[1,2], Aicha Debib[2]*, Ibrahim Yahiaoui[1], Sonia Socaci[3], Ahmed Belouazni[1], Cristina Anamaria Semniec[3], Anca Farcas[3]

[1]*Laboratory of Crop Production and Sustainable Valorization of Natural Resources, University Djilali Bounaama, Khemis Miliana, 44225, Algeria*
[2]*Laboratory of Management and Valorization of Agricultural and Aquatic Ecosystems (LMVAAE), Morsli Abdallah Tipaza University Center, 42000, Algeria*
[3]*Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca 400372, Romania*

ARTICLE INFO

Article history:
Received 05 September 2021
Revised 03 October 2021
Accepted 28 October 2021
Published online 05 December 2021

ABSTRACT

Essential oils (EOs) are known for their medicinal properties which are used in different domains. The current study was conducted to characterize the chemical composition and evaluate the antioxidant and antibacterial efficiency of EOs from Algerian *Juniperus phoenicea* L. Leaves and berries of *J. phoenicea* were collected from five different regions in the northeast of Algeria. EOs were extracted from the various plant samples. The analysis and identification of the components of the leaf-berry combination (Lb) and leaf-only (Lo) EOs were performed using gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of the EOs was tested against seven bacterial strains; two Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and five Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Acinetobacter baumannii*, and *Klebsiella pneumoniae*). To test the antioxidant property of the EOs, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed. Forty-six compounds were identified in the Lb and Lo EOs representing 96.8 to 98.2% and 93.5 to 100%, respectively of the total oil composition. The major monoterpenes compounds were α-pinene with percentage composition of 50 to 85.8% and 42.1 to 83.1% in the Lb and Lo EOs, respectively. The EOs exhibited stronger antibacterial activity against the Gram-positive bacteria compared to the Gram-negative bacteria. Moreover, the Lo EOs showed less significant antioxidant activity in the DPPH radical scavenging assay compared to the Lb EOs for each studied variety. These findings support some of the traditional uses of this plant in food preservation by the Algerian people and also validate its use for protection against infectious diseases.

Keywords: Algerian variety, Antibacterial activity, Antioxidant activity, Essential oils, *Juniperus phoenicea*.

Introduction

The *Juniperus phoenicea* (Cupressaceae) is an aromatic medicinal plant that is well known for its multi-use in modern and traditional medicine. Essential oils (EOs), also referred to as ethereal oils, are natural volatile liquids that can be extracted from different parts of the plant (leaves, seeds, woods, barks, roots, flowers, fruits, and rhizomes).[1] Since ancient times, they have been used in folk medicine. In nature, EOs play an important role in the protection of plants against pathogenic microorganisms and undesirable insects.[2,3] The antimicrobial properties of EOs have been thoroughly investigated in various studies where they were found to be efficient against a broad spectrum of pathogens.[4] The genus *Juniperus* (Cupressaceae) comprises nearly 75 species, widely distributed in the Northern hemisphere, especially in Tunisia, Algeria, and Morocco.[5] Numerous studies have reported the chemical composition and biological activities of the EOs from different species of this genus.[6-8]

*Corresponding author. E mail: a_debib@yahoo.fr
Tel: +213696577219

Citation: Mehira K, Douaoui A, Debib A, Yahiaoui I, Socaci S, Belouazni A, Semniec CA, Farcas A. Chemical Composition, Antioxidant and Antibacterial Efficiency of Essential Oils from Algerian *Juniperus phoenicea* L. against Some Pathogenic Bacteria. Trop J Nat Prod Res. 2021; 5(11):1966-1972. doi.org/10.26538/tjnpr/v5i11.13

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In Algeria, among these species, *Juniperus phoenicea* (Figure 1) is the most widely used for its medicinal properties.[9] Its leaves are used in form of decoction to treat diabetes, diarrhea, and rheumatism. The mixture of leaves and berries of this plant is used as an oral hypoglycemic agent, whereas the leaves are used against broncho-pulmonary disease and as a diuretic agent.[10,11] The dried and powdered fruits can cure skin ulcerations and abscesses.[12] *J. phoenicea* is highly valuable due to the EOs of its leaves and berries. Several studies on their biological activities have been reported in the literature. They have antiseptic, antibacterial,[12-14] antifungal,[15] antioxidant,[6,8,14] and anticancer[15-17] activities.

Figure 1: *Juniperus phoenicea* L. a: Leaves and berries; b: Berries.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Several studies have investigated the chemical characterization of the EOs of *J. phoenicea* L from Algeria.[7,8,10] Their chemical variability according to the geographic region is already reported.[8,18,19] However, to the best of our knowledge, no study has been done on the antioxidant and antibacterial activities of the leaves-berries combination of *J. phoenicea* L growing in the northeast of Algeria. Therefore, this study was aimed at investigating the chemical composition of EOs extracted from the leaves-berries combination of *J. phoenicea* L of distant geographical origins. Also, their antibacterial and antioxidant properties were evaluated.

Materials and Methods

Plant materials and study area

The leaves and berries of *J. phoenicea* were collected during the month of October 2017 in their natural habitats from the mountains. Five different regions located in the northeast of Algeria were chosen; Boutaleb (BO), Ain-Touta (AT), Senhadja-Gerbaz (SG), Djerma (DJ), and Maafa (MA) as depicted in Table 1. They were identified by Mr. Benssasi M., one of the experts of the Belezma national park, and assigned the voucher specimen number PG-06/10-T88.245 before being deposited at the herbarium of the Laboratory of Crop Production and Sustainable Valorization of Natural Resources, University Djilali Bounaama, Khemis Miliana, Algeria.

Sources of bacterial strains

Two Gram-positive bacteria namely, *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* were clinically isolated from patients who were hospitalized in the Hospital Center of Kolea, Tipaza, Algeria. Also, five Gram-negative bacteria; *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens,* and *Acinetobacter baumannii)* were supplied by the Microbiology laboratory of the Algerian Pasteur Institute in Algeria. Each strain was grown in a tube containing 10 mL of sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 24 hours. A pure culture of each bacterial strain was obtained by plating it on appropriate selective media and microscopic examination of the Gram-stained smear (Optika microscope, B-252, M.A.D; Apparecchiature Scientifiche, Milan, Italy).

Confirmation of bacterial identity by MALDI-TOF-MS method

A single colony was "picked" from a fresh overnight culture plate to a "spot" on a MALDI-TOF–MS target plate Microflex LT BIOTYPER (BRUKER)® (BrukerDaltonics, Germany). Then, it was overlaid with 1 µL of matrix solution (10 mg/mL α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and air-dried at room temperature for 5 minutes. After drying, the target plate was placed in the mass spectrometer's ionization chamber. All the spectra were compared with the reference spectrum of the BDAL database and with the main spectrum profiles created. Also, all identifications were reported with the following score values: < 1.7 was interpreted as an unreliable identification; 1.7–2.0 as a probable genus identification; 2.0–2.3 as a secure genus identification and probable species identification; and >2.3 was regarded as highly probable species identification. Only the highest score value of all mass spectra belonging to individual cultures (biological and technical replicates) was recorded.[22]

Extraction of essential oils from Juniperus phoenicea samples

The *J. phoenicea* plant samples were air-dried for 48 hours and then, separately crushed and milled into small pieces and sieved through a 0.5 mm mesh sieve. One hundred grams of the dried plant material were subjected to hydro distillation for 3 h with 500 mL distilled water using a Clevenger-type device (JF 1928). The EOs were collected with a pipette, stored in an Eppendorf tube at 4°C, and protected from light.

GC/MS analysis of essential oils from Juniperus phoenicea

Analysis of the essential oils was performed on a Shimadzu GC-MS QP-2010 system, coupled with a Combi-PAL5000 network mass selective detector system, and a ZB-5ms (30 m x 0.25 mm, film thickness 0.25µm) capillary column. The GC temperature was set at 50^{o}C for 2 min with an increase up to 250°C at a rate of 3°C/min. The temperatures corresponding to the interface, injector, and detector were set at 250^{o}C. Helium 5.0 (carrier gas) was used at a flow rate of 1.5 ml/min. An aliquot of 0.1 µL of EO sample was injected neat at a split ratio of 1:200. The MS detection used for the chemical qualitative analysis was performed with a quadrupole spectrometer operating in full scan (40-400 m/z) electron impact (EI) at ionization energy of 70 eV. The identification of the EOs components was conducted using Shimadzu software by correlating spectra with NIST147 and NIST27 libraries and they were verified and compared with retention indices drawn from flavornet and pherobase databases.[20,21] The results were expressed as a relative percentage from the total peak area.

Screening of essential oils from Juniperus phoenicea for antibacterial activity

The bacterial strains were screened for susceptibility to the EOs of *J. phoenicea* and antibiotics by performing the disc diffusion method on Mueller-Hinton agar medium (Pasteur Institute, Algiers, Algeria) according to the method of Performance Standards for Antimicrobial Susceptibility Testing (M100) of NCCLS (National Committee of Clinical and Laboratory Standards).[23] Petri plates were prepared with 20 mL of sterile Mueller Hinton agar (Sigma, Paris, France). The surface of the medium was inoculated by suspension of the cell (200 µL) adjusted by the McFarland 0.5 method (10^6 CFU/mL). Sterile Whatman paper discs (6 mm; ANTF-009-1K0, PRAT DUMAS, France) were impregnated with 20 µL of the EOs and placed on the media surface. Vancomycin (30µg) and Colistin (10µg) were used as positive controls. Negative controls were performed using paper discs without EOs. The plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs by Vernier calipers.

Antioxidant activity of Juniperus phoenicea essential oils

To measure the antioxidant activity of the test EOs, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was used. The method was carried out as described by Brand-Williams.[24] A fresh solution of 6.10^{-5} mol/L DPPH was prepared in methanol. Then, 3.5 mL of DPPH was mixed with 0.5 mL of oil samples in a test tube.

Table 1: Geographical locations and climatic stages of the five stations of the study area

Variety	Code	Longitude	Latitude	Altitude (m)	Location	Climate
Djerma	DJ	6°16'24.84"	35°40'22.3"	1031	Batna (Belezma National Park)	Semi-arid to cool sub humid
Maafa	MA	5°54'10.06"	35°15'45.81"	957	Batna (Southwest of Batna)	Semi-arid dry and cold
Ain Touta	AT	5°59'13.12"	35°26'21.78"	1005	Batna (stone quarry)	Arid, hot in summer and cold in winter
Boutaleb	BO	5°15'44.75"	35°41'58.22"	1534	Setif (the highlands)	Semi-arid (hot summers & harsh winters
SenhadjaGerbaz	SG	7°12'42.62"	36°55'26.02"	37	Skikda (complete wetlands)	Humid

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

After 30 min, the absorbance of these solutions was read at 517 nm. A triplicate reading was performed for each sample. The radical scavenging activity (RSA) of the DPPH• radical by the samples was calculated according to the formula:

$$RSA(\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol; and Abs_{sample} is the absorbance of DPPH radical + sample.

Statistical analysis

The data obtained were analyzed by using the SPSS Statistical Software for Windows (Version 23.0; Armonk, NY: IBM Corp). All the assays were carried out in triplicates. Quantitative variables were expressed as means ± standard deviation (SD) and qualitative variables as percentages. The ANOVA statistical test was used to analyze the compound data from both the leaf-berry and leaf-only extractions. The selected variables were the mean value of the component's concentration from the two extractions. Then, the main differences in the extracted components were determined using the Fisher LSD post-hoc test. A difference is statistically significant for a *p*-value less than or equal to 0.05.

Results and Discussion

Oil extracts and phytochemical constituents

A viscous liquid with a yellowish color and strong odor of juniper was obtained for the hydro distillation of the essential oil of *J. phoenicea*. As indicated in Table 2, the yield of EOs of *J. phoenicea* leaf-berry combination (Lb) and leaf-only (Lo) collected from five different localities (Table 1) ranged from 0.58 to 0.82% (w/w) and 0.56 to 0.81%, respectively. The Lb yields were higher than those of the Lo. Ennajar *et al.*,[13] obtained a higher yield of EOs from berries compared to the leaves (3.95 and 0.90%, respectively) during their study of Tunisian *J. phoenicea*. In the present study, the most significant yield was observed in the Maafa (MA) Lb sample with 0.82%, while the Ain Touta (AT) Lo sample showed fewer yields (0.56%). The yields and number of compounds from the EOs of *J. phoenicea* from the Lb and Lo differed from each other significantly. Locality and plants' parts significantly affect the yield and the chemical composition. In comparison with the literature data, these yields were lower than those previously investigated.[12,13,16,17] The concentration of the identified EOs compounds in the leaves and berries were expressed as relative abundance based on their retention time, with the results given in Table 3. In total, 54 metabolites were detected among which 15 were unidentified. There was a large difference between *J. phoenicea* Lo and Lb EOs. Both EOs revealed a wide variation in their chemical composition, contents, and the number of identified compounds. Although, Lb EO is richer in compounds compared to Lo oils. The monoterpenes represent the major fraction, in which α-pinene was the main component. The constituents of both EOs was characterized by the presence of terpenes, hence mainly quantitative differences among the samples were observed. According to the results in this study, monoterpenes hydrocarbons were characterized as a major class of compounds in the five samples, followed by oxygenated monoterpenes, and sesquiterpenes. In contrast to Afifi *et al*.,[25] who found that the major constituent of both Lb oils of the Egyptian *Juniperus* was sabinene. It was discovered in the present study that α-pinene was the major constituent of both oils in the samples of the present study. In contrast, the highest contents of α-pinene were observed in the Djerma (DJ) Lb (85.8%) and Maafa (MA) Lo (83.1%) samples (Table 2). On the other hand, the least contents were recorded in both the Senhadja (SG) Lb and Lo oils (50.0 and 42.1%, respectively). Moreover, oxygenated monoterpenes appeared in a significant amount (p<0.05) in both oils of SG and AT samples in contrast to the other samples, where they were detected only in traces. These results agree with those reported on the analysis of other juniper oils from Greece,[26-27] and Tunisia,[13,28,29] as well as Morocco[30] and Algeria.[7,31,32] Furthermore, δ-3-carene, a monotepene hydrocarbon, was also reported as an important metabolite in the samples of the present study, specifically in the Boutaleb (BO) Lb oil sample (19.3%). Also, the β-phellandrene was present in reasonable amounts notably in the SG Lb (23.8%) and Lo (26.9%) samples. Also, β-Pinene and β-myrcene were noted within both the EOs of the five varieties with concentrations ranging from 0.7 to 1.8% and 0.8 to 3.7%, respectively. Δ_3-Carene was present in not negligible amounts in both EOs specifically in the population from the BO Lb sample (19.3%).

In Algeria, Bekhechi *et al.* have focused on the chemical variability of the EOs of *J. phoenicea* var. turbinata collected from eight populations in Algeria. The 50 samples of the EOs that were divided into three clusters in most of the oil samples were dominated by α-pinene (30.2-76.6%), β-phellandrene (up to 22.5%), and α-terpinyl acetate (up to 13.4%). However, five out of the 50 samples exhibited an atypical composition characterized by the predominance of germacrene D (16.7-22.7%), α-pinene (15.8-20.44%), and α-terpinyl acetate (6.1-22.6%).[19]

Antibacterial activity of essential oils from Juniperus phoenicea

Table 4 presents the results of the antibacterial activities of the EOs in the agar diffusion method. All the EO samples showed variable degrees of activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Serratia marcescens* by producing a zone diameter of inhibition from 10 to 30 mm, depending on the susceptibility of the tested bacteria. On the other hand, it was observed that *Acinetobacter baumannii* and *Klebsiella pneumoniae* were resistant to all the EO samples. Also, it was observed that the *Enterococcus faecalis* and *Pseudomonas aeruginosa* strains manifested more sensitivity to the test EOs than the test antibiotics (Colistin and Vancomycin). However, the antibacterial activities of the test EOs against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were moderate. The results obtained are in agreement with the previous studies where Ramdani *et al.*,[32] found that the *Serratia liquefaciens* ATCC 27592 and *Staphylococcus aureus* ATCC 25923 were resistant to Boutaleb EOs. On the other hand, the present observations are in support of the finding by Derwich *et al.*,[33] who indicated that *E. coli* were the most sensitive strain tested toward the leaf oil of Moroccan *J. phoenicea*, while *S. aureus* was found to be sensitive to the same oil.

It was interesting to note that the most remarkable antibacterial activity with inhibition zones higher than 30 mm was noticed for the Djerma Lo EOs against *Enterococcus faecalis* and the Boutaleb and Ain-touta Lb EOs against *Pseudomonas aeruginosa*. Conversely, Serratia marcescens was resistant to all of the samples, with the exception of a mild effect from both of the Ain-touta (AT) EOs. The results of the antibacterial effect of the EOs on *Pseudomonas aeruginosa*in in this study are contrary to the observations made by AL-Khlifeh *et al.* in the Jordanian variety *of J. phoenicea*.[3]

Table 2: Yield and number of compounds identified in the Algerian leaf-only (Lo) and leaf-berry (Lb) of *J. phoenicea* essential oils.

Population	Djerma		Maafa		Ain Touta		Boutaleb		Gerbaz	
	Lo	Lb	Lo	Lb	Lo	Lb	Lo	Lb	Lo	Lo
Yield %	0.63	0.75	0.78	0.82	0.56	0.59	0.81	0.67	0.58	0.65
Number of compounds	9	28	21	22	28	16	20	20	16	13
Total %	98	93.48	0.97	99.3	97.20	99.79	98.97	99.4	99.4	99.8

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Chemical compounds from leaf-only and leaf-berry of *J. phoenicea* essential oils analyzed by GC-MS

	Population	CAS number	Djerma		Maafa		Ain Touta		Boutaleb		Gerbaz	
RT(s)	Compounds		Lb	Lo	Lb	Lo	Lb	Lo	Lb	Lo	Lb	Lo
7. 591	Tricyclene	508-32-1	-	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.1	0.0
7. 964	α.Pinene	80-56-8	85.8	65.3	74.0	83.1	77.3	68.3	68.4	67.7	50.0	42.1
8. 490	Norbornane	471-81-1	0.5	0.5	0.4	0.5	0.5	0.2	1.6	0.5	-	0.2
8. 529	Camphene	79-92-5	-	0.0	0.5	0.4	0.6	0.3	0.6	0.4	0.2	0.0
8. 653	B-Thujene	28634-89-1	-	0.0	-	0.2	0.2	0.2	-	0.0	-	0.0
8. 657	trans-Verbenol	1820-09-3	-	0.0	-	0.4	-	0.0	0.1	0.0	0.0	0.0
9. 290	1.3.8-*p*-Menthatriene	18368-95-1	-	0.0	-	0.0	-	0.0	0.1	0.0	-	0.0
9. 514	β-Pinene	18172-67-3	1.1	0.7	1.1	0.8	0.8	1.3	0.7	0.9	1.5	1.8
9. 941	β-Myrcene	123-35-3	1.9	1.1	1.7	1.2	1.4	3.0	0.8	2.3	5.3	5.7
10. 550	α-Phellandrene	99-83-2	-	0.0	-	0.0	-	1.8	-	1.3	3.8	4.1
10. 646	δ-3-Carene	498-15-7	0.7	0.6	4.7	6.0	3.3	2.5	19.3	6.9	-	0.0
11. 254	*o*-Cymene	527-84-4	-	0.3	1.2	0.0	0.7	0.9	0.6	0.9	0.8	1.2
11. 421	D-Limonene	5989-27-5	-	0.7	-	1.1	0.9	0.0	1.1	0.0	-	0.0
11. 443	Terpinolene	586-62-9	1.5	0.0	-	0.4	-	0.3	-	0.2	-	1.5
11. 481	β-Phellandrene	555-10-2	-	0.3	4.0	0.0	1.3	14.5	-	10.1	23.8	26.9
11. 923	3-Carene	13466-78-9	-	0.0	-	0.0	0.2	0.2	-	0.0	0.4	0.4
13. 572	4-Carene	29050-33-7	-	0.0	-	0.0	-	0.0	0.2	0.2	-	0.0
14. 149	Linalool. formate	115-99-1	4.6	4.0	0.7	1.2	1.0	0.0	0.3	0.3	-	0.0
14. 474	Fenchone	1195-79-5	-	0.0	0.5	0.3	0.6	0.0	0.9	0.0	-	0.0
15. 161	α-Campholenal	4501-58-0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
16. 481	*cis*-Verbenol	18881-04-4	-	0.0	-	0.0	0.2	0.0	1.3	0.0	-	0.0
17. 816	α-Terpineol	98-55-5	-	0.6	1.9	0.3	0.5	0.0	0.5	3.9	-	0.3
18. 959	Berbenone	80-57-9	-	0.0	-	0.0	0.5	0.0	-	0.0	-	0.0
19. 998	p-Menth-1-en-3-one	491-04-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
20. 072	β-Terpinyl acetate	10198-23-9	-	0.0	1.7	0.0	1.1	0.0	-	0.0	1.3	0.0
23. 248	Limonene	5989-27-5	-	0.5	-	0.0	-	0.0	-	0.0	5.5	0.0
23. 319	Terpinolene	586-62-9	0.0	0.0	0.0	0.0	0.0	5.6	0.0	0.0	0.0	0.0
23. 326	Cyclobutane. 1.2-bis(1-methylethenyl)-. trans	19465-02-2	-	0.0	-	0.0	-	0.0	-	0.0	5.3	0.0
23. 346	Dihydrocarvyl acetate. (-)-	20405-60-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.6
24. 382	Copaene	3856-25-5	-	0.4	0.2	0.0	0.3	0.0	-	0.0	-	0.0
25. 899	Caryophyllene	87-44-5	-	0.9	0.5	0.4	0.7	0.2	0.6	0.6	0.3	0.0
26. 426	Thujopsene	470-40-6	-	0.3	-	0.2	-	0.0	0.5	0.0	-	0.0
27. 109	α.Caryophyllene	6753-98-6	-	1.1	0.4	1.2	0.4	0.0	-	0.3	0.3	0.0
27. 753	β-Cubebene	13744-15-5	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27. 941	Germacrene D	23986-74-5	-	0.0	-	0.3	0.1	0.0	0.2	0.6	0.6	0.2
27. 970	Isoledene	95910-36-4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28. 319	Bicyclosesquiphellandrene	54274-73-6	-	2.3	0.3	0.4	0.2	0.0	-	0.0	-	0.0
28. 425	α-Cubebene	24406-05-1	-	0.8	0.3	0.0	0.7	0.0	-	0.0	-	0.0
28. 454	α.Muurolene	10208-80-7	-	1.0	-	0.0	0.5	0.0	-	0.0	-	0.0
28. 729	γ-Cadinene	39029-41-9	0.8	2.1	-	0.0	-	0.0	-	0.0	0.2	0.4
29. 104	δ-Cadinene	483-76-1	-	4.0	0.4	0.0	0.5	0.0	-	0.0	-	0.0
29. 143	α-Cadinene	24406-05-1	1.2	0.3	-	0.6	-	0.0	-	0.7	-	0.0
29. 260	Calamenene	483-77-2	-	3.5	1.5	0.0	1.2	0.0	-	0.0	-	0.0

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

30. 345	Germacrene B	15423-57-1	-	0.2	-	0.0	0.9	0.2	-	0.0	-	0.0
30. 359	γ.Elemene	11029-06-4	-	0.8	0.3	0.7	-	0.0	1.0	1.1	-	0.0
30. 668	Citronellyl propanoate	141-14-0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total monoterpenes		94.6	73.2	87.6	95.1	88.2	98.1	94.0	90.6	85.1	81.3
	Monoterpene hydrocarbons		94.6	72.9	83.6	95.0	86.9	83.6	93.8	80.5	61.3	54.4
	Oxygenated monoterpenes		-	0.3	4.0	0.2	1.3	14.5	0.1	10.1	23.8	26.9
	Total sesquiterpenes		1.5	1.8	0.3	1.4	0.7	0.3	1.2	2.2	0.6	1.8
	Sesquiterpene hydrocarbons		1.5	0.8	0.3	1.4	0.1	0.3	1.1	2.2	0.6	1.7
	Oxygenated sesquiterpenes		-	1.0	-	0.0	0.5	0.0	-	0.0	-	0.0
	Other		2.5	18.4	8.9	2.7	8.3	1.6	3.8	6.6	13.8	16.5
	TOTAL (%)		98.6	93.5	96.8	99.3	97.2	100.0	99.0	99.4	99.4	99.5
	TOTAL (NO)		9	28	21	22	28	16	20	20	16	12

RT: Retention time(s); CAS: Registry number (Chemical Abstracts Service); Lb: Essential oil of leaf and berry; Lo: Essential oil of leaf only; Other: Other chimotype compounds; TOTAL (%): Total of identified compounds in percentage.

Table 4: Mean disk diffusion zone diameters associated with juniper essential oils

		Inhibition zone diameter (mm)						
Location	**Part**	***S. a***	***E. c***	***E.f***	***P.a***	***S.m***	***A. b***	***K.p***
Djerma	*Lb*	16.5 ± 0.23	12.0 ± 0.10	25 ± 0.14	28	00	00	00
	Lo	17.3 ± 0.31	11.9 ± 0.48	30 ± 1.11	28	00	00	00
Maafa	*Lb*	17.3 ± 0.84	10.7 ± 0.01	22	22	00	00	00
	Lo	14.5 ± 0.32	12.0 ± 0.53	25 ± 0.23	24	00	00	00
Ain Touta	*Lb*	15.0 ± 1.79	12.1 ± 0.65	19 ± 0.06	30	12 ± 0.01	00	00
	Lo	14.8 ± 0.523	NI	24	22	16	00	00
Boutaleb	*Lb*	14.6 ± 0.16	14.0 ± 0.67	24 ± 0. 08	30 ± 0.02	00	00	00
	Lo	14.7 ± 0.49	14.2 ± 0.08	24	24	00	00	00
Gerbaz	*Lb*	12.8 ± 0.97	NI	28	12	00	00	00
	Lo	12.7 ± 0.67	10.6 ± 0.82	28	25	00	00	00
Controls	*CS*	00	17 ± 0.04	00	7 ± 0.33	00	00	00
	VA	10 ± 1.22	00	11 ± 0.62	00	00	00	00

Lb: Essential oil of leaf and berry; Lo: Essential oil of leaf only*; S.a: Staphylococcus aureus* (ATCC 25923); E.c: *Escherichia coli* (ATCC 25922); E.f*: Enterococcus faecalis*; P.a: *Pseudomonas aeruginosa*; S.m: *Serratia marcescens;* A.b: *Acinetobacter baumannii;* and K.p: *Klebsiella pneumoniae*.

The results revealed that Gram-negative bacteria were more resistant than Gram-positive strains. These findings are in agreement with the previous studies of Bouzouita *et al.*,[35]and Ait-Ouazzou *et al.*,[36] Moreover, in this study, Lb EO compounds were less effective against *S. aureus*. El-Sawi *et al*.,[16] reported a similar result with the Egyptian variety *of J. phoenicea*. Among the strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis*, the differences in antibacterial activity achieved by the Lb and Lo EOs were extremely significant (p 0.0001).

In this study, the bacterial strain, collection site (geographical variation), and plant parts were found to be significantly correlated. The antibacterial property of EOs is difficult to explain because many factors are known to contribute and cross act within it. For example, the antimicrobial activity of the EOs of *J. phoenicea* could partly be associated with the major constituents of its oil such as α-pinene and β-phellandrene. These components have been reported to display antimicrobial effects.[28,33,36,37] These results are in support of the observations made by Raho *et al.,*[38] who found that the zones of inhibition of *E. coli* and *S. aureus* were 16 and 19 mm, respectively. This activity is probably due to the ability of *J. phoenicea* components of the EOs to complex with both the extracellular proteins and bacterial cell walls. They may also disrupt microbial membranes.[38] Similarly, Angioni *et al*.,[39] also reported that EOs from the leaves of *J. phoenicea* exhibited weak activity against *S. aureus* and there was no activity against *E. coli* or *P. aeruginosa*. Several studies have reported that the behavior of EOs and antimicrobials can be severely modified under the presence of solvents or influence of factors such as pH, the ability of the antibacterial compound to diffuse uniformly through the agar, the volume of EO placed on the paper disks, or thickness of the agar layer.[40,41] As a result, it was difficult to compare the findings to those of other investigations.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Antioxidant activity of essential oils from Juniperus phoenicea

It is well known that free radicals including reactive oxygen species, such as hydroxyl, peroxyl, and superoxide can damage cellular constituents such as DNA, proteins, and lipids and so responsible for many chronic diseases.[42] Therefore, the ability to scavenge free radicals is an important antioxidant property. The antioxidant activity of EOs of the samples is shown in Figure 2. It was evaluated as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The results of the present study showed that all the EOs of *Juniperus phoenicea* exhibited a lower antioxidant activity compared to the ascorbic acid, used as a positive control.

All the samples were able to reduce the stable, purple-coloured radical DPPH into yellow-coloured DPPH-H. It was noted that the radical-scavenging activities of the Lb EOs are significantly higher compared with the radical-scavenging activities of Lo oil, except in the Boutaleb sample. This observation could be influenced by the composition and profiles of terpene compounds. The highest antioxidant activities were observed in the EOs extracted from the Lo Djerma's and Gerbaz's *J. phoenicea* (38.92 and 38.79%, respectively). *α*-Pinene, Δ3-Carene, and *β*- caryophyllene are the most frequently found compounds in the EOs. There was a correlation between these compounds and DPPH scavenging activity. On the other hand, the results of the present study are not in agreement with the observations of Amorati *et al.*,[43] who showed that the antioxidant properties are related to the presence of oxygenated compounds (oxygenated monoterpenes and oxygenated sesquiterpenes). The Lb Gerbaz EO sample, characterized by the lowest α-Pinene rate exhibited the highest antioxidant activity, as observed by Zheljazkov *et al.*,[44] who reported a negative relationship of this compound with the antioxidant activity. The contribution of a single EO compound to their antioxidant activity is still a subject of several debates. Mimica-Duki*et al.*,[45] and Yadegarinia *et al.*,[46] reported that oxygenated monoterpenes act as radical scavenging compounds. However, it is difficult to assign this activity to the only oxygenated compounds because of the chemical complexity of EOs which can generate a synergistic effect between the various compounds.

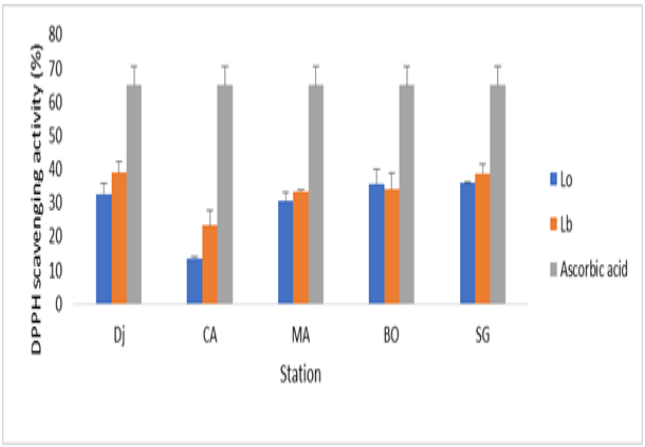

Figure 2: Free radical scavenging activity of Algerian leaf-only and leaf-berry of *J. phoenicea* essential oils.
Lb: Essential oil of leaf and berry; Lo: Essential oil of leaf only; DJ; Djerma ; MA : Maafa ; AT: Ain Touta; BO; Boutaleb; SG: Gerbaz.

Conclusion

The findings of the present study reveal that the EOs of Lb and Lo of *J. phoenicea* L grown in the northeast of Algeria are characterized by a chemical composition rich in monoterpenes hydrocarbon and oxygenated monoterpenes. They showed an important antioxidant activity and a good antibacterial effect on the test bacteria, which can justify the multiple uses of the plant in Algerian traditional medicine. These EOs could be considered as potential alternatives for synthetic antibiotics and natural additives in the food, cosmetic, and pharmaceutical industries. However, the safety and toxicity of these compounds require further investigation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to thank the Directorate-General for Scientific Research and Technological Development (DGRSDT) of the Algerian Ministry of Higher Education and Scientific Research for their financial support.

References

1. Jugreet BS, Suroowan S, Rengasamy RRK, Mahomoodally MF. Chemistry, bioactivities, mode of action and industrial applications of essential oils. Trends Food Sci Technol. 2020; 101(07):89-105.
2. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharm. 2013; 6(12):1451-1474.
3. Iseppi R, Mariani M, Condò C, Sabia C, Messi P. Essential oils: A natural weapon against antibiotic-resistant bacteria responsible for nosocomial infections. Antibiot. 2021; 10(4):417.
4. Essien EE, Ascrizzi R, Flamini G. Characterization of volatile compounds of *Diplazium esculentum*. Chem Nat Compd. 2019; 55(5):958-959.
5. Adams RP. Junipers of the world: The genus Juniperus. (4th ed) Trafford Publishing. USA. 2014; 2p.
6. Cheraif K, Bakchiche B, Gherib A, Bardaweel SK, ÇolAyvaz M, Flamini G, Ghareeb MA. Chemical composition, antioxidant, anti-tyrosinase, anti-cholinesterase and cytotoxic activities of essential oils of six Algerian plants. Mol. 2020; 25(7):1710.
7. Dob T, Dahmane D, Chelghoum C. Essential oil composition of *Juniperus oxycedrus*. growing in Algeria. Pharm Biol. 2006; 44(1):1-6.
8. Harhour A, Brada M, Fauconnier ML, Lognay G. Chemical composition and antioxidant activity of Algerian *Juniperus phoenicea* essential oil. Nat Prod Sci. 2018; 24(2):125-131.
9. Terfaya B, Makhloufi A, Mekboul A, Benlarbi L. Abdelouahid D. *In vitro* antifusarial activity of a tar extracted from the *Juniperus phoenicea* L. Wild in Southwest of Algeria. Phytother. 2019; 17(10):129-133.
10. Dob T, Dahmane D, Chelghoum C. Chemical composition of the essential oil of *Juniperus phoenicea* L. from Algeria. J Essent Oil Res. 2008; 20(1):15-20.
11. Achak N, Romane A, Alifriqui M, Adams RP. Chemical studies of leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). J Essent Oil Res. 2009; 21:337-341.
12. Abdelli W, Bahri F, Höferl M, Wanner J, Schmidt E. Jirovetz L. Chemical composition, antimicrobial and anti-inflammatory activity of Algerian *Juniperus phoenicea* essential oils. Nat Prod Commun. 2017; 12:611-614.
13. Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Savagnac A, Abderraba M. Romdhane M. The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils. J Sci Food Agric. 2010; 90(3):462-470.
14. Ghouti D, Lazouni HA, Moussaoui A, Chabane SD.

Chemical profile, *in vitro* antibacterial and antioxidant activities of *Juniperus phoenicea* L. and *Cotula cinerea* (Del.) essential oils from Southwestern Algeria. Phytother. 2018; 16(1):74-83.
15. Abu-Darwish M, Gonçalves M, Cabral C, Cavaleiro C, Salgueiro L. Chemical composition and antifungal activity of essential oil from *Juniperus phoenicea* sub sp. Phoenicea berries from Jordan. Acta Aliment. 2013; 42(4):504-511.
16. El-Sawi S, Motawae H, Ali A. Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicea* L. grown in Egypt. Afr J Trad Compl Altern Med. 2008; 4(4):417-426.
17. Elmhalli F, Garboui SS, Karlson AKB, Mozūraitis R, Baldauf SL, Grandi G. Acaricidal activity against *Ixodes ricinus* nymphs of essential oils from the Libyan plants *Artemisia herba* alba, *Origanum majorana* and *Juniperus phoenicea*. Vet Parasitol Reg Stud Rep. 2021; 24:100575.
18. Abu-Darwish MS and Efferth T. Medicinal plants from near east for cancer therapy. Front Pharmacol. 2018; 9:56-73.
19. Bekhechi C, Atik Bekkara F, Consiglio D, Bighelli A, Tomi F. Chemical variability of the essential oil of *Juniperus phoenicea*var.turbinata from Algeria. Chem Biodivers. 2012; 9:2742-2753.
20. The Pherobase database of Pheromones and Semiochemicals. [Online]. 2020 [cited 2020 Dec 20] (Online) Available from:**https://www.pherobase.com**
21. Flavornet and Human Odor Space.[Online]. 2020 [cited 2020 Sep 20] (Online) Available from: **http://www.flavornet.org/**
22. Debib A, Menadi S, Sahnouni F, Boukhatem MN, Ouahchia C, Kaced A. Bacterial inhibitory effect of Algerian Pomegranate (*Punica Granatum* L.) extracts (peel, juice, and seed) against multidrug resistant bacteria. J Microb Biotech Food Sci. 2021; e4622.
23. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, CLSI document M100. (31st edition). Wayne, PA; 2021; 30-60p.
24. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995; 28:25–30.
25. Afifi MS, El-Sharkawy SH, Maatoog GT, Sohly M. Rosazza JPN. Essential oils of *Thuja occidentalis, Thuja orientalis, Cupressus sempervirena* and *Juniperus phoenicea*. Mansoura J Pharm Sci. 1992; 8:37-46.
26. Evergetis E, Michaelakis A, Papachristos DP, Badieritakis E, Kapsaski-Kanelli VN, Haroutounian SA. Seasonal variation and bioactivity of the essential oils of two *Juniperus* species against Aedes (Stegomyia) albopictus (Skuse, 1894). Parasitol Res. 2016; 115:2175-2183.
27. Vourlioti-Arapi F, Michaelakis A, Evergetis E, Koliopoulos G, Haroutounian SA. Essential oils of indigenous in Greece six *Juniperus* taxa. Parasitol Res. 2012; 110:1829-1839.
28. Medini H, Elaissi A, Larbi Khouja M, Piras A, Porcedda S, Falconieri D, Chemli R. Chemical composition and antioxidant activity of the essential oil of *Juniperus phoenicea* L. berries. Nat Prod Res. 2011; 25:1695-1706.
29. Aouadi M, Kamel M, Essia S, Wissem AW, Mohamed SA, Hafidh A. J Essent Oil Res. 2021; 33(6):1-10.
30. Fouad B, Abderrahmane R, Youssef A, Rajae H, Fels MA. Chemical composition and antibacterial activity of the essential oil of Moroccan *Juniperus phoenicea*. Nat Prod Commun. 2011; 6:1515-1518.
31. Bouyahyaoui A, Bahri F, Romane A, Höferl M, Wanner J, Schmidt E, Jirovetz, L. Antimicrobial activity and chemical analysis of the essential oil of Algerian *Juniperus phoenicea*. Nat Prod Commun. 2016; 11:519-522.
32. Ramdani M, Lograda T, Silini H, Zeraib A, Chalard P, Figueredo G, Zerrar S. Antibacterial activity of essential oils of *Juniperus phoenicea* from Eastern Algeria. J Appl Pharm Sci. 2013; 3:022-028.
33. Derwich E, Benziane Z, Boukir A. Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. Int J Agric Biol. 2010; 12:199-204.
34. Al-Khlifeh EM, Khleifat KM, Al-Tawarah NAFE., Al-Limoun MO, Abdel-Ghani AH, Alsharafa K, Qaralleh H. Genetic diversity and chemical composition of *Juniperus phoenicea* L; Reflect on its antimicrobial activity. Int J Pharm Res. 2021; 13(1): 3409-3426.
35. Bouzouita N, Kachouri F, Ben Halima M, Chaabouni MM. Chemical composition and antioxidant, antimicrobial and insecticidal activities of the essential oil of *Juniperus phoenicea*. J Soc Chim Tunis. 2008; 10:119-125.
36. Ait-Ouazzou A, Lorán S, Arakrak A, Laglaoui A, Rota C, Herrera A, Conchello P. Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperus longus* essential oils from Morocco. Food Res Int. 2012; 45(1):313–319.
37. Abboud MM, Aljundi IH, Khleifat, KM, Dmour S. Biodegradation kinetics and modeling of whey lactose by bacterial hemoglobin VHb-expressing *Escherichia coli* strain. Biochm Eng J. 2010; 48(2):166-172.
38. Raho GB, Otsmane M, Sebaa F. Antimicrobial activity of essential oils of *Juniperus phoenicea* from Northwestern Algeria. J Med Plant Res. 2017; 1:1-7.
39. Angioni A, Barra A, Russo MT, Coroneo V, Dessí S, Cabras, P. Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. J Agric Food Chem. 2003; 51(10):3073-3078.
40. Burt S. Essential oils: their antibacterial properties and potential applications in foods- a review. Int J Food Microbiol. 2004; 94(3):223–253.
41. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Vuorela P. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 2000; 56(1):3-12.
42. Debib A, Tir-Touil A, Mothana RA, Meddah B, Sonnet P. Phenolic content, antioxidant and antimicrobial activities of two fruit varieties of Algerian *Ficus carica* L. J Food Biochem. 2014; 38(2):207-215.
43. Amorati R and Valgimigli L. Methods to measure the antioxidant activity of phytochemicals and plant extracts. J Agric Food Chem. 2018; 66(13):3324-3329.
44. Zheljazkov VD, Kacaniova M, Dincheva I, Radoukova T, Semerdjieva IB, Astatkie T, Schlegel V. Essential oil composition, antioxidant and antimicrobial activity of the galbuli of six juniper species. Ind Crops Prod. 2018; 124:449–458.
45. Dukić MN, Božin B, Soković M, Mihajlović B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Med. 2003; 69:413-419.
46. Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochem. 2006; 67:1249-1255.