



Chemical Composition and Antioxidant Activity of Algerian *Juniperus Phoenicea* Essential Oil

Aicha Harhour^{1,*}, Moussa Brada¹, Marie-Laure Fauconnier², and Georges Lognay³

¹Laboratory of Natural Substances Valorization, University of Djilali Bounaama, Khemis Miliana, Road of Theniet El-Had, 44225, Algeria

²Agro-Bio Chem Department, General and Organic Chemistry, University of Liege, Gembloux Agro-Bio Tech, 2, Passage of deportees, B-5030 Gembloux, Belgium

³Agro-Bio Chem Department, Analytical Chemistry, University of Liege, Gembloux Agro-Bio Tech, 2, Passage of deportees, B-5030 Gembloux, Belgium

Abstract – Berries and branches essential oil of *Juniperus phoenicea* were obtained by electromagnetic induction heating assisted extraction and by hydrodistillation with a yield varied from (1.2 ± 0.3 to $2.4 \pm 0.7\%$) and from ($0.6 \pm 0.1\%$ to $1.1 \pm 0.1\%$), respectively. forty eight compounds were identified representing (97.2 – 99.7%) of the oil. α -Pinene (40.3 – 67.8%) and δ -3-carene (13.5 – 26.8%) were the main compounds in berries and branches essential oils. Antioxidant activity was evaluated by three means: inhibition of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical, reducing power and β -Carotene/linoleic acid bleaching. The antioxidant activity of essential oils showed IC_{50} ranging from $67.6 \pm 1.02 \mu\text{g/mL}$ to $131.5 \pm 0.8 \mu\text{g/mL}$ for berries and from $98 \pm 1.25 \mu\text{g/mL}$ to $166.8 \pm 0.29 \mu\text{g/mL}$ for the branches. Berries oil show more potent antioxidant activity compared to branches. This result is supported by the three methods investigated in this work.

Keywords – *Juniperus*, Essential oil, Electromagnetic induction heating, Hydrodistillation, Chemical composition, Antioxidant activity

Introduction

The genus *Juniperus* (Cupressaceae) includes many native plants of the Mediterranean regions. In the Algerian flora, five species are present: *J. oxycedrus* L., *J. Sabina* L., *J. thurifera* L., *J. communis* L. and *J. phoenicea* L.¹ This last is commonly known in North Africa as “Al aar- aar” and a preparation of its leaves and cones is used as a hypoglycemic regulator, whereas the leaves are used against broncho-pulmonary diseases and as a diuretic.²⁻³ The *J. phoenicea* oils of extracted from berries as well as wood are used for the treatment of many diseases like leprosy typhoid and tape worm infections.³ While the dried and powdered fruit can heal skin ulcers and abscesses.⁴ In Algeria, it is best known for its antidiarrheal activity.⁵⁻⁶ A lot of reports exists dealing with the chemical composition of *J. phoenicea* essential oils grown in many countries (Table 1). *J. phoenicea* has been characterized

by the occurrence of monoterpenes with α -pinene as major constituent, although its proportion varied drastically from sample to sample.⁵⁻¹³ The geographical and bioclimatic factors of the region, the extraction process and plant parts used, can partly explain the chemical variability of the *J. phoenicea* essential oil compositions. Antioxidant activity of essential oils from different juniper species has been established and the potential of these plants as sources of natural antioxidant has been demonstrated.^{11,14-19}

J. excelsa, *J. oxycedrus* subsp. *oxycedrus*, *J. Sabina* and *J. phoenicea* might be used in the food industry as increasing the shelf-life of raw and processed foods.¹⁷ Leaves of *J. phoenicea* may provide a good source of natural products with interesting medicinal properties.¹⁸ And essential oils which could be potential alternatives to synthetic bactericides and as natural antioxidants for foods.^{11,14-15}

The aims of this study were to further analyze the composition of the essential oils of wild *J. phoenicea* from Ain- Defla (northern Algeria) using GC-FID and GC-MS, as well as to investigate their antioxidant properties. To the authors best knowledge *J. phoenicea*

*Author for correspondence

Aicha Harhour, Laboratory of Natural Substances Valorization, University of Djilali Bounaama, Khemis Miliana, Road of Theniet El-Had, 44225, Algeria.

Tel: +213-782834513; E-mail: aichaharhour@yahoo.fr

Table 1. Major compounds of *J. phoenicea* essential oils (%) from various countries

Country	Author	Plant part	Major compounds								
			α -Pinene	δ , 3-Carene	β -Phellandrene	α -cedrol	α -Terpinolene	α -Terpinemyl acetate	α -Phellandrene	limonene	manoyloxide
Espagne	(7)	L	53.5	1.7	5.9	-	-	-	0.8	T	14.4
	(7)	L	41.2	1.5	4.9	-	-	0.1	0.7	0.6	22
Portugal	(7)	L	34.1	-	19.2	-	-	12.5	3.1	0.2	0.6
	(7)	L	57.8	-	8	-	-	5	1.1	1	-
Italy	(8)	L	48.9	22.82	10.01	-	0.62	2.61	0.08	-	-
		B	84.55	3.61	2.56	-	0.38	0.63	0.02	-	-
Greece	(7)	L	41.8		3.5	-	-	4.6	0.6	3.5	-
Maroco	(9)	L	34	21	-	-	-	6.8	2.2	3.1	-
		B	79.1	5.7	-	-	-	0.9	0.45	14.6	-
	(10)	L	49.15	1.05	0.98	-	-	-	7.39	-	-
Tunisia	(11)	L	55.7	10.7	-	-	-	-	0.8	0.7	-
	(11)	B	80.7	4.5	-	-	-	-	-	-	-
Egypte	(12)	L	38.2	0.99	-	31.2	-	1.01	-	0.74	-
		B	39.3	1.25	4.13	0.47	-	3.36	1.01	-	-
Alegria	(5)D	L	40.2	0.5	t	-	-	T	14.7	-	-
	(6) T	L	34.5	4.7	22.4	-	-	14.7	0.6	1.2	-
	(5) S	L	54	-	7.3	-	-	0.4	-	0.6	-
	(13) Ba	L	36.5	12.4	4.4	-	-	-	-	-	0.2
	(13) Bi	L	59	3	0.8	-	-	-	-	0.7	1.6
	(13) M	L	47.1	-	1.7	-	-	0.4	-	0.8	-

(5) Dob et al, 2008; (6) Mazari et al, 2010; (7) Adams et al, 1996; (8) Angioni et al, 2003; (9) Mansouri et al, 2011; (10) Derwich et al, 2011; (11) Ennadjar et al., 2009; (12) El Sawi et al, 2007; (13) Ramdani et al, 2013; L : Leaves; B : Berries; D : Djelfa; T : Tlemcen; S : Stif; Ba : Batna; Bi : Biskra; M : M'sila .

essential oil from this region has never been studied before. This work will contribute to the knowledge of a local product that could improve the use of Algerian *Juniperus*.

Experimental

General experimental procedures – A pressure cooker (5 L, Chimex, China) was used as container for plant material and water. The EMI heating was performed using hot plate (1800 W, Tristar IK6174, EU). A Clevenger apparatus was used for hydrodistillation. Analyses have been undertaken using GC- FID (Thermo – Trace, Interscience, Belgium), GC/MS operated by HP Chem Station software. All the reference molecules used were from Sigma-Aldrich (Germany).

Plant material – Branches and berries of wild *J. phoenicea* were collected in the first week of June 2013 in Ain Defla region (- latitude: 36°25' N; longitude: 2° 21'7" E; Altitude: 365 m). Voucher specimen was deposited

in the Herbarium of the Agronomic Department of Djilali Bounaama University of Khemis Miliana.

Extraction method – The essential oil was extracted using two different methods, in order to introduce the electromagnetic induction heating assisted extraction as a new extraction approach. Therefore, the yield and chemical composition of *J. phoenicea* extracted by EMI heating were studied and compared with those obtained by classical hydrodistillation.

Hydrodistillation (HD) – 100 g of berries and branches (dried at room temperature ~ 20 °C for 10 days) of *J. phoenicea* were submitted to hydrodistillation with a Clevenger apparatus, and extracted during 1.5 hour. The essential oil was collected by decantation, dried over anhydrous sodium sulfate and stored in closed dark vials at 4 °C until analysis.

Electromagnetic induction heating assisted extraction (EMI) – 400 g of berries or branches was subjected to extraction. The system was equipped with a pressure cooker (5 L capacity), placed on an induction plate

(1800 W), whereas the extraction was carried out in magnetizable conditions. The essential oil was collected by decantation and dried over anhydrous sodium sulfate, and stored at 4 °C in dark glass bottles until use.

Essential Oil analysis – 10 mg of essential oil was dissolved in 5 ml diethyl ether and analyzed by gas chromatography (GC) and by (GC-MS).

GC-FID analysis – The analysis of the oil was carried out by means of an Agilent technology HP GC 6890 system with a flame ionization detector (FID), using a capillary column coated with 5% phenyl-methylpolysiloxane (30 m × 0.25 mm × 0.25 µm film thickness Agilent Technologies, Hewlett-Packard, CA, USA). Temperature program was as follows: 40 °C during 1 min, then raised in a first ramp to 200 °C at 6 °C/min, followed by a second ramp to 280 °C at 30 °C/min, with a final hold at 280 °C during 2 min. Injection (1 µl) was realized in splitless mode at 280 °C. Detector temperature was fixed at 300 °C; Carrier gas was helium at 1 mL/min.

GC-MS analysis – GC/MS was performed with an Agilent HP 6890 GC system coupled with an Agilent HP5973 Network Mass Selective Detector. Agilent HP-5MS capillary column (30 m × 0.25 mm, df=0.25 m), a split-splitless injector at 250 °C (splitless mode), temperature program: from 40° - 250 °C at 6 °C/min, mobile phase: carrier gas was helium at 1 mL/min. The mass spectra have been recorded in EI mode (70 eV), scanned mass range: from 35 to 500 amu. Source and quadrupole temperatures were fixed at 230 °C and 150 °C, respectively. The identification of the components was performed on the basis of chromatographic retention indices and by comparison of the recorded spectra with computed spectral library (Wiley275.L, Adams 2001). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with literature data.²⁰⁻²¹ Retention indices (RI) were calculated according to Joulain and König.²¹

Antioxidant activity determination – Despite numerous and various methods, only one procedure cannot identify all possible mechanisms characterizing a potential antioxidant. Therefore antioxidant activity of *J. phoenicea* essential oils has been determined by using three complementary assays: inhibition of DPPH free radical (i), reducing power (ii), and β - Carotene/ linoleic acid bleaching (ii).

DPPH assay – The stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was used. Briefly fifty µL of various concentrations of the samples (from 25 to 100 µg/ mL) in methanol were added to 2 mL of 60 µM methanol solution of DPPH. After an incubation or 30 min at room tem-

perature (20 degrees), the absorbance was recorded at 517 nm.²²⁻²³ Butylated hydroxyl toluene (BHT) and ascorbic acid were used as positive controls and blanks have been systematically performed. The inhibition percentage of the DPPH radical by the samples was calculated according to the formula:

$$\% \text{ Inhibition} = [(Ab - Aa) / Ab] \times 100 \quad (1)$$

Where:

Ab: is the absorbance of the blank sample and Aa; is the absorbance of the test sample.

The activity was calculated as 50% inhibition concentration (IC₅₀) by plotting the inhibition percentage against the sample concentration. A lower IC₅₀ value indicates greater antioxidant activity. Tests were carried out in triplicate.

Determination of the reducing power – The reducing power of the essential oils was determined according to the method of Hseu et al.²⁴ One mL of different concentrations of the samples (from 25 to 100 µg/mL) were mixed with phosphate buffer (1 mL, 0.2 M, pH = 6.6) and potassium ferricyanide [K₃Fe (CN)₆] (1 mL, 1%). The mixture was incubated at 50 °C for 20 min. One mL of trichloroacetic acid (TCA) (10%) was added to the solution, which was then centrifuged for 10 min at 3000 rpm. The supernatant was and mixed with distilled water (1.5 mL) and FeCl₃ solution (150 µL, 0.1%). The absorbance was measured at 700 nm and compared to the standards. Increased absorbance of the reaction mixture indicated increased reducing power. The assay was carried out in triplicates.

β - Carotene/linoleic acid bleaching assay – The β-carotene/linoleic acid test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O₂). A mixture of β-carotene and linoleic acid was prepared by adding together 0.5 mg of β-carotene in 1 mL chloroform, 25 µL pure linoleic acid and 200 mg tween 40. The chloroform was then completely evaporated under vacuum and 100 mL of pure Hydrogen peroxide was subsequently added to the residue and mixed to form a clear yellowish emulsion. 350 µL of various concentrations of sample (essential oil, BHT and ascorbic acid; 25, 50, 75 and 100 µg/mL) in methanol were added to 2.5 mL of the above emulsion in test tubes and mixed. The sealed tubes were incubated in water bath at 50 °C for 2 h together with a negative control (blank) containing methanol instead of sample. The absorbance values were measured at 470 nm.²⁵ Antioxidant activity was calculated as percentage of inhibition (I %) relative to the control using the following

equation:

$$\% I = \frac{A (\beta\text{-carotene after 2h assay})}{A (\text{initial } \beta\text{-carotene})} \times 100 \quad (2)$$

Where:

A (β -carotene after 2 h assay) is the absorbance value of β -carotene after 2 h assay remaining in the samples and A (initial β -carotene) is the absorbance value of β -carotene in the beginning of the experiment. The activity was calculated as 50% inhibition concentration (IC_{50}). Each assay was made in triplicate.

Statistical analysis – All assays were carried in triplicates and results expressed as means \pm standard deviation. IC_{50} -value (μg extract/ml) is the effective concentration which proves 50% of activity, was calculated for each assay. Statistical comparisons were done with Student's test. Differences were considered to be highly significant at $P < 0.01$ and significant at $P < 0.05$.

Result and Discussion

The essential oils of berries and branches of *J. phoenicea* have been recovered with yields ranging from (1.2 to 2.4%) for EMI heating and from (0.6 to 1.1%) for classical hydrodistillation.

The electromagnetic induction heating assisted extraction showed higher recovery, this innovative process is efficient technique in comparison to hydrodistillation, this efficiency was probably based on the interaction between the speed of the EMI heating and evaporation of the essential oil found in vegetable material.

The results of GC-FID and GC-MS analyses are gathered in Table 2, This table showed that 48 components were identified in berries and branches oil representing (97.2 – 99.7%). α -Pinene ($40.3 \pm 3.6 - 67.7 \pm 4.3\%$) was the major compound in both oils followed by δ -3-carene ($13.5 \pm 1.5\% - 26.8 \pm 2.3\%$) and α -cedrol ($1.5 \pm 0.6 - 7 \pm 1.1\%$). The branches oils were richer in α -pinene than berries. Which consisted mainly of monoterpenoids (hydrocarbons and oxygenated compounds) at (80.6 – 82%). The sesquiterpenoids were presented in berries oils at (15.2 – 16.8%). While branch oils consisted mainly of monoterpenoids and sesquiterpenoids only 9% of the total oil composition. Chemical compositions of essential oils obtained from berries and branches were slightly different. According to the literature, one finds no trace of α -cedrol in *J. phoenicea* essential oil except that from Egypt with α -cedrol proportion of (31.23%).¹² In comparison with literature data, the *J. phoenicea* essential oils compositions were different than those previously investigated

Table 1.⁵⁻¹⁵ This difference can be related with abiotic factors such as specific climate conditions and phenological stages of the collected plants as well as geographical factors such as altitude and nature of the soil.

Higher amounts of hydrocarbon monoterpenes were detected in the *J. phoenicea* essential oil isolated by EMI heating (79.5 – 87.5%) compared to HD (77 – 85.6%). Whereas, the oxygenated monoterpenes were present in high proportions in HD (3.6 – 4.4%) compared to EMI heating (2.5 – 2.6%) essential oil, α -Pinene was the main component in the *J. phoenicea* essential oil but the relative amounts differed for the two isolation methods: ($44.81 \pm 3.4\% - 67.7 \pm 4.3\%$) for EMI heating and ($40.3 \pm 3.6 - 50.5 \pm 4.2\%$) for HD oils respectively. δ -3-carene and α -cedrol were the two other main components in *J. phoenicea*. The highest proportion of δ -3-carene was found in EMI heating essential oil ($13.5 \pm 1.5 - 26.8 \pm 2.3\%$) compared with HD essential oil ($14.5 \pm 1.8 - 20.1 \pm 2.4\%$) and α -cedrol is more abundant in HD ($4.5 \pm 0.8 - 7 \pm 1.1\%$) against ($1.5 \pm 0.6 - 4.2 \pm 1.2\%$) in EMI heating. Therefore, the EMI heating, highly accelerated the extraction process, without major difference in proportions of compounds. In *J. phoenicea* from eastern Algeria.¹³ Three chemotypes were identified: α -pinene / terpinolene (i), α -pinene / δ -3-carene (ii) and α -pinene / β -phellandrene (iii). The findings of this study show that the chemical composition of our samples belongs to the second chemo type: α -pinene / δ -3-carene with a significant amount of α -cedrol.

The results of the antiradical capacity of *J. phoenicea* essential oils and of the standards (ascorbic acid, BHT) are expressed as an inhibition percentage of the DPPH radical at different concentrations (25, 50, 75 and 100 $\mu\text{g}/\text{mL}$). Abilities of the tested samples to scavenge DPPH• were assessed on the basis of their IC_{50} values which were inversely related to their antioxidant capacities, (Table 3). Berries essential oil ($IC_{50} = 67.6 \pm 1.02 \mu\text{g}/\text{mL}$) has shown a weak antioxidant activity compared to BHT ($IC_{50} = 18.3 \pm 0.8 \mu\text{g}/\text{mL}$) and acid ascorbic ($IC_{50} = 14.9 \pm 1.7 \mu\text{g}/\text{mL}$). Similarly, the branches essential oil has also a weak efficiency with an $IC_{50} = 98 \pm 1.25 \mu\text{g}/\text{mL}$. Therefore, when compared to BHT and ascorbic acid, both oil samples were clearly less effective than these synthetic antioxidants. The low antioxidant activity of the examined oils in DPPH test may be partially due to the dominance of α -pinene (40.3 – 50.5%) and δ -3-carene (14.5-20.1%) which are considered as weak antioxidants.¹⁶ These different results might be due differences in the chemical composition and possible interactions between the volatile constituents.

Table 2. Chemical composition of *J. phoenicea* essential oils (mean of three triplicates \pm SD)

Compound	RI _a	RI _b	Area %			
			HD		EMI	
			Berries	branches	Berries	Branches
Tricyclene	927	918	tr	tr	0.1 \pm 0.1	tr
α -Thujene	930	924	tr	0.2 \pm 0.1	0.1 \pm 0.2	0.2 \pm 0.1
α -Pinene	939	933	40.3 \pm 3.6	50.5 \pm 4.2	44.8 \pm 3.4	67.7 \pm 4.3
α -Fenchene	935	943	1.9 \pm 0.2	1.9 \pm 0.2	1.6 \pm 0.7	1.1 \pm 0.4
Sabinene	975	970	0.4 \pm 0.1	0.5 \pm 0.1	0.7 \pm 0.4	0.1 \pm 0.1
β -Pinene	979	973	1.5 \pm 0.2	2.2 \pm 0.2	1.6 \pm 0.6	1 \pm 0.3
β -Myrcene	991	989	2.8 \pm 0.4	3.9 \pm 0.3	2.6 \pm 0.7	1.4 \pm 0.4
δ -2-Carene	1001	998	/	tr	tr	tr
α -Phellandrene	1003	1002	0.8 \pm 0.1	0.9 \pm 0.2	0.1 \pm 0.1	0.6 \pm 0.2
δ -3-Carene	1011	1010	20.1 \pm 2.4	14.5 \pm 1.8	26.8 \pm 2.3	13.5 \pm 1.5
α -Terpinene	1017	1015	0.3 \pm 0.1	0.4 \pm 0.1	0.1 \pm 0.1	0.3 \pm 0.2
p- Cymene	1025	1023	tr	0.3 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1
β -Phellandrene	1030	1027	4.1 \pm 0.8	6.5 \pm 0.9	0.4 \pm 0.3	0.4 \pm 0.3
(E)- β -Ocimene	1050	1055	tr	tr	0.1 \pm 0.1	0.3 \pm 0.2
γ -Terpinene	1060	1057	0.3 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.2	0.2 \pm 0.1
α -Terpinolene	1089	1087	4.5 \pm 1.2	3.8 \pm 1.1	0.2 \pm 0.2	0.6 \pm 0.3
Terpinen-4-ol	1177	1178	0.2 \pm 0.1	0.6 \pm 0.2	0.4 \pm 0.2	0.6 \pm 0.2
α -Terpineo	1189	1191	tr	0.5 \pm 0.1	0.9 \pm 0.4	0.2 \pm 0.1
Carvacrolmethylether	1245	1244	/	tr	0.1 \pm 0.1	0.1 \pm 0.1
Bornylacetate	1289	1286	0.7 \pm 0.3	1.0 \pm 0.4	0.5 \pm 0.3	0.2 \pm 0.2
transCarvylacetate	1337	1340	/	0.2 \pm 0.1	0.2 \pm 0.2	0.9 \pm 1.1
α -Terpinylacetate	1349	1350	2.7 \pm 0.9	2.1 \pm 0.7	0.4 \pm 0.3	0.6 \pm 0.3
β -Elemene	1391	1393	0.3 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.2	0.6 \pm 0.2
β -Funebrene	1415	1415	0.2 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.2
β -Caryophyllene	1419	1422	1.9 \pm 0.5	0.1 \pm 0.1	0.6 \pm 0.3	0.5 \pm 0.3
Thujopsene	1431	1434	0.3 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.1	0.2 \pm 0.1
α -Humulene	1455	1456	1.6 \pm 0.6	0.9 \pm 0.3	0.8 \pm 0.3	0.1 \pm 0.1
α -Amorphene	1485	1481	tr	0.2 \pm 0.1	tr	0.2 \pm 0.2
Germacrene- D	1485	1484	0.2 \pm 0.1	0.4 \pm 0.1	4.9 \pm 0.8	1.2 \pm 0.6
β -Selinene	1490	1489	tr	/	0.1 \pm 0.1	0.6 \pm 0.3
α -Selinene	1498	1498	tr	0.1 \pm 0.1	0.1 \pm 0.3	0.7 \pm 0.4
α -Muurolene	1500	1502	tr	tr	0.1 \pm 0.2	0.5 \pm 0.2
EE- α -Farnesene	1506	1509	0.2 \pm 0.1	0.1 \pm 0.1	0.3 \pm 0.2	0.3 \pm 0.2
Germacrene- A	1509	1514	tr	tr	0.5 \pm 0.5	0.1 \pm 0.1
γ -Cadinene	1514	1517	tr	tr	0.2 \pm 0.2	0.3 \pm 0.3
δ -Cadinene	1523	1525	0.3 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.3	0.9 \pm 0.3
Elemol	1550	1553	1.6 \pm 0.6	0.5 \pm 0.2	0.2 \pm 0.3	0.3 \pm 0.2
Germacrene B	1558	1561	0.2 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.5	0.4 \pm 0.3
Germacrene D-4-ol	1576	1580	0.3 \pm 0.1	/	0.1 \pm 0.3	/
Caryophylleneoxide	1583	1588	0.3 \pm 0.2	0.4 \pm 0.2	0.2 \pm 0.1	0.3 \pm 0.2
α -Cedrol	1601	1602	7.0 \pm 1.1	4.5 \pm 0.8	4.2 \pm 1.2	1.5 \pm 0.6
epi-Cedrol	1611	1615	tr	tr	tr	0.1 \pm 0.1
Fonenol	1621	1625	tr	/	tr	/
γ -Eudesmol	1630	1636	0.5 \pm 0.2	0.1 \pm 0.1	0.3 \pm 0.2	0.1 \pm 0.1
τ , Muurolol	1646	1646	0.1 \pm 0.1	tr	0.2 \pm 0.1	tr
β -Eudesmol	1651	1650	0.2 \pm 0.1	/	0.4 \pm 0.2	/
α -Eudesmol	1652	1656	0.4 \pm 0.2	0.9 \pm 0.3	0.3 \pm 0.1	0.3 \pm 0.2
α -Cadinol	1654	1659	1 \pm 0.3	0.2 \pm 0.1	0.4 \pm 0.3	tr
Monoterpenes			77	85.6	79.5	87.5
Oxygenated monoterpenes			3.6	4.4	2.5	2.6
Sesquiterpenes			5.1	2.4	8.2	6.6
Oxygenated sesquiterpenes			11.7	6.4	7	3
Total identification			97.4	98.8	97.2	99.7

tr : traces (< 0.1%)

RI_a : Retention indices (Adams)RI_b : Retention indices relative to C7-C20 on the HP-5MS capillary column

Table 3. IC₅₀ values (µg/mL) of *J. phoenicea* essential oil, BHT and ascorbic acid.

Assays	Essential oil of the branches	Essential oil of the berries	BHT	Ascorbic acid
DPPH	98 ± 1.25	67.6 ± 1.02	18.3 ± 0.8	14.9 ± 1.7
Reducing power	166.8 ± 0.29	131.5 ± 0.82	9.7 ± 1.23	5.7 ± 0.02
β-Carotene bleaching	119.2 ± 1.01	98.6 ± 1.12	99.7 ± 0.02	24.2 ± 0.08

Table 3 shows the plot of reducing power of *J. phoenicea* essential oils in comparison with ascorbic acid and BHT as standards. At the concentration of 100 µg/mL, the absorbance values of essential oils are 0.305 and 0.386 for berries and branches, respectively. The berries oil has a reducing activity slightly higher than that the branches. Compared to BHT (9.7 ± 1.23 µg/mL) and ascorbic acid (5.7 ± 0.02 µg/mL), the oil samples were less effective than the standards.

This poor performance can be explained by their chemical profile characterized by a very low content of phenolic compounds generally associated to antioxidant activity.¹⁴⁻¹⁵

The result of lipid peroxidation inhibitory activity of the essentials oils, assessed by the β-carotene bleaching test are shown in Table 3. The extracts reduced the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.²⁵ *J. phoenicea* essential oils of the branches (IC₅₀ = 119.2 ± 1.01 µg/mL) and berries (IC₅₀ = 98.6 ± 1.12 µg/mL) showed moderate antioxidant activity (Table 3). Nevertheless they were less efficient than ascorbic acid (IC₅₀ = 24.2 ± 0.08 µg/mL) and BHT (IC₅₀ = 99.7 ± 0.02 µg/mL). This activity can be explained by the presence of monoterpenoids indeed the antioxidant activity of an essential oil may be attributed to a complex interaction between different constituents, which may provide additive, synergistic or antagonistic effects, even for those present at low concentrations.¹⁴

Conclusion

In this study, the EMI assisted heating was presented as an extraction method suitable for essential oil extraction. It resulted a reduced extraction time. Moreover, alteration of essential oil constituents is surely limited in comparison with classical hydrodistillation process (Clevenger apparatus). After 55 min of EMI assisted extraction at 140°C, it was possible to collect almost all the existing essential oils from the three samples with a yield of (1.2 ± 0.3 – 2.4 ± 0.7%, w/w). The essential oil of *J. phoenicia* was rich in monoterpenes belongs to α-pinene / δ-3-carene chemo type with a significant amount of α-cedrol. Overall,

the results of the antioxidant activity of the *J. phoenicea* essential oils evaluated by three various tests indicate that these oils offered limited potentialities as antioxidant. The highest antioxidant activity was exhibited by the berries oil. However, complementary investigations are necessary to assess the effectiveness of this oil in food system.

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