

A Comparative Study of the Chemical Composition of the Leaves Volatile Oil of *Juniperus phoenicea* and *Juniperus oxycedrus*

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Abstract: Juniper species from the Cupressaceae family are widely distributed in Morocco. This study was designed to compare the phytochemistry of the essential oil obtained from leaves of *Juniperus phoenicea* and *Juniperus oxycedrus* collected than Atlas median region in Morocco. The essential oil was extracted by hydro-distillation and analysed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry system (GC/MS) for their chemical composition. Twenty three constituents were identified in leaves oil of *Juniperus phoenicea* representing 81.87% of the total oil composition. The yield was 1.62% and the major constituent in leaves was α -pinene (49.15%) followed by α -phyllandrene (7.39%), mycene (5.24%), B-pinene (3.58%), linalool (2.54%), piperitone (1.56%), γ -terpinene (1.28%), Trans-pinocarveole (1.23%), ρ -cymene (1.10%), α terpineol (1.02%) and γ -cardinene (1.01%). Forty constituents were identified in leaves oil of *Juniperus oxycedrus* representing 83.92% of the total oil and the yield was 1.66%. The leaves oil was characterised by high contents of α -pinene (31.25%) followed by sabinene (5.21%), limonene (5.02%), B-pinene (4.58%), caryophyllene oxide (4.12%), myrcene (3.56%), ρ -cymene (3.21%), B phellandrene (3.01%), γ -terpinene (2.19%), terpinen-4-ol (2.01%), germacrene D (1.57%), (E)-caryophyllene (1.25%) and δ -ocimene (1.09%).

Key words: *Juniperus phoenicea* • *Juniperus oxycedrus* • Chemical composition • α -pinene

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases, because they contain chemical components of therapeutic value [1]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [2] and the WHO based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,000 [3]. Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances [4] and pharmaceutical industries [5].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition [6].

Juniper species from the Cupressaceae family are widely distributed in Morocco. Aromatic oils from junipers have been used since antiquity for fragrance, flavouring, medicinal, Antimicrobial, insecticidal and cosmetic purposes [7-8-9].

Juniperus phoenicea. (Cupressaceae family) is the species found in Morocco. It extends to Egypt [10] and Central Arabia [11]. The leaf essential oil of *Juniperus phoenicea* has been reported in varying details from Saudi Arabia [12], France [13-14] and from Greece and Spain. Also, there are some reports on the analysis of fruit essential oils [15-16-17]. *Juniperus phoenicea*, is small tree that is native to the northern lands bordering the Mediterranean Sea from Portugal to Palestine. It is also native to North Africa in Algeria, Morocco and Canary Islands [18]. *Juniperus phoenicea* has been used for centuries as a steam inhalant for bronchitis and to control arthritis. The oil is also irritating to microbes, so much so that it kills many of them [19-20].

Juniperus oxycedrus (Cupressaceae family and the Gymnospermae division) is the species found in Morocco and is widely used as traditional folk medicine for treatment of different infectious diseases. It extends to Turkey [21-22], Colombia [23], Spain [24] and Greece [25-26]. The leaf essential oil of *Juniperus oxycedrus* has been reported in varying details from Lebanon [27], Corsica [28] and from Croatia [29]. The oil extracted from *Juniperus oxycedrus* was used in dermatology to treat chronic eczema and other skin diseases while the rectified oil was used as a fragrance component in detergents, soaps, creams and lotions [30]. The boiled fruit extract of *Juniperus oxycedrus* has widely been used in the treatment of gastrointestinal disorders, common colds, as expectorant in cough, to treat calcinosis in joints and as diuretic to pass kidney stone, against urinary inflammations and haemorrhoids [31-32]. The essential oil of *Juniperus oxycedrus* has been the object of several studies of antioxidant activities [33], antinociceptive [34], antifungal [35] and anti-cancer [36].

In the light of this work we have determined and comparative the chemical composition of leaves essential oils of *Juniperus phoenicea* and *Juniperus oxycedrus* collected in the region of Boulmane in Morocco, where the inhabitants frequently use these plants in traditional medicine.

MATERIALS AND METHODS

Vegetal Material and Essential Oil Extraction:

The leaves of *Juniperus phoenicea* and *Juniperus oxycedrus* have been collected during March 2009 in the region of Boulmane, 90 km in the south east of Fez city (latitude: 25° 31 '11" longitude: 5° 22' 21"; altitude: 2100 m); the climate is semi-humid with strong continental influence having an annual average temperature of 20°C, east of Fez city. The collected leaves were then dried in the open air for fifteen days. The leaves were then isolated from the other specimen and conserved for extraction.

The essential oils were extracted by hydro-distillation using an apparatus of Clevenger type. The extraction took 3 h for mixing 250 g of leaves in 1600 mL of distilled water. The essential oil was determined and separated from water; the resulted essential oil was dehydrated over anhydrous sodium sulphate and kept at the refrigerator till GC and GC-MS analysis.

Gas Chromatography Analysis (GC-FID and GC/MS):

The chemical composition of leaf oil from *Juniperus phoenicea* and *Juniperus xycedrus* in Morocco was determined by GC-FID and GC-MS using a CP-SIL 5 HP fused silica column.

The GC (Trace GC ULTRA, Thermo Fischer) analysis equipped with flame ionisation detector (GC-FID), Varian capillary column (CP-Sil 5 HP, 60 m length, 0.32 mm of diameter and Film thickness 0.25 µm). The column temperature was programmed from 40 to 280°C for 5°C/min. The temperature of the injector was fixed to 250°C and the one of the detector (FID) to 260°C. The debit of gas vector (nitrogen) was fixed to 1 mL/min. The volume of injected specimen was 0.5 µL of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

The identification of different chemical constituents was done by gas phase chromatography (Ultra GC Trace) coupled with mass spectrometry (PolarisQ, Thermo Fischer) (GC/MS). The utilized column was; Varian capillary column (CP-Sil 5 HP; 60 m length, 0.32 mm of diameter and Film thickness 0.25 µm). The column temperature was programmed from 50 to 280°C for 3°C/min. The temperature of the injector was fixed to 240°C and the one of the detector to 200°C. Electrons impact: 70ev. The debit of gas vector (Helium) was fixed to 1.5 mL/min. The volume of injected specimen was of 1 µL of diluted oil in hexane solution (10%).

The constituents of essential oils were identified in comparison with their Kovats Index, calculated in relation to the retention time of a series of lineary alkanes (C₄-C₂₈) with those of reference products and comparison with their Kovats Index with those of the chemical components gathered by [37] and in comparison with their spectres of mass with those gathered in a library of (NIST-MS) type.

RESULTS AND DISCUSSION

The chemical composition of essential oils of the different studied plants (*Juniperus phoenicea* and *Juniperus oxycedrus*) is presented in Table 1.

The constituents of *Juniperus phoenicea* from Morocco are listed in order of their elution on the CP-Sil 5 HP column, Figure (A). In total twenty three volatile constituents, representing 81.87% of the total composition, were identified in the leaves oils (Table 1).

Table1: Chemical composition of essential oils of *Juniperus phoenicea* and *Juniperus oxycedrus*

*KI	**Mass range (m/z)	Method of identification	Compounds	***Area (%)	
				<i>Juniperus phoenicea</i>	<i>Juniperus oxycedrus</i>
924	(136),93,91,136,121,77,92,79,43,41,105	KI, GC/MS	B-pinene	3.58	4.58
933	(136),93,79,91,77,41,121,80,94,107,39	KI, GC/MS	camphene	0.28	0.61
938	(136),93,91,136,121,77,92,79,43,41,105	KI, GC/MS	α -pinene	49.15	31.25
948	(136),41,93,69,39,27,53,79,77,67,91	KI, GC/MS	myrcene	5.24	3.56
958	(136),93,41,27,39,79,80,77,43,29,91	KI, GC/MS	δ -ocimene	-	1.09
964	(136),93,77,91,136,79,94,41,80,92,39	KI, GC/MS	B phellandrene	-	3.01
973	(136),93,41,91,77,79,39,27,69,94,43	KI, GC/MS	B-thujene	-	1.07
983	(136),93,41,91,77,79,39,27,69,94,43	KI, GC/MS	sabinene	-	5.21
988	(136),93,91,121,77,92,79,43,41,105	KI, GC/MS	γ -terpinene	1.28	2.19
1018	(136),68,93,39,67,41,27,53,79,94,92	KI, GC/MS	limonene	-	5.02
1506	(220),43,41,79,93,91,95,69,55,67,81	KI, GC/MS	caryophyllene oxide	0.45	4.12
1032	(134),119,134,91,120,117,41,77,39,65,115	KI, GC/MS	ρ -cymene	1.10	3.21
1137	(154),71,111,93,43,86,41,69,55,68,154	KI, GC/MS	terpinen-4-ol	-	2.01
1548	(152),82,110,39,41,27,95,137,109,54,152	KI, GC/MS	piperitone	1.56	-
1505	(204),161,105,91,41,119,79,81,93,77,27	KI, GC/MS	germacrene D	0.68	1.57
1984	(204),93,133,91,41,79,69,105,107,120,77	KI, GC/MS	E-caryophyllene	-	1.25
1001	(138),81,96,95,55,41,67,43,39,68,82	KI, GC/MS	sabina ketone	-	1.06
1062	(152),110,81,95,67,68,41,69,109,55,70	KI, GC/MS	B-thujone	-	1.05
1136	(150),79,107,108,106,77,91,41,105,39,27	KI, GC/MS	myrtenal	-	1.04
1191	(152),79,91,108,41,93,43,119,77,39,67	KI, GC/MS	myrtenol	-	1.03
1126	(125),109,41,94,81,39,69,55,91,43,57	KI, GC/MS	verbenol	0.31	1.02
1114	(150),81,53,108,41,69,107,79,39,27,150	KI, GC/MS	pinocarvone	-	1.01
1494	(204),93,133,91,41,79,69,105,107,120,77	KI, GC/MS	B-caryophyllene	0.98	-
1206	(152),91,119,77,134,117,92,39,109,65,93	KI, GC/MS	carveol	-	0.95
954	(136),93,77,91,136,79,94,41,80,92,39	KI, GC/MS	α -phellandrene	7.39	0.89
1128	(154),95,41,110,93,55,67,139,121,96,69	KI, GC/MS	borneol	0.36	0.83
1572	(204),121,93,41,107,67,79,81,105,91,119	KI, GC/MS	germacrene B	0.65	-
1004	(136),93,91,79,77,92,121,80,136,94,105	KI, GC/MS	3-carene	1.05	0.62
1224	(194), 92,91,81,41,134,55,109,79,43,53	KI, GC/MS	sabinenyl acetate	-	0.58
1267	(196), 95,43,93,436,121,41,80,55,108,69	KI, GC/MS	bornyl acetate	0.53	0.53
1344	(204),161,105,119,41,81,91,120,93,55,204	KI, GC/MS	B-cubebene	-	0.51
1333	(196),43,121,93,136,68,41,59,67,81,79	KI, GC/MS	α -terpinyl acetate	-	0.46
1221	(204),161,119,105,93,41,91,92,81,120,204	KI, GC/MS	B-copaene	-	0.44
1578	(204),93,80,41,121,92,43,55,67,91,147	KI, GC/MS	B-humulene	-	0.42
1440	(204),161,189,204,105,91,133,119,95,41,81	KI, GC/MS	cadinene-3,9-diene	-	0.40
1583	(222),59,149,43,41,108,93,79,81,67,164	KI, GC/MS	B-eudesmol	0.36	-
1710	(222),69,81,41,93,95,68,109,67,55,107	KI, GC/MS	farnesol	-	0.36
1228	(154),69,41,68,29,93,123,67,70,84,55	KI, GC/MS	geraniol	-	0.32
1059	(154),43,93,81,71,69,84,68,108,41,55	KI, GC/MS	1,8-Cineole	-	0.30
1543	(222),95,150,151,43,41,81,69,55,107,93	KI, GC/MS	α -Cedrol	-	0.26
719	(136),93,91,79,41,39,77,121,136,27	KI, GC/MS	tricyclene	0.21	-
1321	(152),92,91,70,55,41,83,79,134,69,119	KI, GC/MS	trans-pinocarveol	1.23	0.21
1133	(154),59,93,121,136,81,43,68,95,67,41	KI, GC/MS	α -terpineol	1.02	0.19
1430	(204),161,189,204,105,91,119,133,27,55	KI, GC/MS	γ -cadinene	1.01	0.11
1042	(136),93,121,91,136,79,77,105,39,41,107	KI, GC/MS	terpinolene	0.91	0.09
Total Identified Compounds				81.87	83.92
Essential oil (%)				1.62	1.66

*RI: Retention index was determined by GC-FID on a CP-Sil 5 HP column.

**Mass range (m/z) was determined by mass spectrometry (PlarisQ).

***Area (%) was determined by mass spectrometry (PlarisQ).

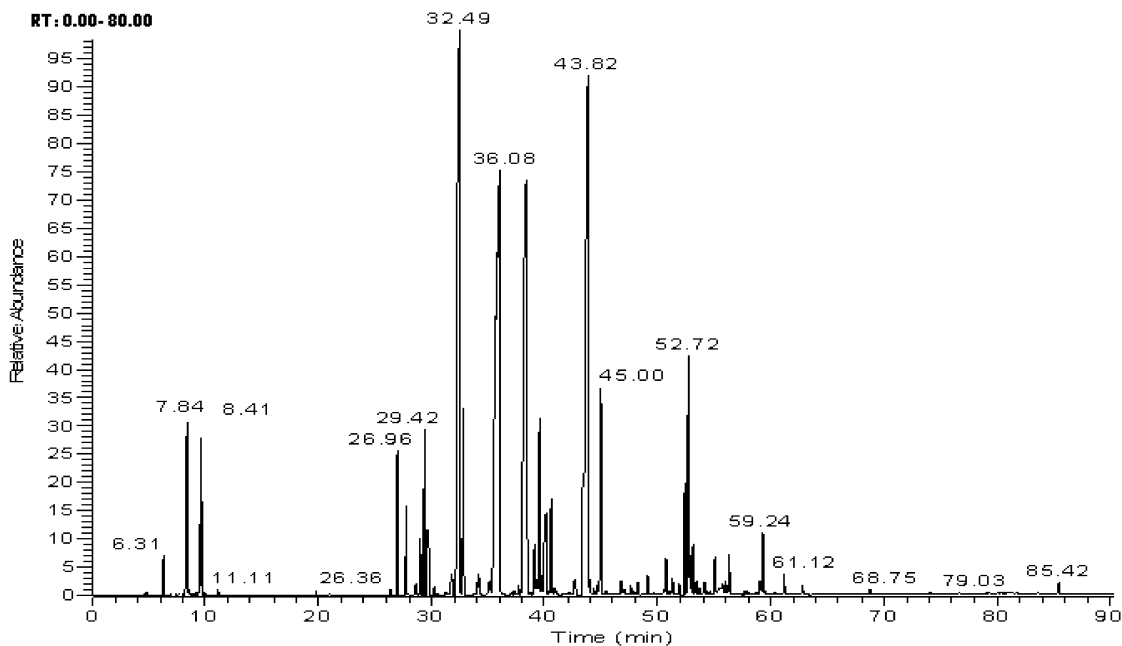


Fig. A: Chromatogram of *Juniperus phoenicea*

Monoterpene hydrocarbons (70.19%) were found to be the major group of constituents, the main one being α pinene (49.15%) followed by α -phellandrene (7.39%), mycene (5.24%), B-pinene (3.58%), linalool (2.54%), piperitone (1.56%), γ -terpinene (1.28%), Trans-pinocarveol (1.23%) ρ -cymene (1.10%), α terpineol (1.02%) and γ -cadinene (1.01%). The essential oils yield of *Juniperus phoenicea* collected from region of Boulmane (Morocco) was 1.62%. It is relatively higher than other plants industrially exploited as a source of essential oils: *Artemisia* (0.65%) [38], *Thymus* (1%) [39], lavender (0.8-2.8%), menthe (0.5-1%), neroli (0.5-1%) and laurel (0.1-0.35%) [40], *Mentha rotundifolia* (1.54%) [41] and *tetraclinis* (0.22%) [42]. In this study the yield is low to those of *Laurus nobilis* essential oils analyzed in Morocco by [43], which the yield was 1.86%.

The chemical compositions revealed that this leaves had compositions similar to those of other *Juniperus phoenicea* essential oils analyzed in USA by [44], which the major component was α -pinene. Contrary it's different to the composition of essential oil of leaves of *Lavandula dentate* studied in Morocco, which the major component were 1, 8 cineol (41.28%) and sabinene (13.69%) [39]. Intensive research on the chemical characteristics has been conducted on this species [37-45]. The leaves essential oil of *Juniperus phoenicea* has been reported in varying detail [46].

In this study the yield of essential oils of *Juniperus phoenicea* collected from region of Boulmane (Morocco), where 1.62%. The yield of essential oils of leaves of *Juniperus phoenicea* is relatively higher than other plants studied in Spain (0.66%); Portugal (0.41%) and Greece (0.52%) [44]. Contrary to the yield of essential oils of leaves of *Juniperus phoenicea* studied in Egypt, which was (1.96%) [10].

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree: Robert *et al.* [44] studied the composition of *Juniperus phoenicea* oil collected from the Portugal, Spain and Greece, they reported that the total oil constituents obtained were 98.3, 99 and 88%, respectively and the composition is characterized by a high content of α -pinene (34.1, 53.5 and 41.8%), B-phellandrene (19.2, 5.9 and 3.5%) and B-caryophyllene (0.22, 1.0 and 0.5%). In our previous studies on the chemistry of Egyptian *Juniperus phoenicea* [10], considerable differences were observed in the essential oil composition between leaves and berries: α -pinene (38.22 and 39.30%), (α -cedrol 31.23% and sabinene 24.29%), respectively. Furthermore the essential oils obtained from flower, leave and stems from basil (*Ocimum basilicum* L.) from Mersin province (Bu"yu"keceli-Gu" Inar) in Turkey contained: estragole (58.26, 52.60 and 15.91%), limonene (19.41, 13.64 and 2.40%) and pcymene (0.38, 2.32 and 2.40%) [47]. In previous studies on the chemistry of Uruguay [48],

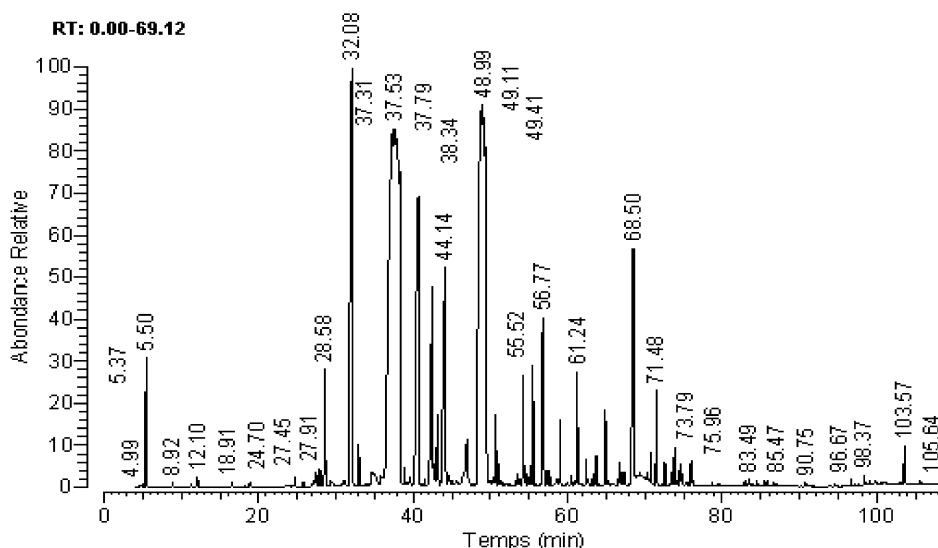


Fig. B: Chromatogram of *Juniperus oxycedrus*

considerable differences were observed in the essential oil composition between *Mentha rotundifolia* and *M. pulegium*: Piperitenone (80.8%) and Pulegone (73.4%) and the total constituents identified was 93.5 and 99.3%, respectively.

The constituents of *Juniperus oxycedrus* from Morocco are listed in order of their elution on the CP-Sil 5 HP column, Figure (B). In total, forty volatile constituents, representing 83.92 % of the total composition, were identified in the leaves oils Table (1). The most abundant components found in the leaf oil were α -pinene (31.25%) followed by sabinene (5.21%), limonene (5.02%), B-pinene (4.58%), caryophyllene oxide (4.12%), myrcene (3.56%), p -cymene (3.21%), B-phellandrene (3.01%), γ -terpinene (2.19%), terpinen-4-ol (2.01%), germacrene D (1.57%), (E)-caryophyllene (1.25%) and δ -ocimene (1.09%). The essential oils yield of *Juniperus oxycedrus* collected from Atlas median region (Taferdoust), Morocco is of 1.66%. It is relatively higher than other plants industrially exploited as a source of essential oils: Tetraclinis (0.22%) [42], *Artemisia herba-alba* (0.59%), *Artemisia absinthium* (0.57%) and *Artemisia pontica* (0.31%) [49], lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%), laurel (0.1-0.35%) [40] and its low from yield of *Juniperus occidentalis* studied by Adams [50], which is 2.3% and of *Juniperus oxycedrus* in Pindos from Greece which the yield is 2.21% [26].

The chemical compositions revealed that the leaves had compositions similar to those of other *Juniperus oxycedrus* essential oils analyzed in Lebanon by Loizzo *et al.* [27] and in Europe by Milos and Radonic [29], which the major component was α -pinene. Adams *et*

al. [51] studied the Cryptic speciation between *Juniperus deltoids* and *Juniperus oxycedrus* in the Mediterranean collected from Morocco, Portugal, Spain, France, Italy, southern Greece, Northern Greece and Turkey. They reported that the major compounds were α -pinene (45.3%, 47.3%, 40.9%, 53.2%, 19.3%, 19.7%, 27.4% and 32.7%) respectively. Contrary to the composition of essential oil of wood of *Juniperus oxycedrus* studied Spain, France and Italy which the major component was δ -cadinene [52]. The berries oil of *Juniperus oxycedrus* studied in Greece from two different locations: Holomontas and Pindos which the major constituents were α -myrcene (23.4%) and citronellol (26.8%) [26]] and of *Juniperus occidentalis* which the major commercially important compounds identified as α -cedrene (8.8 %), B-cedrene (2.6 %), thujospene (18.9 %), cuparene (1.5 %), cedrol (38.9 %) and widdrol (1.6%) [50]. Marongiu *et al.* [53] have analysed samples collected from Sardinia and observed the presence, as the most abundant components, besides δ -cadinene, of 1-epi-cubenol (12.5%), cubenol (10.5%), α -muurolol (4.8%), α -cadinol (3.7%) and α -humulene (3.2%). Intensive research has been conducted on this species [54-55-56]. In this study the yield and total oil composition of essential oils of *Juniperus oxycedrus* collected from Atlas median region of Morocco were 1.66% and 83.92%. The yield of essential oils of leaves of *Juniperus oxycedrus* is relatively higher than other plants studied in Italy (Sardinia) (0.04-2.54%) [57] and Algeria (0.1%) [58].

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree: Milos and Radonic [29] studied the essential

oils composition in fresh needles, green and mature berries of *Juniperus oxycedrus* collected from Croatia and reported that the number of compounds were 36, 15 and 22 and the total oil obtained were 94.90, 94.33 and 90.94%, respectively. In Portugal Cavaleiro *et al.* [35], studied the composition and variability of the essential oils of the leaves and berries from *Juniperus navicularis* and reported that the composition is characterized by α -Pinene (6.3-38.0%), limonene (7.0-34.6%), α -phellandrene (2.2-13.1%) and *p*-cymene (4.8-10.3%) were the major constituents of the oils from leaves and B-myrcene (25.8%) and α -pinene (24.4%) were the major ones of the oil from berries. In other studies on the chemistry of *Juniperus oxycedrus* from Lebanon [27], considerable differences were observed in the essential oil composition between berries and wood which obtained: α -pinene (27.4%) and δ -cadinene (14.5%) respectively. Furthermore, the essential oils, obtained from flower, leaves and stems from basil (*Ocimum basilicum* L.) from Mersin province (Bu'yu'keceli-Gu Inar) in Turkey contained: estragole (58.26, 52.60 and 15.91%), limonene (19.41, 13.64 and 2.40%) and *p*-cymene (0.38, 2.32 and 2.40%) [47]. In other studies on the chemistry of tree *Artemisia* from Morocco [49], considerable differences were observed in the total oil composition between *Artemisia herba-alba* (83.10%), *Artemisia absinthium* (80.72%) and *Artemisia pontica* (43.95%). On the other hand, the essential oils, obtained from berries and leaves of *Juniperus excelsa* in Turkey obtained α -pinene (34.0%), cedrol (12.3%), L-verbenol (5.4%) and D-verbenol (4.4%) from berries. The leaves essential oil showed the major constituents was α -pinene (29.7%), cedrol (25.3%), α -muurolene (4.4%) and 3-carene (3.8%) [59]. Intense research reveals that the variation in the quantitative and qualitative composition of the leaf and mainly the berry oil, had been the subject of several studies [60 61 62].

CONCLUSION

The present work has been concerned with determining and comparing the chemical composition of essential oils extracted from the leaves of *Juniperus phoenicea* and *Juniperus oxycedrus*, collected in the Moroccan middle Atlas region of Boulmane. The yield and the total oil of essential oils for *Juniperus phoenicea* and *Juniperus oxycedrus* were 1.62 and 81.87% and 1.66 and 83.92%, respectively. The chemical analyses, by GC/MS and GC-FID, have allowed us to identify around 81.87% of the total volatile products for *Juniperus phoenicea* and the major compound in leaves

was α -pinene (49.15%). The total volatile product for *Juniperus oxycedrus* was 83.92% and the major compound in leaves was α -pinene (31.25%). In this study, the yield and the total oil of the leaves essential oil extracted from *Juniperus phoenicea* and *Juniperus oxycedrus* are similar.

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