Qualitative, Quantitative Analysis and Chiral Characterization of the Essential Oils of *Juniperus phoenicea* L. and *Juniperus oxycedrus* L.

Dahmane Dahmane[1],*, Fahima Abdellatif Dahmane[1], Tahar Dob[1], and Chaabane Chelghoum[2]

[1]*Laboratory of Research on Bio-active Products and Valorization of Biomasse, Faculty of Chemistry,*
Ecole Normale Supérieure. El Bachir El Ibrahimi. B.P. 92, ENS, Kouba-Algiers, Algeria.
[2]*Laboratory of Chromatography, Faculty of Chemistry, Houari Boumediene University of Sciences and Technology,*
USTHB, El Alia, B.P. 32, Bab Ezzouar, Algiers, Algeria

Abstract − Isolation of oils from leaves of *Juniperus phoenicea* and *Juniperus oxycedrus* was obtained by steam distillation extraction method. The compositions of essential oils (EOs) were studied by means of GC-MS and GC-FID, using the internal standard method and relative response factors. Around ninety eight compounds were determined in total, representing 98.25 g/100 g of EO of *J. phoenicea* and 98.48 g/100 g of EO of *J. oxycedrus*, respectively. The volatile leaf oils were dominated by the terpenic hydrocarbon fractions (79.87 g/100 g) and (61.27 g/100 g) characterized by high contents of α-pinene (64.6 g/100 g) and (54.0 g/100 g) in *J. phoenicea* and *J. oxycedrus*, respectively, as the main component. Also, the enantiomeric distribution of α-pinene, sabinene, camphene, δ-3-carene, β-pinene, limonene, linalool, terpinen-4-ol, bornyl acetate, and borneol in both oils is presented for the first time.

Keywords − *Juniperus phoenicea, Juniperus oxycedrus,* Steam distillation, GC quantitative analysis, enatio-GC-FID, GC-MS

Introduction

The genus *Juniperus* (Cupressaceae) consists of approximately 75 species, all of which grow in the northern hemisphere. The genus is divided into three sections: *Caryocedrus* (one species, *J. drupacea* Labill.); *Juniperus* (= *Oxycedrus*, 14 species) and *Sabina* (60 species).[1] The flora of Algeria lists five native *Juniperus* species: *J. communis, J. phoenicea, J. oxycedrus, J. sabina* and *J. thurifera*. Among them, *J. phoenicea* and *J. oxycedrus* commonly known as "Arar" and "Taga", respectively.[2]

Several studies on the chemical composition of the oils in leaves from *J. oxycedrus* and *J. phoenicea* subspecies (or varieties) have been reported in the literature, these studies concerned plants from various origins all around the Mediterranean basin; Portugal,[3-8] Spain,[3-5,7,9-11] France,[3,4,12-14] Italy,[7,15,16] Croatia,[17] Greece,[3,4,7,9,18] Turkey,[7,13,14,19,20] and North African; Tunisia,[21-27] Algeria[28-32] and Morocco.[7,33-35] The most of the studies concern to oils obtained by hydrodistillation. Few reports have been investigated on the chemical composition of essential oils leaf of *J. phoenicea* [22,33,36-38] and *J. oxycedrus* [5,7,9,13,39] isolated by steam distillation. In agreement with results obtained by other authors, the chromatographic analysis of essential oils of their species showed that α-pinene chemotype was the component present in the greatest percentage.

In Algeria, Bekhechi et *al*. have been focused on the chemical variability of the essential oil of *J. phoenicea* var. *turbinata* collected from eight population in Algeria, the 50 samples of essential oils have been divided into three clusters, in most 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%).[31] The volatile leaf oils of *J. phoenicea* var. *phoenicea* and *J. phoenicea* subsp. *turbinata*, have been recently examined from throughout the Mediterranean region.[36-37] Recently, one study on the essential oil from berries and branches of *J. phoenicea* collected in Ain-Defla region (northern Algeria) analysed by GC and GC-MS showed the presence of α-pinene (40.3-67.8%) and δ-3-carene (13.5-26.8%) as main components.[28]

*Author for correspondence
Dahmane Dahmane, Laboratory of Research on Bio-active Products and Valorization of Biomasse, Ecole Normale Supérieure. El Bachir El Ibrahimi. B.P. 92, ENS, Kouba-Algiers, Algeria.
Tel: +213-21-287-981; E-mail: dahmane12@gmail.com

Adams *et al*. studied leaf terpenoids of *J. phoenicea* from the Canary Islands (Spain) and Madeira Island (Portugal), the volatile oils were dominated by α-pinene (50.3-76.0%).[38]

To our knowledge, there is only one report studied enantiomeric and non-enatiomeric distribution of mono-terpenes fraction in the oil of *J. oxycedrus*.[30] In previous works, we have studied the essential oils composition of the Algerian Juniper.[28-32]

The demand for quantitative data in the EO field is mainly due to their increased economic importance and to the continual increase in controls to verity, safety and biological activity.[40] The quantitative composition of most EOs is very often reported in the literature in terms of relative percentage abundances, although this approach can unfortunately only give an approximate indication of the ratio between components in the sample under investigation.

The aims of this study were to investigate the detailed chemical composition and determined enantiomeric distri-bution of monoterpenes of the essential oils of two medicinal plant species *J. phoenicea* and *J. oxycedrus* grown in Bouira region of Algeria.

Experimental

Plant material - The leaves of *J. phoenicea* and *J. oxycedrus* were collected from a single location in a reserved garden of Lakhdaria city, Bouira Province, 69 Km South-East of Algiers (36°N, 3°E). The two species were identified by direct comparison with herbarium sample, Higher National Agronomic School (ENSA, Algiers).

Chemicals – For the measurement of response factors, the following standard compounds, available in the laboratory, were used: α-pinene (99%), β-pinene (99%), α-phellandrene (95%), δ-3-carene (98.5%) and limonene (99%) for monoterpene hydrocarbons; (*E*)-caryophyllene (98.5%) and α-cedrene (95%) for sesquiterpene hydro-carbons; linalool (98.5%), borneol (99%), terpinen-4-ol (95%), α-terpineol (98.5%) and myrtenol (98.5%) for monoterpene alcohols; fenchone (99.5%), α-thujone (96%), camphor (95%), verbenone (99%) and carvone (98.5%) for monoterpene ketones; citronellal (96%), neral (95%) and geranial (95%) for aldehydes; bornyl acetate (98.5%) and menthyl acetate (99%) for esters; α-cedrol (99%) and α-bisabolol (95%) for sesquiterpene alcohol; and caryophyllene oxide (99%) for sesquiterpene oxide. Standard solutions in hexane of these compounds were prepared at different concentration and spiked with internal standard (*n*-octane >99%, Aldrich) in order to

have a final concentration of 0.7 mg/mL *n*-octane. Each standard solution was analysed for three consecutive GC runs. All Standards used were of analytical reagent grade. GC grade standards of enantiomeric and non-enantio-meric terpenes were obtained from Sigma-Aldrich and Fluka (Germany) in the highest available purity. For determination of retention indices, a hydrocarbon mixture C_8-C_{20} (Fluka) was used. These pure standards were used to optimize the separation conditions, determine the elution order of enantiomer pairs and provide positive identification of the terpenes present in the plant species. The chiral compounds were diluted 1:100 (v/v) in *n*-hexane prior to analysis in all applications.

Steam distillation apparatus and procedure – The leaves of *J. phoenicea* and *J. oxycedrus* were steam distilled for 3 h using a circulatory Clevenger- type apparatus,[41] and the oils were dried over anhydrous sodium sulfate and stored at 4 °C in the dark. Each essential oil was prepared at a concentration 0.7 g/100 g of *n*-octane internal standard and diluted 1:10 (v/v) in *n*-hexane prior to GC injection.

Gas Chromatography with Flame Ionisation Detection – Analyses were carried out using on a GC 7890A system (Agilent Technologies, USA), equipped with a G4513A auto sampler and a split/splitless injector using fused silica capillary column with stationary phase HP5-MS column (30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Agilent Technologies, USA) The temperature program was 60 °C for 3 min then 3 °C/min to 240 °C for 3 min; injection temperature, 250 °C; Helium was used as carrier gas at a constant flow; flow rate, 1 mL/min in the split mode 1:50, with an injection volume of 1.0 μL; detection temperature 300 °C; H_2 flow, 30 mL/min; air flow, 400.0 mL/min; make-up flow; (He) 25 mL/min. Data were processed through GC solution software (Agilent ChemStation Rev.B.04.03).

Quantitative data was obtained from electronic integra-tion of area percentages without the use of correction factors. In order to determine linear retentions indices (LRIs), a series of *n*-alkane (C_8-C_{20}) mixtures were analyzed under the same operative conditions on HP5-MS column, the linear retention indices were calculated following Van den Dool and Kratz.[42]

Enantio-GC Analysis – The GC chiral analyses were carried out using an Agilent Technologies a GC 7890A apparatus equipped with FID and fused silica capillary column internally coated with 20% *β*-cyclodextrin in 35% phenyl methyl polysiloxane HP-chiral 20*β* (30 m × 0.32 mm, 0.25 μm film thicknesses, J&W Scientific, Agilent Technologies, USA). The oven temperature was pro-

grammed as follows: 40 °C (5 min), 40 °C-130 °C (1 min) at 1 °C/min, 130 °C-200 °C (3 min) at 2 °C/min. Inlet temperature (split: 1/100) was 250 °C and detector temperature was 300 °C. Carrier gas was helium (1 mL/min). Injected volume was 0.1 μL. The quantitative composition of each sample was obtained from normalized peak areas.

Gas Chromatography-Mass Spectroscopy (GC-MS) – Gas chromatography-Mass spectroscopy (GC-MS) analysis of *J. phoenicea* and *J. oxycedrus* volatile components were performed on TRACE GC Ultra coupled with DSQ II mass spectrometer equipped with a HP-5MS fused-silica column (30 m × 0.32 mm i.d., 0.25 μm film thicknesses). It was programmed from 60 °C (3 min) to 240 °C (3 min) at 3 °C/min with helium carrier gas at a flow rate of 1 mL.min⁻¹ and injector heater 250 °C. The MS conditions were EI source, electron energy 70 eV and source temperature 250 °C. Acquisition mass range, m/z = 40-450.

The GC-MS chiral analysis was performed with a Hewlett Packard GC (HP5890 series II) /quadripole MS system (model HP MSD5971), equipped with an electronic impact source at 200 °C , fitted with a same column HP-chiral 20β (30 m × 0.32 mm, 0.25 μm film thickness, J&W Scientific, Agilent Technologies, USA). The chromatographic conditions were the same with GC chiral analysis, the electron impact spectra were recorded at an ion voltage of 70 eV over a scan range of 40-450 uma.

Component Quantification – Quantification of essential oils components was carried out using peak-area internal standardization with response factors (RFs), according to the IOFI guidelines for the quantitative gas chromatography of volatile flavoring substances.[43] Owing to the large number of compounds present, the response factors were calculated for eight different chemical classes and then by their functional groups to have the same quantitative GC correction factor. RFs and calibration curves were determined by diluting each standard solution (see chemicals) in hexane, at five concentrations, with each specimen containing *n*-octane as internal standard (IS) (0.7 mg/mL).

The response factors (RFs) were calculated according to the following formula:[44]

$$RF = (C_{anal}/C_{is}) \times (A_{is}/A_{anal})$$

Where C_{anl}: is the concentration of the standard compound, A_{anal}: is its absolute peak area, A_{is}: is the octane absolute peak area and C_{is}: is the concentration (0.7 mg/100 mL).

The average RFs obtained for each standard compound within a chemical class are used as a correction factor specific for each chemical class.

Once calculated, response factors were used for the absolute quantification of volatiles, based on the following equation:

$$C_{comp} = \{[A_{comp}/A_{is}) \times C_{is} \times RF]/W_{oil}\} \times 100$$

Where: C_{comp} is the concentration expressed as g/100 g, of the target volatile compound and A_{comp}: its absolute peak area, W_{oil} is the weight of the oil (g) and other terms are as previously reported.

Component Identification – Essential oil components identification was based on comparison of their GC linear retention indices (LRI) on HP-5MS column, with those from literature or to the reference compounds available in our laboratory, and by comparing mass spectral with those compiled in Nist 8, Wiley 9 mass spectrum libraries or reported in literature.[45,46]

Retention time confirmation of individual chiral monoterpenes was performed by analyzing pure standards under the same conditions chromatographic. The elution order of the enantiomers was assigned using enantiomerically pure reference compounds of definite chirality. To determine the elution order, we injected each enantiomer, then we injected the standard mixture to see the proper separation.

Results and Discussion

The essential oils isolated by steam distillation from leaves of two cited species were studied by means of GC-MS/IE and GC-FID, quantities are calculated from the peak areas according to the IOFI guidelines for the quantitative gas chromatography of volatile flavoring substances.[43] Each response factor was calculated by means of a one-point calibration, using standard compounds representative of the eight chemical classes; therefore, for class of compounds following values were obtained: 1.31 for alcohols; 1.30 for ketones; 1.28 for aldehydes; 1.38 for esters; 1.52 for sesquiterpene oxides; 1.02 for monoterpene hydrocarbons; 1.05 for sesquiterpene hydrocarbons and 1.21 for oxygenated sesquiterpenes (Table 1). The best response toward FID detector is given by hydrocarbons (RF = 1), while a different trend occurs for oxygenated compounds, giving greater values of the response factors. All the standard curves were linear over the concentration range, with the correlation coefficients of linear regression (R^2) in the range 0.9952-0.9999 (Table1).

For instance, Fig. 1 shows calibration curve of α-pinene, the analysis was performed on the basis of the

Table1. Measurements of response factors (RFs) for the different chemical groups

Standard compounds	Calibration curve equation	Correlation coefficient (R^2)	Mean ± SD	RFs
Monoterpene hydrocarbons				
α-Pinene	y = 0.9823x + 0.0174	0.9982	1.01 ± 0.03	
β-Pinene	y = 1.0085x + 0.0045	0.9993	1.01 ± 0.02	
α-Phellandrene	y = 1.0369x - 0.0034	0.9990	1.02 ± 0.02	1.02 ± 0.01
δ-3-Carene	y = 1.0379x - 0.0022	0.9979	1.04 ± 0.04	
Limonene	y = 0.9938x + 0.0145	0.9987	1.01 ± 0.03	
Sesquiterpene hydrocarbons				
(*E*)-Caryophyllene	y = 0.9850x + 0.2501	0.9968	1.04 ± 0.04	1.05 ± 0.02
α-Cedrene	y = 1.0383x + 0.1045	0.9999	1.07 ± 0.02	
Oxygenated monoterpenes				
Alcohols				
Linalool	y = 1.2822x + 0.0278	0.9994	1.33 ± 0.09	
Borneol	y = 1.2604x - 0.0132	0.9994	1.26 ± 0.06	
Terpinen-4-ol	y = 1.2807x - 0.0590	0.9952	1.27 ± 0.10	1.31 ± 0.04
α-Terpineol	y = 1.3448x - 0.0504	0.9993	1.32 ± 0.05	
Myrtenol	y = 1.4047x - 0.1453	0.9993	1.37 ± 0.06	
Ketones				
Fenchone	y = 1.2968x - 0.4253	0.9991	1.23 ± 0.05	
α-Thujone	y = 1.3437x - 0.4353	0.9997	1.29 ± 0.04	
Camphor	y = 1.3662x + 0.4493	0.9979	1.36 ± 0.02	1.30 ± 0.05
Verbenone	y = 1.3393x - 0.2252	0.9991	1.25 ± 0.05	
Carvone	y = 1.3666x - 0.2792	0.9991	1.28 ± 0.05	
Aldehydes				
Citronellal	y = 1.2019x + 0.4416	0.9972	1.28 ± 0.05	
Neral	y = 1.2120x + 0.5779	0.9976	1.29 ± 0.05	1.28 ± 0.02
Geranial	y = 1.2251x + 0.5226	0.9967	1.29 ± 0.05	
Esters				
Bornyl acetate	y = 1.5670x - 0.0858	0.9994	1.49 ± 0.05	1.47 ± 0.04
Menthyl acetate	y = 1.5393x - 0.5601	0.9990	1.44 ± 0.06	
Oxygenated sequiterpenes				
α-Cedrol	y = 1.0637x + 0.2664	0.9960	1.12 ± 0.05	1.21 ± 0.14
α-Bisabolol	y = 1.2301x + 1.3582	0.9995	1.31 ± 0.05	
Sesquiterpene oxides				
Caryophyllene oxide	y = 1.5019x + 0.1331	0.9953	1.52 ± 0.10	1.52 ± 0.10

y = 0,982x + 0,017
R^2 = 0,998
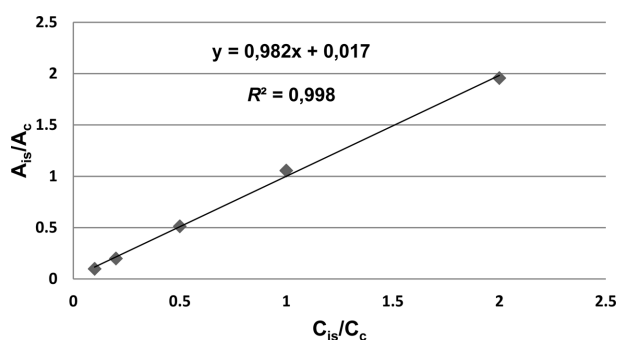
Fig. 1. Calibration curve of α-pinene used to quantify the samples.

mean of three calibration curve for α-pinene (y = 0.9823x + 0.0174, R^2 = 0.9982).

Table 2 reports qualitative and quantitative data obtained (relative chromatographic area A% and amounts g/100 g) from GC-MS and GC-FID analysis. Relative amounts of individual components of *J. phoenicea* and *J. oxycedrus* oils were calculated on the basis of their GC peak areas on an HP5-MS capillary column without FID response factor correction. Fig. 2 shows a comparison of the GC-FID profiles of *J. phoenicea* and *J. oxycedrus* oils, with numbered peaks for interpretation of identity. In order to provide the real concentration of oils constituents,

Table 2. Qualitative and quantitative analysis of chemical composition of *Juniperus phoenicea* and *Juniperus oxycedrus* essential oils from steam distillation procedures

Peak	LRI[a]	RI[b]	Compounds		*J. phoenicea*		*J. oxycedrus*	
				RF[c]	%A[d]	g/100g[e]	%A	g/100g
1.	924	921	Tricyclene	1.02	0.37	0.36	0.59	0.58
2.	928	924	α-Thujene	1.02	0.03	0.03	-	-
3.	938	932	α-Pinene	1.02	65.7	64.6	54.5	54.0
4.	948	645	α-Fenchene	1.02	0.39	0.39	0.46	0.46
5.	950	946	Camphene	1.02	0.51	0.50	0.82	0.81
6.	955	953	Thuja-2,4(10)-diene	1.02	0.08	0.08	0.10	0.10
7.	971	961	Verbenene	1.02	0.06	0.06	0.43	0.42
8.	974	969	Sabinene	1.02	0.05	0.05	0.09	0.08
9.	978	974	β-Pinene	1.02	0.95	0.94	0.71	0.70
10.	992	988	Myrcene	1.02	2.11	2.08	0.47	0.47
11.	1002	1001	δ-2-Carene	1.02	0.03	0.03	-	-
12.	1006	1002	α-Phellandrene	1.02	0.18	0.17	-	-
13.	1012	1008	δ-3-Carene	1.02	6.64	6.53	0.73	0.72
14.	1018	1014	α-Terpinene	1.02	0.04	0.04	0.03	0.03
15.	1025	1020	*p*-Cymene	1.02	0.40	0.40	0.87	0.86
16.	1030	1024	Limonene	1.02	3.04	2.99	1.63	1.62
17.	1051	1044	(*E*)-β-Ocimene	1.02	-	-	0.03	0.03
18.	1059	1054	γ-Terpinene	1.02	0.18	0.18	0.03	0.03
19.	1071	1065	*cis*-Sabinene hydrate	1.31	-	-	0.08	0.10
20.	1087	1083	Fenchone	1.30	0.03	0.04	0.07	0.09
21.	1089	1086	Terpinolene	1.02	0.45	0.44	0.36	0.36
22.	1101	1095	Linalool	1.31	0.26	0.33	0.59	0.75
23.	1108	1106	*cis*-Rose oxide	1.1	0.03	0.03	1.77	1.90
24.	1118	1114	*endo*-Fenchol	1.31	-	-	0.10	0.13
25.	1127	1122	α-Campholenal	1.28	0.05	0.06	1.19	1.49
26.	1137	1132	*cis*-Limonene oxide	1.1	-	-	0.11	0.11
27.	1140	1135	*trans*-Pinocarveol	1.31	0.07	0.09	0.83	1.06
28.	1146	1141	Camphor	1.30	0.56	0.70	4.28	5.41
29.	1150	1145	Camphene hydrate	1.31	0.03	0.04	0.13	0.16
30.	1157	1155	Isoborneol	1.31	-	-	0.04	0.05
31.	1162	1158	*trans*-Pinocamphone	1.30	0.04	0.05	0.12	0.15
32.	1164	1160	Pinocarvone	1.30	-	-	0.24	0.30
33.	1167	1165	Borneol	1.31	0.16	0.21	0.41	0.52
34.	1174	1172	*cis*-Pinocamphone	1.30	-	-	0.02	0.03
35.	1176	1174	Terpinen-4-ol	1.31	0.02	0.03	0.28	0.35
36.	1179	1179	*p*-Cymen-8-ol	1.31	0.05	0.07	0.13	0.16
37.	1186	1186	α-Terpineol	1.31	0.03	0.04	0.29	0.37
38.	1192	-	Unknown	-	0.12	-	0.16	-
39.	1198	1195	Myrtenal	1.28	0.05	0.06	0.49	0.61
40.	1212	1204	Verbenone	1.30	0.02	0.03	0.87	1.11
41.	1222	1218	*endo*-Fenchyl acetate	1.38	0.08	0.10	0.49	0.66
42.	1225	1223	Citronellol	1.31	-	-	0.15	0.20
43.	1229	1226	*cis*-Carveol	1.31	0.15	0.19	0.17	0.21
44.	1242	1235	*trans*-Chrysanthenyl acetate	1.38	-	-	0.05	0.07
45.	1246	1239	Carvone	1.30	0.02	0.03	0.15	0.19

Table 2. continued

Peak	LRI[a]	RI[b]	Compounds	RF[c]	%A[d]	g/100g[e]	%A	g/100g
					J. phoenicea		*J. oxycedrus*	
46.	1254	1249	Piperitone	1.30	0.08	0.10	0.23	0.29
47.	1257	1254	Linalool acetate	1.38	0.68	0.91	0.82	1.11
48.	1277	1275	Isopulegyl acetate	1.38	0.24	0.32	0.48	0.64
49.	1288	1287	Bornyl acetate	1.38	1.36	1.82	3.62	4.86
50.	1292	1289	*p*-Cymen-7-ol	1.31	0.02	0.03	0.33	0.42
51.	1301	1295	3-Thujanol acetate	1.38	-	-	3.14	4.21
52.	1309	1306	Dihydro carveol acetate	1.38	-	-	0.62	0.84
53.	1315	1315	(*2E, 4E*) Decadienal	1.28	0.20	0.24	2.02	2.52
54.	1324	1319	(*2E, 4E*) Decadienol	1.31	0.03	0.04	0.49	0.63
55.	1340	1335	δ-Elemene	1.05	0.14	0.14	0.07	0.07
56.	1342	1339	*trans*-Carvyl acetate	1.38	0.05	0.07	0.39	0.53
57.	1351	1346	α-Terpenyl acetate	1.38	0.98	1.30	0.85	1.14
58.	1361	1359	Neryl acetate	1.38	-	-	0.03	0.04
59.	1374	1373	α-Yalangene	1.05	-	-	0.29	0.3
60.	1378	1374	α-Copaene	1.05	0.08	0.08	0.33	0.34
61.	1385	1379	Geranyl acetate	1.05	0.04	0.04	0.08	0.11
62.	1387	1387	β-Bourbonene	1.05	0.06	0.06	0.10	0.11
63.	1394	1389	β-Elemene	1.05	0.26	0.26	0.26	0.26
64.	1408	1409	α-Gurjunene	1.05	0.05	0.05	0.17	0.17
65.	1409	1410	α-Cedrene	1.05	-	-	0.05	0.05
66.	1422	1417	β-Caryophyllene	1.05	0.82	0.83	0.19	0.19
67.	1425	1424	2,5-dimethoxy-*p*-Cymene	1.05	0.04	0.04	0.07	0.07
68.	1436	1434	γ-Elemene	1.05	0.28	0.29	0.08	0.08
69.	1453	-	Unknown	-	0.11	-	-	-
70.	1456	1452	α-Humulene	1.05	0.48	0.49	0.14	0.15
71.	1476	-	Unknown	-	0.24	-	-	-
72.	1479	1478	γ-Muurolene	1.05	0.20	0.20	0.19	0.19
73.	1484	1483	α-Amorphene	1.05	0.78	0.79	0.03	0.03
74.	1489	1484	Germacrene D	1.05	0.10	0.10	0.18	0.18
75.	1495	-	Unknown	-	0.32	-	-	-
76.	1497	1493	*epi*-Cubebol	1.21	0.19	0.22	0.23	0.27
77.	1503	1500	α-Muurolene	1.05	0.27	0.27	0.22	0.23
78.	1510	1511	δ-Amorphene	1.05	0.03	0.03	-	-
79.	1514	1513	γ-Cadinene	1.05	0.16	0.16	0.12	0.12
80.	1522	1521	*trans*-Calamenene	1.05	0.26	0.26	0.34	0.34
81.	1526	1522	δ-Cadinene	1.05	1.44	1.46	0.28	0.29
82.	1535	1531	(*Z*)-Nerolidol	1.21	0.07	0.09	-	-
83.	1538	-	Unknown	-	0.16	-	-	-
84.	1546	1544	α-Calacorene	1.05	0.19	0.06	0.10	0.10
85.	1552	1548	Elemol	1.21	0.45	0.52	0.35	0.41
86.	1559	1559	Germacrene B	1.21	0.07	0.08	0.13	0.13
87.	1563	1561	(*E*)-Nerolidol	1.21	0.84	0.99	0.03	0.04
88.	1565	-	Unknown	-	0.09	-	0.04	-
89.	1577	1574	Germacrene D-4-ol	1.21	0.10	0.12	0.13	0.15
90.	1586	1582	Caryophyllene oxide	1.52	0.36	0.52	1.14	1.69
91.	1604	1600	Cedrol	1.21	0.06	0.07	0.06	0.07

Table 2. continued

Peak	LRI[a]	RI[b]	Compounds		J. phoenicea		J. oxycedrus	
				RF[c]	%A[d]	g/100g[e]	%A	g/100g
92.	1613	1608	Humulene epoxide II	1.52	0.24	0.35	0.68	0.80
93.	1622	1618	Junenol	1.21	0.14	0.16	0.05	0.06
94.	1632	1627	1-epi-Cubenol	1.21	0.81	0.94	0.47	0.55
95.	1635	1630	γ-Eudesmol	1.21	0.09	0.11	-	-
96.	1645	1640	epi-α-Muurolol	1.21	0.41	0.48	0.23	0.27
97.	1649	1644	α-Muurolol	1.21	0.10	0.11	0.08	0.10
98.	1654	1649	β-Eudesmol	1.21	0.15	0.18	0.16	0.19
99.	1657	1652	α-Eudesmol	1.21	0.50	0.58	0.27	0.31
100.	1663	1652	α-Cadinol	1.21	0.05	0.05	0.15	0.17
101.	1677	1675	Cadalene	1.05	0.06	0.07	0.10	0.10
102.	1684	1685	α-Bisabolol	1.21	0.08	0.09	0.08	0.09
103.	1689	1687	Eudesma-4(15),7-dien-1β-ol	1.21	0.11	0.13	0.06	0.08
104.	1721	1714	(2E ,6Z)-Farnesol	1.21	0.12	0.14	0.23	0.27
Monoterpene hydrocarbons					81.21	79.87	61.85	61.27
Oxygenated monoterpenes					5.37	6.99	26.22	28.20
Sesquiterpene hydrocarbons					5.73	5.68	3.37	3.45
Oxygenated sesquiterpenes					4.87	5.84	4.44	5.56
Total					97.5	98.25g	96.32	98.48g

[a] Linear retention indices as determined on a HP5-MS column.
[b] Retention indices reported by Adams library [45].
[c] Response factor.
[d] Relative area was given according to FID area percentage data.
[e] Values considered are g/100 g.

we carried out a methodology based on the calculation of response factors for all chemical groups determined.

The comparison between relative and absolute quantitative data (A% peak area vs. g/100 g) generally shows a reduction of the total hydrocarbons, followed by an increase of the oxygenated compounds in the absolute quantitative values.[47,48] As shown in Table 2, significant quantitative differences were registered. According to chromatographic peak area the total identified fraction accounts for 97.5% and 96.32% of total oils of *J. phoenicea* and *J. oxycedrus*, respectively; in particular, the highest amounts of volatiles were dominated by monoterpene hydrocarbons (79.87 g/100 g, 61.27 g/100 g) characterized by α-pinene (64.6 g/100 g, 54.0 g/100 g) as a major compound in *J. phoenicea* and *J. oxycedrus* oils, respectively. The amounts of oxygenated monoterpenes fraction were slightly higher (28.20 g/100 g) in total oil of *J. oxycedrus*, with camphor (5.41 g/100 g), bornyl acetate (4.86 g/100 g) and (2E,4E) decadinal (2.57 g/100 g) were observed at main compounds. In *J. phoenicea*, other monoterpene compounds are present with a moderate mass percent, such as δ-3-carene (6.53 g/100 g), limonene (2.90 g/100 g) and myrcene (2.8 g/100 g). Oxygenated and

hydrocarbons sesquiterpenes gave a smaller contribution, with 5.56-5.84 g/100 g and 3.45-5.68 g/100 g in both oils, respectively (Table 2). The sesquiterpene in two oils content were low (0.05-1.44%) confirming results reported by related literature.[30,34-38]

The values of % peak areas in agreement with data found in the literature; Barrero *et al.*[33] determined as major components of *J. phoenicea* oil steam distilled, α-pinene (45.5%, vs. 65.7% found here), and δ-3-carene (13.0% vs. 6.64%). According to Adams *et al.*,[38] the leaf essential oils steam distilled from the Canary Islands and Maderia were dominated by α-pinene (57.3-76.0%), with β-phellandrene (0.5-8.0%), myrcene (2.3-3.3%), α-terpinyl acetate (trace-5.0%), β-caryophyllene (0.4-1.4%), and *trans*-totarol (0.1-2.1%) were present a moderate amounts. On the other hand, the high content of α-pinene in our results (65.7%) is consistent with similar finding in the essential oil hydrodistilled from the branches of *J. phoenicea* in Ain-Defla region of Algeria (50.5%) [28] and Morocco (65.4%).[37]

For *J. oxycedrus*, there are only four reports on the phytochemical studies of the leaf essential oil steam distilled of *J. oxycedrus* growing in other parts of the
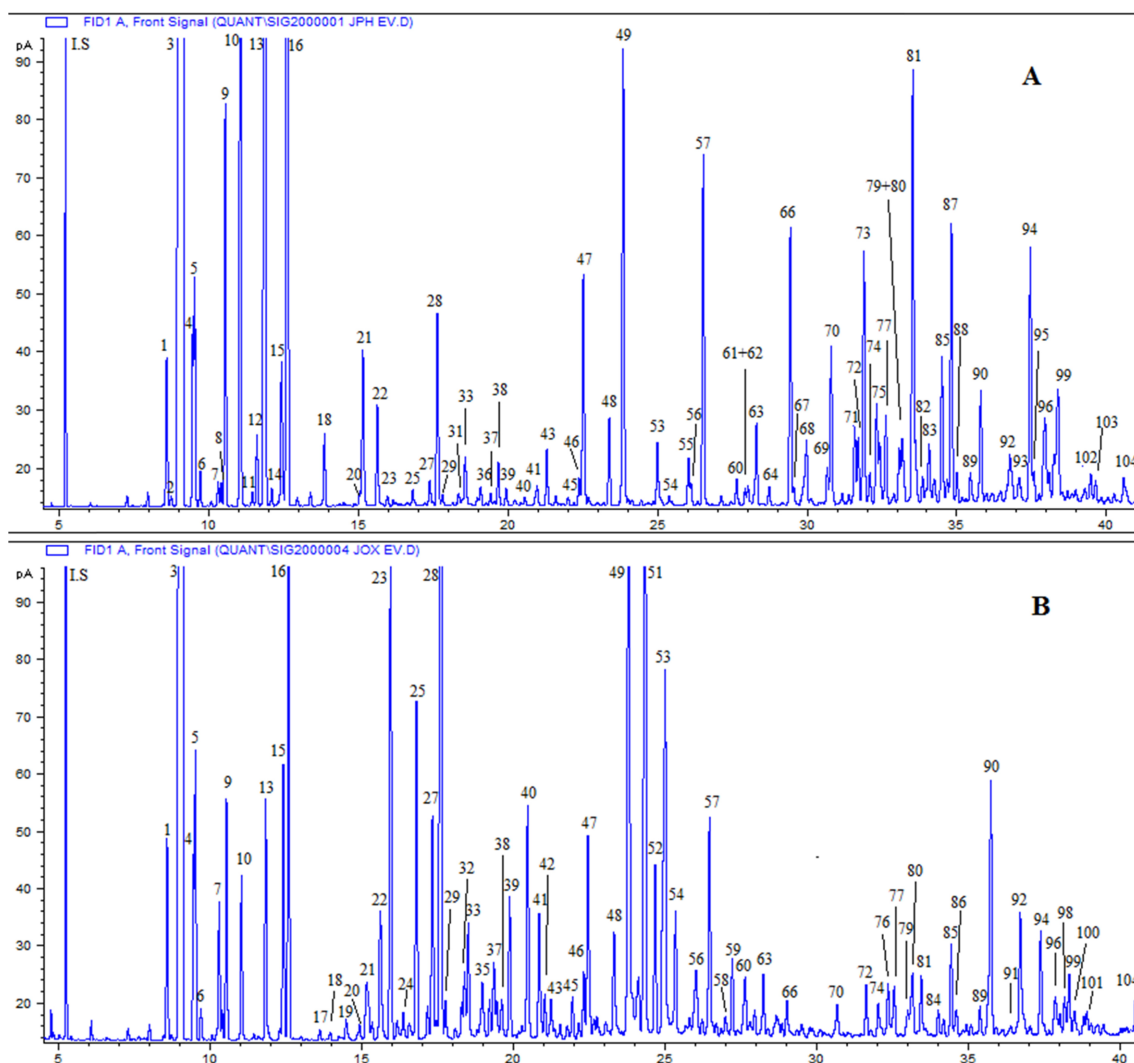
Fig. 2. GC-FID chromatographic profiles of *J. phoenicea* (**A**) and *J. oxycedrus* (**B**). Numbers refer to compounds identified in Table 1.

Mediterranean regions (Morocco, Portugal, Spain, France, Italy and Greece).[5,7,9,39] A report indicated the presence of α-pinene (54.8%), limonene (17.11%) and germacrene D (6.85%) in the leaves steam distilled oil of *J. oxycedrus* ssp. *oxycedrus* growing in Eastern Athens of Greece.[39] A similar result was obtained by Adams and other, in his study of France *J. oxycedrus* when the fresh leaves were treated by steam distillation and found that the largest group of constituents in the essential oil was mono-terpenes (75.5%), the major components were α-pinene (53.2%), δ-3-carene (5.1%) and limonene (3.5%) in monoterpene fraction. Also, in Moroccan leaf essential oil of *J. oxycedrus* ssp. *oxycedrus*, α-pinene (45.3%) and δ-3-carene (13.9%) were the major constituents, followed by C_{10}-dienol acetate (5.8%).[7]

For the sake of comparison, we have reported in (Fig.

3) the profile chromatographic of the standard mixture used for the identification of enantiomeric compounds. To illustrate the differences existing in terpene levels among the samples are expressed as the relative amount of each chiral terpene with respect to the total peak area sum in each chromatogram recorded by FID. In all cases, the enantiomeric excesses were calculated from peak areas obtained from FID signals and excess of predominant enantiomer was expressed as a percentage, ee = [(predo-minant enantiomer − minor enantiomer)/predominant enantiomer + minor enantiomer)] ×100.

Table 3 reports the average values of enantiomeric ratios found for the components analysed, and Fig. 4 reported the chromatographic profile of essential oils of *J. phoenicea* (A) and *J. oxycedrus* (B), the enantiomeric distribution of α-pinene, sabinene, camphene, δ-3-carene,
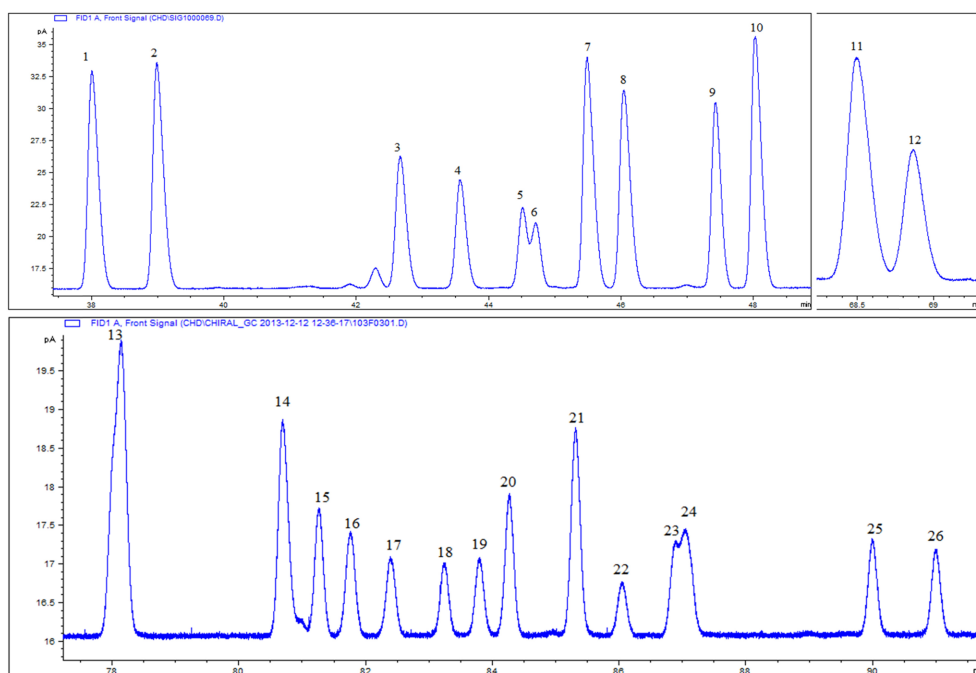
Fig. 3. Enantio-GC-FID analysis of the standard monoterpenes chiral; (**1**): (-)-α-pinene, (**2**): (+)-α-pinene, (**3**): (-)-camphene, (**4**): (+)-camphene, (**5**): (+)-δ-3-carene, (**6**): (-)-α-phellandrene, (**7**): (+)-β-pinene, (**8**) :(-)- β-pinene, (**9**) : (-)-limonene, (**10**): (+)-limonene, (**11**): (-)-linalool, (**12**): (+)-linalool, (**13**): (±)-camphor, (**14**):(+)-α-fenchol, (**15**): (-)-terpinene-4-ol, (**16**): (+)-pulegone, (**17**): (-)-pulegone, (**18**): (+)-menthol, (**19**): (-)-menthol, (**20**): (+)-bornyl acetate, (**21**): (-)-verbenone, (**22**): (+)-carvone, (**23**) and (**24**): (±)- citronellol, (**25**): (-)-borneol, (**26**): (+)-borneol.
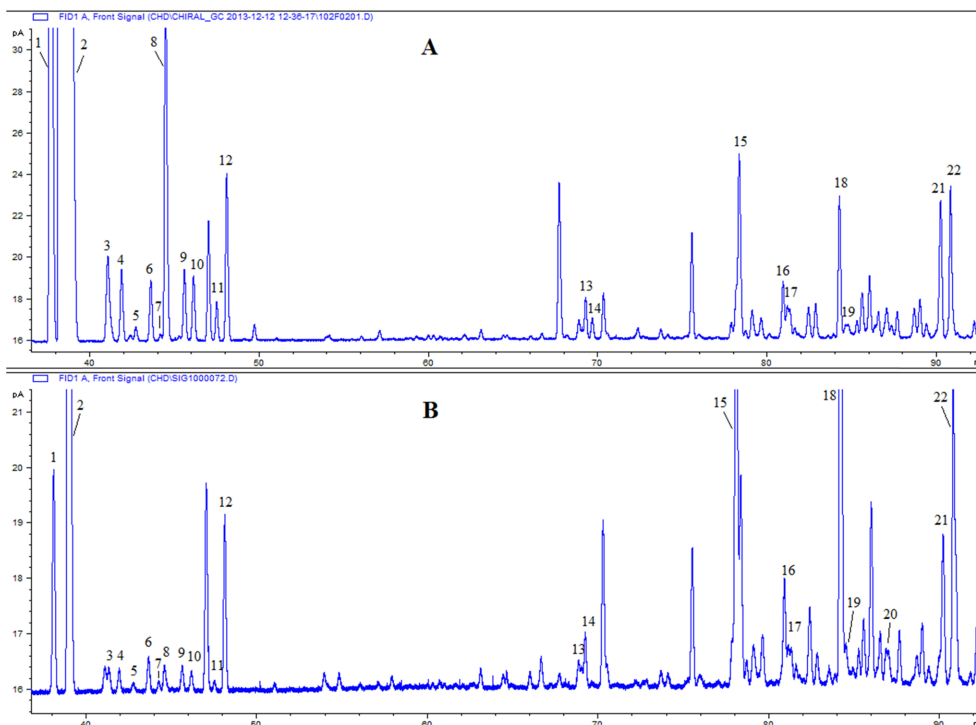
Fig. 4. Enantio-separation of the essential oils of *J. phoenicea* (**A**) and *J. oxycedrus* (**B**) by means of GC-FID; (**1**): (-)-α-pinene, (**2**): (+)-α-pinene, (**3**): (-)-sabinene (**4**): (+)-sabinene, (**5**): (-)-camphene, (**6**): (+)-camphene, (**7**): (-)-δ-3-carene, (**8**): (+)-δ-3-carene, (**9**): (+)-β-pinene, (**10**) :(-)- β-pinene, (**11**) : (-)-limonene, (**12**): (+)-limonene, (**13**): (-)-linalool, (**14**): (+)-linalool, (**15**): (±)-camphor, (**16**):(-)-terpinene-4-ol, (**17**): (+)-terpinene-4-ol, (**18**): (+)-bornyl acetate, (**19**): (-)-bornyl acetate, (**20**): (±)- citronellol, (**21**): (-)-borneol, (**22**): (+)-borneol.

Table 3. Enantiomeric distribution of chiral components of essential oils of *J. phoenicea* and *J. oxycedrus* from Algeria.

Compounds	Enantiomer compound[a]	%[b]	ee (%)[c]	%[b]	ee (%)[c]	Identification methods[d]
		J. phoenicea		*J. oxycedrus*		
α-Pinene	1S, 5S-(-)	10.87	78.26	11.40	77.2	1, 2
	1R, 5R-(+)	89.13		88.60		1, 2
Sabinene	1S, 5S-(-)	60.35	20.70	50.94	10.88	2
	1R, 5R-(+)	39.65		40.06		2
Camphene	1S, 4R-(-)	19.92	60.16	20.93	58.14	1, 2
	1R, 4S-(+)	80.08		79.07		1, 2
δ-3-Carene	1S-(-)	1.40	97.20	25.71	48.58	2
	1R-(+)	98.60		74.29		1, 2
β-Pinene	1R, 5R-(+)	51.74	3.48	56.41	12.82	1, 2
	1S, 5S-(-)	48.26		43.59		1, 2
Limonene	4S-(-)	18.43	63.14	4.88	90.24	1, 2
	4R-(+)	81.57		95.12		1, 2
Linalool	R-(-)	26.92	46.16	63.41	26.82	1, 2
	S-(+)	73.08		36.59		1, 2
Camphor[e]	(±)	100	100	100	100	1, 2
Terpinen-4-ol	4R-(-)	68.52	37.04	77.46	54.92	1, 2
	4S-(+)	31.48		22.54		1, 2
Bornyl acetate	1R-(+)	91.49	82.92	94.47	88.94	1, 2
	1S-(-)	8.51		5.53		2
β-Citronellol[e]	(±)	-	-	100	100	1, 2
Borneol	1S, 4S-(-)	50.26	0.52	31.07	37.86	1, 2
	1R, 4R-(+)	49.74		68.93		1, 2

[a] The order of elution of the different compounds and their enantiomers from the chiral column was as indicated in the table.
[b] Relative content of enantiomeric pairs.
[c] Enantiomeric excess
[d] Identification methods: 1; Co-GC and 2; GC/MS-Chiral
[e] Enantiomeric pairs no separated in column HP-Chiral 20β

β-pinene, limonene, linalool, terpinen-4-ol, bornyl acetate, camphor, citronellol and borneol present in both oils.

R-(+)-α-pinene (88.6-89.13%), *R*-(+)-bornyl acetate (91.49-94.74%) on both oils and *R*-(+)-limonene (95.12%) of *J. oxycedus* and *R*-(+)-δ-3-carene (98.60%) of *J. phoenicea* were detected with high enantiomeric purity, therefore, these chiral compounds are suitable in the authenticity control of juniper oil. (+)-Antipode of α-pinene, camphene, δ-3-carene, limonene and bornyl acetate were largely dominated over (-)-antipode in both juniper oils. However, camphene (79.07-80.08%) in both oils and linalool (73.08%) of *J. phoenicea* were again exclusively a high percentage as a *S*-(+) enantiomer, while sabinene, β-pinene and borneol were present a racemate mixture (40-60%).

Only one studies of the enantiomeric distributions of monoterpenes fraction in *J. oxycedrus* needles and berries obtained by SPME, Foudil-Cherif and Yassaa determined three enantiomeric pairs α-pinene, camphene and β-

pinene, and three absolute enantiomers (+)-sabinene, (+)-limonene and (+)-β-phellandrene (100%) of needles oil of *J. oxycedrus*,[30] three compounds (α-pinene, camphene and β-pinene enantiomers) of them also separated in the present study (Table 3). α-Pinene (88.6%), camphene (79.07%), δ-3-carene (74.29%), β-pinene (56.41%), limonene (95.12%), bornyl acetate (94.47%) and borneol (68.93%) showed a dominant (+)-absolute configuration, while sabinene (50.94%), linalool (63.41%) and terpinene-4-ol (77.46%), were present in (-)-absolute configuration. However, the enantiomeric pair (±)-β-citronellol and (±)-camphor no separated by conventional ES-GC in this column HP chiral-20B (Fig. 3.). In order to confirm the identity of enantiomers and eliminate possible interferences, chiral analysis was carried out, also using an MS detector.

The determination of the enantiomeric excess is an efficient method of authentication when asymmetric molecules remain unaltered by the extraction process.[49]

In conclusion, the essential oils composition of *J.*

phoenicea and *J. oxycedrus* were determined by means of advanced analytical techniques and methods. This quantification procedure has led to determination of around 98 components, distributed among the both oils investigated. The results here reported are intended as an updating of the data available in the literature. The major components for *J. phoenicea* were α-pinene (64.6 g/100 g), δ-3-carene (6.53 g/100 g) and limonene (2.99 g/100 g), while α-pinene (54.0 g/100 g), camphor (5.41 g/100 g) and bornyl acetate (4.86 g/100 g) were the major components found in the leaf oils of *Juniperus oxycedrus*. The enantiomeric distribution within chiral volatiles was determined, leading to the separation of ten enantiomeric pairs.

References

(1) Adams, R. P. Junipers of the World: The Genus Juniperus: 4th ed.; Trafford Publisher: U.S. **2014**, p 2.

(2) Quezel, P.; Santa, S. Nouvelles Flores d'Algérie et des Régions Désertiques Méditerranéennes : tome I ; Centre National de la Recherche Scientifique: France, **1963**, pp 34-38.

(3) Adams, R. P.; Barrero, A. F.; Lara, A. *J. Essent. Oil Res.* **1996**, *8*, 367-371.

(4) Rezzi, S.; Cavaleiro, C.; Bighelli, A.; Salgueiro, L.; Pronça da Cunha, A.; Casanova, J. *Biochem. Syst. Ecol.* **2001**, *29*, 179-188.

(5) Adams, R. P. *Biochem. Syst. Ecol.* **1998**, *26*, 637-645.

(6) Cavaleiro, C.; Salgueiro, L. R.; da Cunha, A. P.; Figueiredo, A. C.; Barroso, J. G.; Bighelli, A.; Casanova, J. *Biochem. Syst. Ecol.* **2003**, *31*, 193-201.

(7) Adams, R. P.; Morris, J. A.; Pandey, R. N.; Schwarzbach, A. E. *Biochem. Syst. Ecol.* **2005**, *33*, 771-787.

(8) Machado, M.; Santoro, G.; Sousa, M. C.; Salgueiro, L.; Cavaleiro, C. *Flavour Frag. J.* **2010**, *25*, 156-160.

(9) Adams, R. P.; Altarejos, J.; Fernandez, C.; Camacho, A. *J. Essent. Oil Res.* **1999**, *11*, 167-172.

(10) Adams, R. P. *Biochem. Syst. Ecol.* **2000**, *28*, 515-528.

(11) Salido, S.; Altarejos, J.; Nogueras, M.; Sanchez, A.; Pannecouque, C.; Witvrouw, M.; Clercq, E. D. *J. Ethnopharmacol.* **2002**, *81*, 129-134.

(12) Boti, J. B.; Bighelli, A.; Cavaleiro, C.; Salgueiro, L.; Casanova, J. *Flavour Frag. J.* **2006**, *21*, 268-273.

(13) Adams, R. P.; Terzioglu, S.; Mataraci, T. *Phytologia* **2010**, *92*, 156-166.

(14) Adams, R. P.; Mataraci, T. *Phytologia* **2011**, *93*, 293-303.

(15) Angioni, A.; Barra, A.; Russo, M. T.; Coroneo, V.; Dessi, S.; Cabras, P. *J. Agric. Food Chem.* **2003**, *51*, 3073-3078.

(16) Valentini, G.; Bellomaria, B.; Maggi, F.; Manzi, A. *J. Essent. Oil Res.* **2003**, *15*, 418-421.

(17) Milos, M.; Radonic, A. *Food Chem.* **2000**, *68*, 333-338.

(18) Stassi, V.; Verykokidou, E.; Loukis, A.; Harvala, A.; Philianos, S. *J. Essent. Oil Res.* **1995**, *7*, 675-676.

(19) Sezik, E.; Kocakulak, E.; Baser, K. H. C.; Ozek, T. *Chem. Nat. Compd.* **2005**, *41*, 352-354.

(20) Hayta, S.; Bagci, E. *Acta Bot. Gallica* **2014**, *161*, 201-207.

(21) Ennajar, M.; Bouajila, J.; Lebrihi, A.; Mathieu, F.; Savagnac, A.; Abderraba, M.; Raies, A.; Romdhane, M. *J. Sci. Food Agric.* **2010**, *90*, 462-470.

(22) Ennajar, M.; Afloulous, S.; Romdhane, M.; Ibrahim, H.; Cazaux, S.; Abderraba, M.; Raies, A.; Bouajila, J. *J. Food Sci.* **2011**, *76*, C224-C230.

(23) Medini, H.; Marzouki, H.; Chemli, R.; Marongiu, B.; Piras, A.; Porcedda, S.; Tuveri, E. *J. Essent. Oil Bear. Plant* **2008**, *11*, 233-241.

(24) Medini, H.; Elaissi, A.; Farhat F.; Khouja, M. L.; Chemli, R.; Harzallah-Skhiri, F. *Chem. Biodivers.* **2009**, *6*, 1378-1387.

(25) Medini, H.; Elaissi, A.; Larbi Khouja, M.; Piras, A.; Porcedda, S.; Falconieri, D.; Marongiu, B.; Chemli, R. *Nat. Prod. Res.* **2011**, *25*, 1695-1706.

(26) Medini, H.; Elaissi, A.; Khouja, M. L.; Chraief, I.; Farhat, F.; Hammami, M.; Chemli, R.; Harzallah-Skhiri, F. *Chem. Biodivers.* **2010**, *7*, 1254-1266.

(27) Riahi, L.; Chograni, H.; Ziadi, S.; Zaouali, Y.; Zoghlami, N.; Mliki, A. *J. Essent. Oil Res.* **2013**, *25*, 324-329.

(28) Harhour, A.; Brada, M.; Fauconnier, M. L.; Lognay, G. *Nat. Prod. Sci.* **2018**, *24*, 125-131.

(29) Dob, T.; Dahmane, D.; Chelghoum, C. *Pharm. Biol.* **2006**, *44*, 1-6.

(30) Foudil-Cherif, Y.; Yassaa, N. Food Chem. **2012**, *135*, 1796-1800.

(31) Bekhechi, C.; Atik Bekkara, F.; Consiglio, D.; Bighelli, A.; Tomi, F. *Chem. Biodivers.* **2012**, *9*, 2742-2753.

(32) Dob, T.; Dahmane, D.; Chelghoum, C. *J. Essent. Oil Res.* **2008**, *20*, 15-20.

(33) Barrero, A. F.; Herrador, M. M.; Arteaga, P.; Quilez del Moral, J. F.; Sanchez-Fernandez, E.; Akssira, M.; Aitigir, M.; Mellouki, F.; Akkad, S. *J. Essent. Oil Res.* **2006**, *18*, 168-169.

(34) Achak, N.; Romane, A.; Alifriqui, M.; Adams, R. P. *J. Essent. Oil Res.* **2009**, *21*, 337-341.

(35) Mansouri, N.; Satrani, B.; Ghanmi, M.; El ghadraoui, L.; Aafi, A.; Farah, A. *Phytotherapie* **2010**, *8*, 166-170.

(36) Adams, R. P.; Altarejos, J.; Arista, M. *Phytologia* **2014**, *96*, 110-116.

(37) Adams, R. P.; Arista, M.; Boratynski, A.; Houari, H. H.; Leschner, H.; Liber, Z.; Minissale, P.; Sciandrello, S.; Mataraci, T. *Phytologia* **2014**, *96*, 149-158.

(38) Adams, R. P.; Rumeu, B.; Nogales, M.; Fontinha, S. S. *Phytologia* **2009**, *91*, 40-53.

(39) Vourlioti-Arapi, F.; Michaelakis, A.; Evergetis, E.; Koliopoulos, G.; Haroutounian, S. A. *Parasitol Res.* **2012**, *110*, 1829-1839.

(40) Rubiolo, P.; Sgorbini, B.; Liberto, E.; Cordero, C.; Bicchi, C. *Flavour Frag. J.* **2010**, *25*, 282-290.

(41) Adams, R. P. *Essential Oils Waxes* **1991**, *12*, 159-173.

(42) Van den Dool, H.; Kratz, P.D. *J. Chromatogr.* **1963**, *11*, 463-471.

(43) IOFI Working Group on Methods of Analysis. *Flavour Frag. J.* **2011**, *26*, 297-299.

(44) Costa, R.; Zellner, B. D.; Crupi, M. L.; De Fina, M. R.; Valentino, M. R.; Dugo, P.; Dugo, G.; Mondello, L. *Flavour Frag. J.* **2008**, *23*, 40-48.

(45) Adams, R. P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy; Allured Publishing Corporation: U.S.A, **2007**, p 804.

(46) Terpenoids Library List. Available online: http://massfinder.com/wiki/Terpenoids_Library_List (accessed on 12 January 2020).

(47) Bicchi, C.; Liberto, E.; Matteodo, M.; Sgorbini, B.; Mondello, L.; Zellner, B. D.; Costa, R.; Rubiolo, P. *Flavour Frag. J.* **2008**, *23*, 382-391.

(48) Cicchetti, E.; Merle, P.; Chaintreau, A. *Flavour Frag. J.* **2008**, *23*, 450-459.

(49) Bisht, D.; Chanotiya, C. S.; Rana, M.; Semwal, M. *Ind. Crop Prod.* **2009**, *30*, 422-426.