Biodiversity Journal, 2022,13 (4): 885–894 https://doi.org/10.31396/Biodiv.Jour.2022.13.4.885.894

Chemical composition of essential oils of *Juniperus phoenicea* L. (Cupressales Cupressaceae) from two localities in West-Algeria

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ABSTRACT The Phoenician juniper, *Juniperus phoenicea* L. (Cupressales Cupressaceae) fits between steppe formations at low altitudes (coastal dunes) and forest formations. Our work consists in contributing to the study of the phytochemical screening of the essential oils of *J. phoenicea* collected from two sites: the first in inland Mountains (continental), Djebel Mekther Wilaya of Naama, and the second in coastal area, Stedia Wilaya Mostaganem, in western Algeria. The essential oils of the aerial part of the dried plant, obtained by hydro distillation, revealed significant yields of 0.329% and 0.283%, respectively, Djebel Mekther and Stedia. The results of the GC/MS chromatographic analysis of the two essential oils possess a large chemical variation with 64 constituents representing more than 72.5% for the Stedia sample and 77.3% for the sample of Djebel Mekther, were identified. A comparison was made between essential oil components of *J. phoenicea* in our two regions and 14 samples from the literature. The Principal Component Analysis (PCA) reveals a significant chemical variability making it possible to define three types of chemical profiles of *J. phoenicea* and we have a significant dissimilarity between the samples of Djebel Mekther all the other regions.

KEY WORDS *Juniperus phoenicea*; Essential oils; Chemical composition; Djebel Mekther; Stedia; Algeria.

Received 18.06.2022; accepted 12.11.2022; published online 30.12.2022

INTRODUCTION

The Cupressaceae family (Cupressales) is widely represented among all Gymnosperm, occurring in various habitats on all continents except Antarctica. Most of the generic diversity is localised in the southern hemisphere, but the largest genus *Juniperus* L. is mainly north-temperate (Silba, 1986; Van Royen, 1979) with the exception of *J. procera* Hochst. ex Endl., which also grows along the Rift Mountains in East Africa in the southern hemisphere (Adams, 2008).

This genus listed 67 species and 34 varieties (using the most widely accepted variety category

instead of the category subspecies) which shows strongly relictual distributions, and a large number of localised, rare and/or endangered taxa.

The genus is divided into three sections: *Juniperus* (10 species), *Caryocedrus* Enciricher (1 specie) and *Sabina* (Miller) Spach (56 species) (Adams, 2008).

All *Juniperus* species have been assigned to six major clades of which the fifth clade contains only *J. phoenicea* L., native to the Mediterranean area and the only ancient species in the world with leaves with serrated edges (Kangshan et al., 2010) (Fig. 1). *Juniperus phoenicea* (section *sabina*) is a small tree native to North Africa, Algeria and Morocco as well as the Canary Islands (Adams, 2004). These small evergreen trees or shrubs, monoecious or dioecious, with fleshy seed cones in which the cone scales are fused resembling 'berries'. These seed cones are fully ripe only in the second year, globose to ovoid, blackish when very young, later green or yellowish and slightly pruinose, dark red when ripe (Adams, 2008). *Juniperus phoenicea* is divided in two subspecies, *J. phoenicea* subsp. *phoenicea* and *J. phoenicea* subsp. *turbinara* (Guss.) Nyman. The first subspecies is characterized by female globose cones and obtuse or subacute scale-leaves, seed cones globose and is widespread in a greater geographical area, the second has cones turbinate seed and a more restrict distribution (Franco, 1986; Meloni et al., 2006).

MATERIAL AND METHODS

Study area and samples

The plant materials, *Juniperus phoenicea*, were collected in October 2017 from two sites located at the western Algeria: inland mountains (continental): in the region of Ain sefra, 32°41'23.15"N 0°31'43.06" W, 1929 m, Wilaya of Naama, characterized by a Saharan climate with cool winter; coastal (littoral): in the region of Stidia, 35°48'19.10"N 0°4'23.07"W, 11 m, Wilaya of Mostaganem, characterised by a semi-arid climate with moderate winter.

Essential oil extraction

After weighing the total weight of the harvested plant, the plant material is divided into test portions of 100g, each one will undergo hydro distillation using a Clevenger-type device and boiled for 2 hours. The oil is decanted, filtered and stored in an airtight bottle at 4 °C (Dubey, 2003). The yield of the plant is measured according to the dry weight (Dj. Mekther: 1838 g, Stedia: 2237 g), and the oils obtained (6.065 g–6.33 g).

Gas chromatography–mass spectrometry

Analysis of essential oils by gas chromatogra-

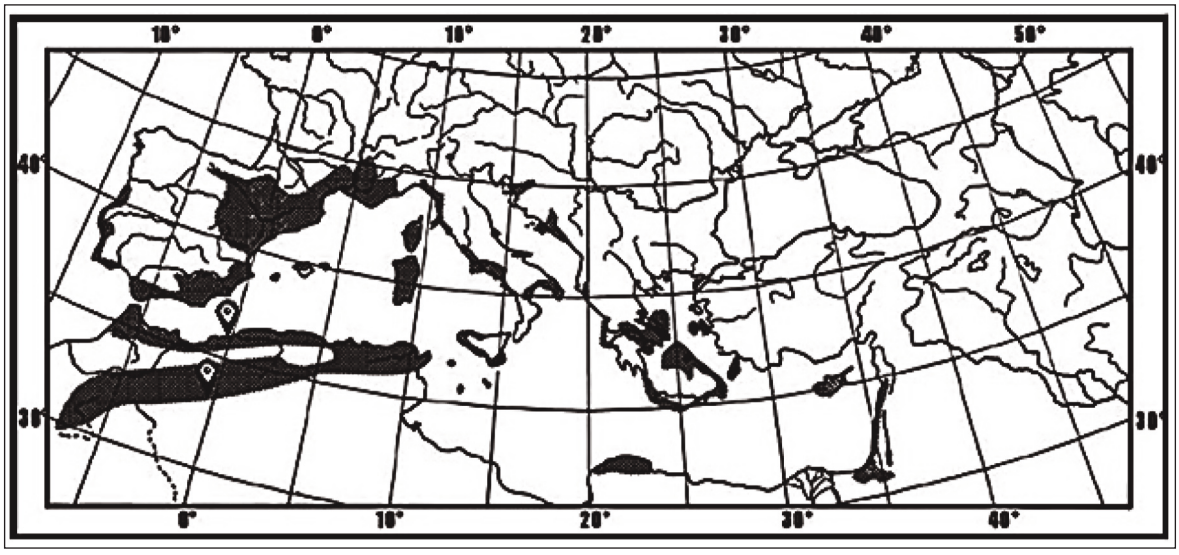

Figure 1. Distribution of of *Juniperus phoenicea* (Boratyński et al., 1992; Quezel et al., 1992).

phy-mass spectrometry (GC-MS) was performed with a GCMS-TQ8030 (Shimadzu Co., Japan) equipped with a DB-5 capillary column (30 m × 0. 25 mm ID, film thickness 0.25 µm). The oven temperature was programmed (60 to 230 °C at 5 °C/min) and kept isothermal at 250 °C for 10 min; ion source and transfer line temperature, 250 °C; carrier gas, He (1 ml/min). The mass spectrometer type (MS-5973 N) [(GC/MS)] was acquired over the mass range of 40 to 850 a.m.u. with an ionisation energy of 70 eV.

Characterization

Essential oil components were identified based on their Kovats retention indexes (KRIs) (determined by reference to a homologous series of alkanes), and by comparison of their fragmentation patterns by mass spectrometry with those reported in the literature (Davies, 1990; Adams, 2007). The identification of the different components was assigned by matching their mass spectra with data from the Wiley and NIST libraries (NIST, 2014).

Statistical analysis

Principal component analysis (PCA) was performed with R software (v.4.0.4) and carried out on the cumulative data corresponding to our samples taken for the two sites and those from bibliographical data (Rezzi et al., 2001, Achak et al., 2008, Adams et al., 2014). These data consist of 16 specimens: 2 samples collected from the 2 sites, the first in inland Mountains, continental area, as Djebel Mekther Wilaya of Naama, and the second in coastal area, Stedia Wilaya Mostaganem in the western Algeria; and 14 samples from the literature: 2 samples from Corsica (Rezzi et al., 2001), 2 samples collected from Amassine-Ourika in Atlas mountains, Morocco (Achak et al., 2008), and for Adams et al. (2014), 10 samples 5 in Spain (Grazalema, Penone, Tarifa sands, Madeira, Canary Islands); 2 in Italy (Crotone, Sicily), 1 in Egypt (Sinai) 1 in Turkey and 1 in Morocco (Oukaimeden in Atlas Mountains).

There are 22 variables which are compounds that seem to distinguish taxa (Adams et al., 2014). This analysis was also carried out in order to study the variability of the chemical composition for each of the 16 sites. Principal Component Analysis (PCA) in order to highlight a possible variability in the chemical composition of the essential oils of the leaves of *J. phoenicea*.

RESULTS AND DISCUSSION

The essential oils of the aerial part of the dried plant, obtained by hydro distillation revealed significant yields of 0.329% for the sample of Djebel Mekther (Wilaya of Naama) and 0.283% for the sample from Stidia (Wilaya of Mostaganem), these values are higher than those obtained on samples from Greece (0.21%) and Spain (0.30%) (Adams et al., 1996) and are also lower than those obtained on samples from Portugal (0.41%) (Adams et al., 1996) and Egypt (0.36%) (El-Sawi et al., 2007). This variation in yield can be attributed to the particular geographical location of this species which greatly influences plant yield (Caissard et al., 2004), (Figueredo et al., 2007).

The results of the GC/MS chromatographic analysis of the two essential oils extracted from the leaves of *J. phoenicea* is described in Table 1. The essential oils possess a large chemical variation.

In our study, a total of 64 constituents representing, more than 72.5% for the Stedia samples and 77.3% for the samples of Djebel Mekther, were identified based on the comparison of their mass spectra with those of authentic compounds reported in the literature and show that all essential oils of *J. phoenicea* contain a predominance of monoterpene hydrocarbons, with α-pinene as the major constituent, followed by oxygenated monoterpenes (Afifi et al., 1992; Rezzi et al., 2001; Angioni et al., 2003; El-Sawi et al., 2007; Ennajar et al., 2009; Mazari et al., 2010).

Indeed, the monoterpene fraction of the essential oils of Djebel Mekther (continental) and Stedia (coastal) is more important and represents respectively approximately 49.14% and 71.83% of the overall chemical composition of the oil, including:

1. Monoterpene hydrocarbons (37.75% for Djebel Mekther and 62.24% for Stedia), the main major constituents which characterise the essential oils of *J. phoenicea* from the sample of Djebel Mekther (continental) are: α-Pinene (18.22%), δ-3-Carene (6.72%), Myrcene (3.79%), Limonene (3.11%), β-Pinene (1.88%). and those for the Stedia sample (costal) are : α-Pinene (19.07%), γ-terpinene

IK	Nom commun	STD-2-A	MKT-1-A	IK	Nom commun	STD-2-A	MKT-1-A
921	Tricyclene	0.00	0.38	1254	linalyl acetate	0.25	2.96
932	**Pinene <α->**	**19.07**	**18.22**	1275	Isopulegyl acetate	0.00	0.84
946	Camphene	0.12	1.36	1319	Decadienol <(2E.4E)->	0.21	0.00
969	Sabinene	0.21	0.14	1345	Cubebene <α->	0.00	0.42
974	Pinene <β->	0.71	1.88	**1346**	**Terpinyl acetate <α->**	**0.15**	**1.44**
988	**Myrcene**	**3.55**	**3.79**	1373	Ylangene <α->	0.00	0.47
989	Heptanol <2.6-dimethyl-2-> Heptaminol	0.19	0.00	1374	Copaene <α->	0.00	0.30
1001	Carene <δ-2->	0.00	0.23	**1410**	**Cedrene <α->**	**0.00**	**0.12**
1002	**Phellandrene <α->**	**2.42**	**0.00**	1415	Undecadienal <(2E.4E)->	0.00	0.23
1008	**Carene <δ-3->**	**0.00**	**6.72**	1417	Caryophyllene <(E)->	0.12	1.48
1014	Terpinene <α->	0.00	0.12	1419	Cedrene <β->	0.00	2.15
1020	Cymene <ρ->	0.79	0.80	1452	Humulene <α->	0.00	1.07
1024	Limonene	1.92	3.11	1454	Farnesene <(E)-β->	0.00	0.64
1025	**Phellandrene <β->**	**15.47**	**0.77**	1475	Cadina-1(6).4-diene <trans->	0.00	0.96
1044	Ocimene <(E)-β->	0.00	0.23	1484	Germacrene D	0.12	2.10
1086	Terpinolene	0.23	0.00	1493	Zingiberene <α->	0.00	0.15
1095	Linalool	0.14	1.63	1496	Valencene	0.00	0.17
1114	Fenchol <endo->	0.16	0.00	1498	Selinene <α->	0.00	0.91
1118	Menth-2-en-1-ol <cis-ρ->	0.12	0.00	1500	Muurolene <α->	0.00	0.97
1122	α-Campholenal	0.00	0.23	1504	Cuparene	0.00	0.24
1128	Ocimene <allo->	0.00	0.50	1505	Bisabolene <β->	0.00	0.39
1141	Camphor	0.00	0.13	1513	Cadinene <γ->	0.00	0.53
1054	γ-terpinene	17.56	0.00	1521	Sesquiphellandrene <β->	0.00	0.87
1165	Borneol	0.52	0.00	1521	Calamenene <trans->	0.00	0.51
1172	Pinocamphone <cis->	0.00	0.33	1522	Cadinene <δ->	0.00	1.73
1174	Terpinen-4-ol	0.00	0.23	1559	Germacrene B	0.11	0.00
1186	**Terpineol <α->**	**8.04**	**1.21**	1559	Dodecanoic acid	0.17	0.42
1192	Dihydro carveol	0.00	0.32	1565	Spathulenol	0.15	0.00
1204	Verbenone	0.00	0.39	1582	Caryophyllene oxide	0.00	0.40
1215	Carveol. trans-	0.00	0.24	**1600**	**cedrol**	**0.00**	**9.87**
1218	Fenchyl acetate <endo->	0.00	0.37	**1652**	**Cadinol <α->**	**0.00**	**0.83**
1249	Geraniol	0.00	0.15	**1978**	**manoyl oxide**	**0.00**	**0.65**

Table 1. Composition of essential oils derived from leaves of *Juniperus phoenicea* from Djebel Mekther (Wilaya of Naama) and Stedia (Wilaya of Mostaganem) in West-Algeria. KI = linear Kovats Index on DB-5 column with reference libraries. and from the literature. Compositional values Unidentified and less than 0.1% are reported 0.00.

(17.56%), β-Phellandrene (15.47%), Myrcene (3.55%) , α-Phellandrene (2.42%) and Limonene (1.92%).

2. Oxygenated monoterpenes represent 11.39% for the sample of Djebel Mekther whose fractions are: linalyl acetate (2.96%); linalool (1.63%); α-terpinyl acetate (1.44%); α-Terpineol (1.21%), and 9.59% of the Stedia sample, the fractions of which are : α-Terpineol (8.04%); Borneol (0.52%); linalyl acetate (0.25%). However, the sesquiterpene fraction appeared in low proportion for the two studied stations (22.49% for Djebel Mekther and 9.74% Stedia) including:

Sesquiterpene hydrocarbons (12.16% for Dj. Mekther and 0 .4% for Stedia). In the sample of Dj. Mekther we found β-Cedrene (2.15%) and Germacrene (2.1%), in the sample of Stedia we found dodecanoic acid (0.17%), germacrene D (0.12%), E-Caryophyllene (0.12) and Germacrene B (0.11%).

Oxygenated Sesquiterpenes (11.1% for Dj. Mekther and 0.15% for Stedia), the compounds in the sample of Djebel Mekther are: Cedrol which is the main constituent of this fraction with 9.87%, α-Cadinol (1.07%) and Caryophyllene Oxide (0.4%), in the Stedia sample we found Spathulenol with 0.15%. For the diterpine fraction, only manoyl oxide was found in the sample of Djebel Mekther with 0.65%.

Likewise, a study conducted by Adams et al. (2014) on the essential oils of different populations of *J. phoenicea* (Canary Islands, Sinai, Morocco, Madeira, Turkey, Sicily, sand dunes of Tarifa, Italy: Crotone, Spain: El Penon, Grazalema) gave as majority compounds α-pinene ranging from 17.7% to 67.9% and β-phellandrene ranging from 0.5 to 31.5%. The composition of the oils of *J. phoenicea* var. *turbinata* was also dominated by monoterpenes, a sample from Morocco was characterised by a very high α-pinene content (74.0%) (Mansouri et al., 2011). In contrast, two oil samples from the Spanish coast and the Portuguese coast were characterised by good amounts of α-pinene (28.3 and 34.1%) as well as b-phellandrene (25.3 and 19 .2%) and α-terpinyl acetate (15.5 and 12.5%) (Adams et al., 1996).

For the choice of significant axes to be interpreted, the study is based on the eigenvalues and the rate of inertia calculated for each axis (Table 2).

The first axis generally carries the maximum information in terms of variance which is 37.37%, the second and the third axis carry much less and are respectively 19.67% and 14.93% (Fig. 2).

Main axis 1 is essentially constructed from the opposition of 2 groups of variables:

(i) Camphene, Cedrol, α.cedrene, α.cadinol and β.pinene which have a negative contribution (-0.841, -0.747, -0.746, -0.689, -0.61 respectively). (ii) Trans-pinocarveol and β.phellandrene which have a positive contribution (+0.748) and (+0.716) respectively.

Thus, the Stedia samples, which has a negative contribution on A1, shows significant amounts of β-phellandrene (15.47%) and low amounts of camphene and β-pinene, and zero in trans-pinocarveol, α-cedrene, Cedrol and α-cadinol.

The samples of Djebel Mekther is characterised by a negative contribution to A1, as they contain significant amounts of camphene, β-pinene, α-cedrene, cedrol and α-cadinol (1.36; 1.88; 0.12; 9.87; 0.83 respectively) and very low in β-phellandrene (0.77%) and trans-pinocarveol (0%).

Component 2, which is essentially constructed from the positive contributions of the trans-β-farnesene and β-caryophyllene variables, does not intervene in the discrimination.

Figure 3 represents the map of the samples according to the first two components (A1 and A2), explaining 57% of the variance. This representation forms 3 groups of individuals which are as follows:

(i) The samples from Stedia gathered on the right of the map with Madeira, Turkey, Sicily, Tarifa sands and Italy (Crotone) where the joint analysis of the maps of individuals and variables allows to associate higher compositions in β-phellandrene and trans-pinocarveol (15.47% at Stedia up to 31.5% at Tarifa sands) and lower in Camphene, β-pinene, α-cadinol (0.12%, 1.3% and 0.4%, respectively), and zero in α-cedrene and cedrol.

(ii) The samples gathered in the negative coordinates of the second component of the map (Corsica 1, Ourika 1300 m DL, Canary Islands, Sinai, Morocco 940 m) where the joint analysis of the maps of individuals and variables makes it possible to associate the higher compositions in α-cadinol (1%) and lower in Camphene, β-pinene, β-phellandrene, trans-pinocarveol (0.6%, 1.6%, 4.9% and 0.5%, respectively), and zero in α-cedrene, cedrol, except for sinai which has 0.3% cedrol.

(iii) On the left of the map, the sample originating from Djebel Mekther has a negative score on this component or the joint analysis of the maps makes it possible to associate higher compositions in Camphene, β-pinene, α-cedrene; cedrol, α-cadinol (1.36%, 1.88%, 0.12%, 9.87% and 0.83%, respectively), lower in β-phellandrene (0.77%), and nil in trans-pinocarveol. The PCA reveals a significant chemical variability making it possible to de-

Axes	Eigenvalues	Variance (%)	Cumulative variance (%)
Axis 1	7.101	37.373	37.373
Axe 2	3.737	19.671	57.044
Axe 3	2.837	14.932	71.976

Table 2. Eigenvalues and variances of the three-factorial axes. (Boratyński et al., 1992; Quezel et al., 1992).

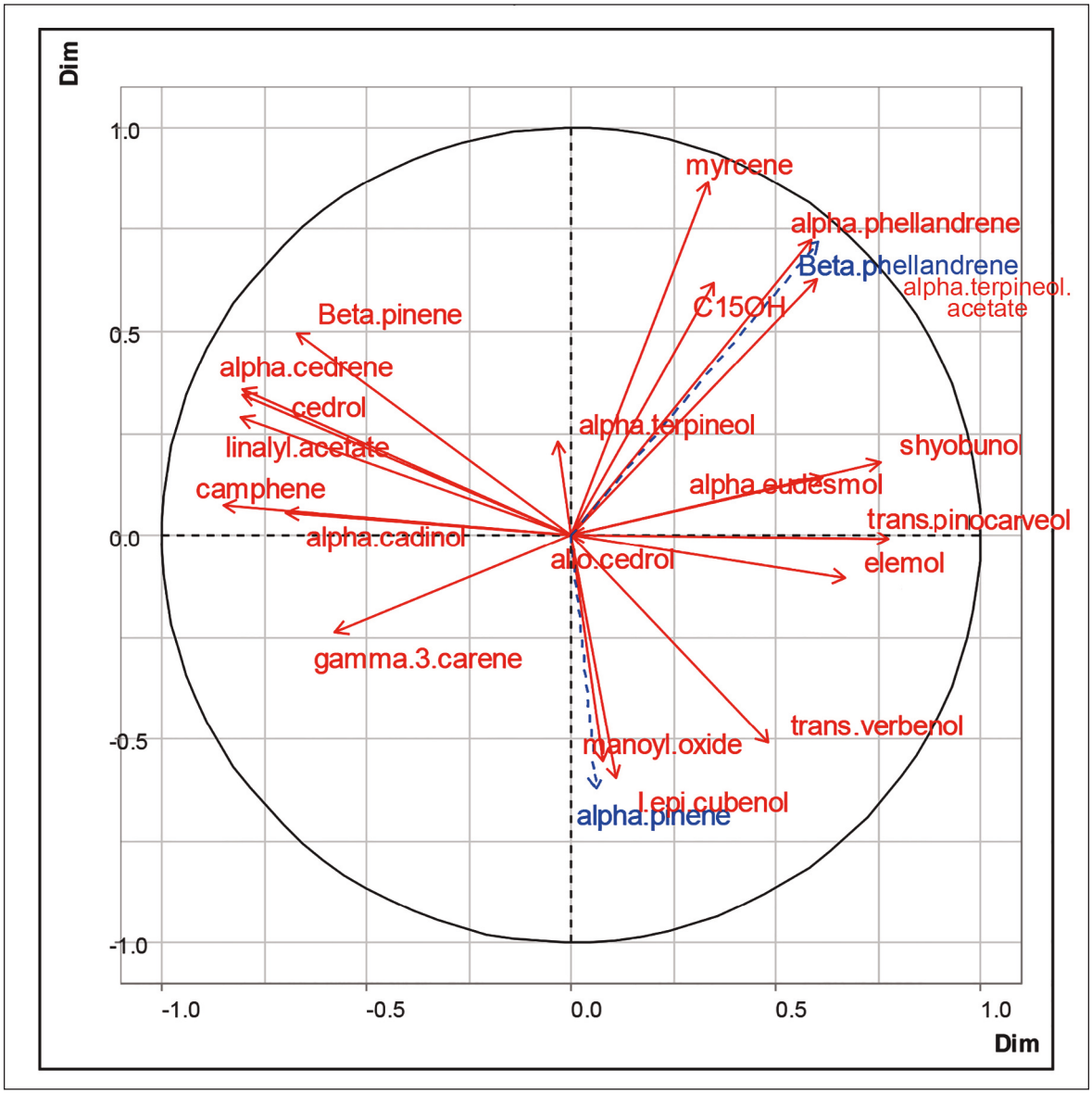

Figure 2. Map of variables according to components A1 and A2.

fine three types of chemical profiles of *J. phoenicea* defined by compounds which have positive and negative contributions whose absolute values are greater than 0.6 found in variable quantities depending on the site.

The chemical type containing β-phellandrene and trans-pinocarveol groups the six regions originated from the coastal area (Stedia, Madeira, Turkey, Sicily, Tarifa sands and Italy: Crotone). The ß-phellandrene is the second product; the population of Tarifa in Spain is individualised by a rate of 31.5% (Adams et al., 2014), and the Algerian population (Stedia) with a rate of 15.47% compared to other regions which are characterised by high altitudes - 520 m in Canary Islands up to 1300 m in Morocco (Ourika) or even 1929 m in Djebel Mekther - where the β-phellandrene oscillates between 0.05 and 4.9%, even in the forests of Assif Almal, Aghbar and Tifnoute, with respective altitudes of 1400, 1500 and 1550 m where β-phellandrene represent 0.89%, 1.62% and 0.79%, respectively.

For trans-pinocarveol, Abu-Darwish et al. (2013) found in Tafilah province (1260 m) and Shoubak province (1365 m) an amount of 3.5% and 2.0%, respectively. While, a study on *J. phoenicea* of the garden of Lakhdaria city, Bouira (low altitude) found an amount of 0.09% trans-pinocarveol (Dahmane et al., 2020). The chemical type containing α-cadinol groups the last five samples of group 2 were collected at higher altitudes (Corsica 1, Ourika 1300 m DL, Canary Islands, Sinai, Morocco 940 m) having higher amounts (0.2% to 1.0%) even at Djebel Mekther (0.83%) compared to the first samples group at low altitudes (Stedia, Madeira, Turkey, Sicily, Tarifa sands and Italy: Crotone) (0% to 0.4%).

The chemical type containing Camphene, β-pinene, α-cedrene; cedrol and α-cadinol which groups only the sample of Djebel Mekther in G1 comes from the highest altitude among all the samples. The amounts are 1.36%, 1.88%, 0.12%, 9.87% and 0.83%, respectively. Even at the forests of Assif Lmal (1400 m), Aghbar (1500 m) and Tifnoute (1550 m), Camphene are 0.62%, 1.15% and 0.35%, respectively, β-pinene are 0.76%, 0.87% and 0.87%, respectively, α-cadinol are 0%, traces (< 0.05%)., 0%, respectively (Mansouri et al., 2010). In Tafilah province (1260 m) and Shoubak province (1365 m), camphene is 0.7%, β-pinene 1.4% vs 1.5% and cedrol is 3.1% vs 2.0%, At mid-altitude Camphene is 0.51%, Cedrol is 0.06%, α-Cadinol 0.05% and β-Pinene is 0.95% (Dahmane et al., 2020).

The use of the Jaccard similarity index reveals the following conclusions:

a significant dissimilarity between the samples of Djebel Mekther (1929 m) and the other regions which varies between 38% and 68%;

the region of Stidea reveals values of median dissimilarity (50%) compared to the other maritime regions;

the recognition of strong differences between the two population groups suggests a long period of isolation, indicating their origin from other Pleistocene refuge or, better, other ecological refuge areas. Ecologically, *J. phoenicea* is a pioneer, demanding in light and relatively resistant to a dry climate (Zohary, 1973; Quézel & Pesson 1980; Quézel

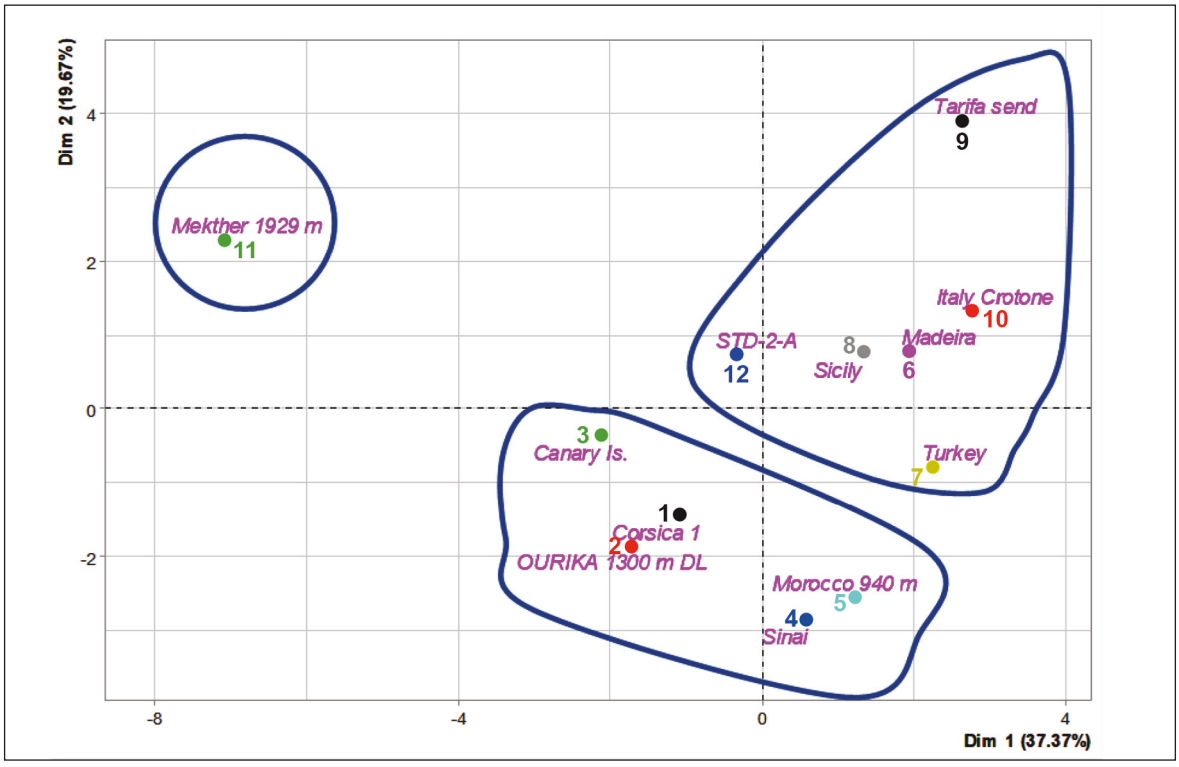

Figure 3. PCA - Map of samples according to components A1 and A2.

& Barbero, 1981; Auclair 1996; Charco, 2001; Tzedakis 2004; Petit et al., 2005). *Juniperus phoenicea* from Djebel Mekther shows a distribution on the steep slopes, in a disseminated and sporadic way on the overhanging slopes of the Sahara region, while the chemical type containing Camphene, β-pinene, α-cedrene, cedrol and α-cadinol takes refuge in high altitudes.

CONCLUSIONS

Analysis of the chemical composition of the essential oil of *J. phoenicea* made it possible to identify 64 compounds representing more than 72.5% for the Stedia sample and 77.3% for the sample of Djebel Mekther,

According to the results obtained from a PCA, three groups could be distinguished on the basis of the β-phellandrene, trans-pinocarveol, Camphene, β-pinene, α-cadinol, α-cedrene and cedrol contents.

The PCA reveals a significant chemical variability making it possible to define three types of chemical profiles of *J. phoenicea* defined by compounds which have positive and negative contributions. The samples from Stedia gathered on the right of the map with Madeira, Turkey, Sicily, Tarifa sands and Italy (Crotone) where the joint analysis of the maps of individuals and variables allow to associate higher compositions in: β-phellandrene and trans-pinocarveol; and lower in: Camphene, β-pinene, α-cadinol and zero in α-cedrene and cedrol. The samples gathered in the negative coordinates of the second component of the map (Corsica 1, Ourika, Canary Islands, Sinai, Morocco 940 m) where the joint analysis of the maps of individuals and variables makes it possible to associate the higher compositions in: α-cadinol; and lower in: Camphene, β-pinene, β-phellandrene, trans-pinocarveol and zero in α-cedrene, cedrol, except for sinai which has cedrol. The sample originating from Djebel Mekther has a negative score on this component or the joint analysis of the maps makes it possible to associate higher compositions in: Camphene, β-pinene, α-cedrene; cedrol, α-cadinol and lower in: β-phellandrene, and nil in trans-pinocarveol.

The use of the Jaccard similarity index reveals the following conclusions: a significant dissimilarity between the samples of Djebel Mekther and the other regions that varies between 38% and 68%. The region of Stidea reveals values of median dissimilarity (50%) compared to the other maritime regions. For this reason and for its status as a relict species, *J. phoenicea* deserves protection here.

ACKNOWLEDGMENTS

This work was supported by the Laboratory of Physical Chemistry of Macromoleculars and Biological Interfaces (LPC/MBI), University Mustapha Stambouoli of Mascara, Algeria. The authors are grateful to Pr. Bouhadda for his help and assistance.

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