Chemical Studies of Leaf Essential Oils of Three Species of *Juniperus* From Tensift Al Haouz-Marrakech Region (Morocco)

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Abstract

Volatile components from fresh and air-dried leaves of *Juniperus thurifera* var. *africana*, *J. phoenicea* and *J. oxycedrus* (Cupressaceae) were isolated by direct water distillation (Clevenger-type apparatus) and analyzed using GC (FID) and GC/MS. The essential oils from leaves of *J. thurifera* var. *africana* were characterized by a high sabinene content (16.5–21.8%), γ-terpinene (9.3–11.5%), and α-pinene (7.6–9.1%). The major components for *J. phoenicea* were α-pinene (38.2–58%) and δ-3-carene (7.6%), while 13-epi-manoyl oxide (12.5–13.2%), (Z)-6-pentadecen-2-one (11.5–12.2%), and α-pinene (8.5–17.1%) were the major components found in the leaf oils of *J. oxycedrus*. The fresh leaves of *J. thurifera* var. *africana* contained 1.46% oil, while the yield was 1.14% from the dried leaves. The oil yields from the dried leaves of *J. phoenicea* and *J. oxycedrus* were 0.94% and 0.01%, respectively. Air-drying moderately effected the qualitative and quantitative composition of the oils.

Key Word Index

Juniperus thurifera var. *africana*, *Juniperus phoenicea*, *Juniperus oxycedrus*, Cupressaceae, essential oil composition, sabinene, γ-terpinene, 13-epi-manoyl oxide, (Z)-6-pentadecen-2-one, α-pinene.

Introduction

Juniperus is the second most diverse genus of the conifers. The genus *Juniperus* L. consists of approximately 67 species and 28 varieties (1). Several of the Mediterranean *Juniperus* species *J. oxycedrus* L., *J. phoenicea* L. and *J. thurifera* L. grow in the mountains of the northern part of Africa (Morocco, Algeria) (2). The genus *Juniperus* is divided into three sections: *Caryocedrus*, *Juniperus* (= *oxycedrus*), which includes the needle-like leafed junipers such as *Juniperus oxycedrus*, and *Sabina*, the scale-like leaf junipers, which includes *Juniperus phoenicea* and *Juniperus thurifera*.

Juniperus phoenicea (Cupressaceae) is a small tree that is native to the areas bordering the Mediterranean Sea from Portugal to Israel. It is also native to North Africa, Algeria, and Morocco, as well as the Canary Islands (3).

Recently, Rezzi et al. (4) reported, on infraspecific variation in the leaf essential oils of *J. phoenicea* var. *turbinata* (Guss.) Parl. from Corsica, two chemical types: high α-pinene, low β-phellandrene, low α-terpinyl acetate-type (Cluster I, 35 indvs.) and low α-pinene, high β-phellandrene, high α-terpinyl acetate-type (Cluster II, 15 indvs.).

Cavaleiro et al. (5) showed an infraspecific chemical variability of the leaf oil of *J. phoenicea* var. *turbinata* from Portugal. Three chemical types were found: two Clusters (A and B) were differentiated in the basis of their α-pinene, β-phellandrene, and α-terpinyl acetate ratios. The ratio of the three compounds of Cluster A was close to 2:1, but the ratio of Cluster B was close to 1. The oils of Cluster C were dominated by α-pinene (average 81.5%). This extremely high content of α-pinene has not yet been reported for *J. phoenicea* leaf oil, but only is described for oils isolated from the berries (6–10).

Thuriferous juniper is only found in isolated parts of the western Mediterranean: France (Alps, Pyrenees and Corsican highlands), Spain, Algeria and Morocco. This tree constitutes an important element of the forestall biodiversity in temperate and Mediterranean countries.

More recently a study (11) using proanthocyanidins and the number of seeds per cone resulted in the naming of a new

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subspecies (*J. thurifera* subsp. *africana* Maire) and 3 chemivars (*hispanica* (Spain), *gallica* (France) and *corsicana* (Corsica)). In a recent treatment of the conifers, authors recognized *J. thurifera* and treated *J. africana* and *J. thurifera* var. *africana* as synonyms and reported that the leaf oil of *J. thurifera* from central Spain contained a large amount of limonene (51.5%) and moderate amounts of linalool, piperitone, linalyl acetate and α-terpinyl acetate (12).

More recently, a study (11) analyzed geographic variation in the oils from different Moroccan populations of thuriferous juniper (Tizi-n'Ait-Imi (M1, M2), Oukaimeden (OM), Tizi-n'Tichka/Kasbah Telouet (TM)). In the Tizi-n'Ait-Imi population, individuals M1 and M2 exhibited the extremes of the samples analyzed: δ-3-carene was not detected in M1, but was about 1.7–3.1% in the other Moroccan samples, and individual M2 had smaller amounts of sesquiterpenes such as γ- and δ-cadinene, elemol, germacrene B, germacrene D-4-ol, α- and β-eudesmol and α-cadinol, but the oil was quite different in several major compounds (α-pinene, sabinene, γ-terpinene, *cis*-sabinene hydrate, terpinen-4-ol, elemol and cedrol) with sabinene representing the high content of the Moroccan leaf oil.

A recent study analyzed the effect of the leaf drying and geographic sources on the oil composition of *Juniperus thurifera* var. *africana* from different Moroccan populations of thuriferous juniper (13,14). The fresh leaf oil of *J. thurifera* var. *africana* from Ait Lkak and Plateau of Matat was clearly dominated by sabinene (21.1% and 35.9%, respectively), but much less in Forêt Islane (10.1%). Population Forêt Islane had a large amount of δ-cadinene (12.4%) compared to Ait Lkak (0.6%) and Plateau of Matat (0.8%)(13).

Juniperus oxycedrus is one of 10 to 11 species that comprise the section *Juniperus* (= *oxycedrus*) of the genus *Juniperus* throughout the world (1). This shrub or tree has a typical Mediterranean distribution and three subspecies have been recognized in the Iberian Peninsula: subsp. *oxycedrus*, subsp. *macrocarpa* (Sibth. et Sm.) Ball and subsp. *badia* (H. Gay) Debeaux, with the first one being the most abundant (15). However, in the recent monograph of *Juniperus* (Adams, 2004), treats *J. oxycedrus* subsp. *macrocarpa* as *J. macrocarpa* Sibth. et Sm. and recognizes *J. oxycedrus* subsp. *badia* as *J. oxycedrus* var. *badia* H. Gay.

The oil of *J. oxycedrus* leaves has been reported by Stassi et al. (16), Boti et al. (17) and Adams (18). The leaf oil was dominated by α-pinene and cedrol with moderate amounts of dihydro-p-cymen-8-ol, α-terpineol and δ-cadinene.

Milos and Radonic (18) reported that the major compounds were α-pinene (41.37%) and manoyl oxide (12.29%) in the fresh needle oil, and the chemical composition of the oils isolated from green and mature *J. oxycedrus* berries was higher in the α-pinene and sesquiterpene hydrocarbons.

Sofia Solido et al. (20) reported that the main components of the leaf oil of *J. oxycedrus* subsp. *badia* were α-pinene (39.8%) and the diterpene manoyl oxide (10.2%), while the berry oil was dominated by α-pinene (65.1%) and myrcene (4.2%).

In this paper, the leaf oils are reported and examined for populations of *J. thurifera* var. *africana*, *J. phoenicea* and *J. oxycedrus* from Tensift Al Houz- Marrakech region. The samples of oils produced by water distillation (Clevenger-type apparatus) have been analyzed by GC/MS and GC-FID. With this investigation both fresh and air-dried leaves were used.

Experimental

Plant material: The plant materials were collected from Tensift Al Haouz area of Marrakech. *Juniperus oxycedrus* and *J. phoenicea* were collected from Amassine-Ourika (1300 m, 12/22/2003), Atlas Mtns, Morocco, and *J. thurifera* from Ait Lkak Oukaimden (2700 m, 12/22/2003), Atlas Mtns, Morocco. The voucher specimens have been deposited at the Laboratory of Applied Organic Chemistry, Faculty of Science Semlalia, Cadi Ayyad University (Morocco).

A portion of the leaves from each of the trees (per population) were air-dried for 16 days at room temperature (ca. 22°C) to produce the dried leaf samples.

Isolation of essential oils: The fresh and air-dried leaves (1000 g fresh wt. and 1000 g air-dried wt.) of the each sample were water-distilled for 4 h. The plant material was isolated by water distillation (Clevenger-type apparatus), produced in the still, and the oil samples were stored at -20°C until analyzed. The exhausted leaves were oven-dried (4 h, 105°C) for determination of oils yields.

Essential oil analysis: The essential oils were analyzed by gas chromatography using a Varian 3800 gas chromatograph fitted with a 30 m x 0.25 mm BP-5 fused silica capillary column with a 0.25 μm coating thickness directly coupled to a Varian Saturn 2000 MSD mass spectrometer. The GC/MS was operated under the following conditions: injector temperature, 220°C; transfer line, 240°C; oven temperature programmed 60–240°C (3°C/min); carrier gas, He 1 mL/min; injection, 0.1 μL (10% solution); split 1:10, 500 ng on column. EI mass spectra were collected at 70 eV ionization voltage over the mass range *m/z* 40–450. Identifications were made using combined MS and RI data from authentic compounds (21). Quantitation was by FID on Varian 3400 gas chromatograph using the Varian chemstation software.

Table I. Oil yields of the leaf oils of *Juniperus thurifera*, *J. phoenicea* and *J. oxycedrus*

	Juniperus thurifera var. *africana*		*Juniperus phoenicea*		*Juniperus oxycedrus*	
	FL	DL	FL	DL	FL	DL
Yield (%), 1000 g 'as is' basis	0.73	1.03	0.21	0.85	0.01	0.01
Yield (%), oven dry weight basis	1.46	1.14	0.42	0.94	0.02	0.01

FL: 1000 g fresh leaves (ca. 50% moisture), DL: 1000 g air dried leaves (ca. 10% moisture).

Results and Discussion

The oil yields (mL/100 g) of the fresh and dried leaf oils of three species of *Juniperus* obtained from different provenances are given in Table I.

The yield of the leaf oil obtained from a tree of *J. phoenicea* was lower (0.21%) for the fresh leaves than the dried leaves (0.85%). The chemical composition of oils prepared from plant material is summarized in Table II where the constituents are listed in order of their elution from silica capillary column BP-5 and their identification has been carried out by means of GC and GC/MS analyses in combination with retention indices. *Juniperus phoenicea* oil was clearly dominated by α-pinene (38.2% from fresh leaf and 58% from dried leaf); δ-3-carene (7.6%) was the second major component, followed by others such as β-caryophyllene (2.1%), γ-cadinene (3%), limonene (1%) and epi-13-manoyl oxide (1.3%). A comparison of the chemical composition of leaf oils of *J. phoenicea* var. *turbinata* from Corsica revealed that the oil was very similar to cluster I of this study (high α-pinene, low β-phellandrene, and low α-terpinyl acetate), but the oils of *J. phoenicea* var. *turbinata* from Spain and *J. phoenicea* subsp. *eu-mediterranea* from Portugal showed two major compounds: α-pinene (28.3% and 34.1%, respectively) and α-terpinyl acetate (15.5% and 12.5%, respectively)(3–10).

The oil yield of the leaf oils of *J. thurifera* var. *africana* obtained from Oukaimden area are given in Table I. As can be seen, it was higher for the dried leaves (1.03%) and lower for the fresh leaves (0.73%). The identified components and their percentages are given in Table II. An immediate observation in this composition was the presence of a high level of sesquiterpenes, although there were remarkable differences concerning the main components—high sabinene (16.5–21.8%), γ-terpinene (9.3–11.5%), α-pinene (7.6–9.1%) and lower percentages of components such as α-thujene (1.4–2.6%), α-fenchene (1.1–1.2%), α-terpinene (1.5–2.2%), terpinen-4-ol (1.4–2.3%), elemol (1.4–1.2%), α-eudesmol (1.6–4.0%), and oplopenone (1–3.2%). From comparison of other leaf oils, some qualitative differences in chemical composition can be deduced. Thus, the leaf oil composition of Spanish and French oils was higher in limonene (30–75%) and lower in sabinene (11). However, the composition of the oils from population Ait Lkak, Atlas Mtns (Marrakech) was similar to those reported for oils of *J. thurifera* from Moroccan populations, Atlas Mtns (Marrakech).

Table II. Percentage composition of the leaf oil isolated from fresh and dried leaves of *Juniperus phoenicea, J. thurifera* var. *africana*, and *J. oxycedrus*

		Juniperus thurifera var. *africana*		*Juniperus phoenicea*		*Juniperus oxycedrus*	
RI	**Compound**	**FL**	**DL**	**FL**	**DL**	**FL**	**DL**
926	tricyclene	0.4	0.8	0.4	0.2	t	-
931	α-thujene	1.4	2.6	-	-	-	-
939	α-pinene	7.6	9.1	58.0	38.2	8.5	17.1
953	α-fenchene	1.1	1.2	0.4	0.7	0.3	0.6
953	camphene	0.8	t	0.6	0.5	0.2	0.2
967	verbenene	-	-	-	-	0.1	0.6
976	sabinene	21.2	16.5	-	-	-	0.2
980	β-pinene	0.2	0.3	0.7	1.1	0.2	0.5
991	myrcene	0.5	1.1	1.2	0.7	t	t
1005	α-phellandrene	t	t	-	-	t	t
1011	δ-3-carene	0.9	1.0	7.5	7.6	0.6	2.0
1018	α-terpinene	2.2	1.5	-	-	-	-
1026	p-cymene	0.7	0.3	1.5	1.7	0.1	0.5
1031	limonene	0.7	0.7	1.0	1.1	-	-
1031	β-phellandrene	t	0.4	-	t	0.2	0.4
1037	(Z)-β-ocimene	t	0.5	-	-	-	-
1050	(E)-β-ocimene	0.2	0.3	-	-	-	-
1062	γ-terpinene	9.3	11.5	0.3	0.4	-	-
1068	*cis*-sabinene hydrate	0.1	0.2	-	-	-	0.5
1088	terpinolene	t	0.1	-	-	0.5	-
1091	p-cymenene	0.1	0.2	-	-	0.1	0.2
1097	*trans*-sabinene hydrate	1.2	1.6	-	-	-	-
1098	linalool	-	-	0.1	0.2	-	-
1099	α-pinene oxide	-	-	0.1	0.2	-	-
1102	nonanal	-	-	0.2	0.2	-	-
1102	*cis*-thujone (= β-thujone)	0.4	0.4	-	-	-	-
1114	*trans*-thujone (= α-thujone)	1.5	1.0	-	-	-	-
1125	α-campholenal	-	-	-	-	0.4	0.7
1139	*trans*-pinocarveol	t	1.2	0.2	0.2	-	-
1163	pinocarvone	-	t	-	-	t	0.3
1165	borneol	-	-	0.3	0.1	t	t
1171	umbellulone	0.3	0.3	-	-	-	-
1173	isopinocamphone	0.2	0.6	-	-	0.2	0.6
1177	terpinen-4-ol	1.4	2.3	-	-	0.5	1.3
1180	m-cymen-8-ol	-	-	-	t	t	0.9

Table II. Continued

		Juniperus thurifera var. *africana*		*Juniperus phoenicea*		*Juniperus oxycedrus*	
RI	**Compound**	**FL**	**DL**	**FL**	**DL**	**FL**	**DL**
1183	p-cymen-8-ol	0.5	0.8	0.1	0.3	t	t
1189	α-terpineol	0.4	0.6	0.3	0.3	0.2	0.3
1191	myrtenol	-	-	0.1	t	t	0.3
1193	(Z)-4-decenal	0.3	-	-	-	-	-
1196	myrtenal	-	-	0.1	t	t	0.3
1204	verbenone	-	-	0.2	0.9	0.1	1.0
1217	*trans*-carveol	-	t	0.1	0.2	0.1	0.5
1228	citronellol	-	-	0.4	0.2	-	-
1250	car-3-ene-2-one	-	-	-	-	t	0.3
1257	linalyl acetate	0.8	1.0	0.1	-	-	-
1269	p-menth-2-ene-1,4-diol	0.6	0.9	-	-	-	-
1273	isopulegyl acetate	-	-	0.7	0.8	-	-
1277	pregeijerene B	0.3	0.4	-	-	-	-
1285	bornyl acetate	0.7	1.1	-	0.1	0.5	1.3
1300	terpinen-4-yl acetate	0.4	0.3	-	-	-	-
1312	unknown(FW 43,57,95,68,152)	1.0	-	-	-	-	-
1350	α-terpinyl acetate	0.3	0.7	0.1	-	0.9	-
1375	β-ylangene	-	-	-	-	0.1	0.3
1376	α-copaene	0.3	0.4	0.2	t	-	-
1383	β-bourbonene	t	t	-	-	1.6	1.7
1391	β-elemene	t	t	0.6	0.8	-	-
1418	β-caryophyllene	-	-	2.1	-	-	-
1429	*cis*-thujopsene	-	-	-	0.2	-	-
1437	γ-elemene	-	-	0.3	t	-	-
1477	γ-muurolene	0.2	0.2	-	-	1.6	1.2
1480	germacrene D	0.5	0.9	-	-	2.6	-
1485	α-amorphene	-	-	1.4	1.0	-	-
1490	β-selinene	-	-	0.3	-	-	-
1491	*trans*-murrola-4(14),5-diene	-	-	0.5	-	-	-
1494	2-tridecanone	-	-	-	-	6.6	3.7
1499	α-muurolene	2.3	1.8	0.4	0.2	0.2	0.8
1513	γ-cadinene	2.0	2.6	-	3.0	2.0	3.7
1521	*cis*-calamenene	-	-	0.3	-	-	-
1524	δ-cadinene	0.4	t	0.4	t	0.1	0.3
1529	*trans*-calamenene	-	-	1.7	1.7	-	-
1538	α-cadinene	0.4	0.5	-	-	-	-
1546	α-calacorene	-	-	0.3	t	-	-
1549	elemol	4.0	1.8	0.5	0.3	-	-
1556	germacrene B	0.1	0.2	-	-	-	-
1563	(E)-nerolidol	-	-	0.1	0.1	-	-
1581	caryophyllene oxide	0.3	1.1	0.3	1.1	3.2	2.6
1595	salvial-4(14)-en-1-one	-	-	-	-	0.6	0.8
1596	cedrol	0.6	0.7	-	-	-	-
1606	humulene epoxide II	-	-	t	-	1.8	1.6
1608	β-oplopenone	0.7	0.8	-	-	-	-
1627	1-epi-cubenol	-	-	0.6	2.2	0.5	0.7
1640	epi-α-cadinol	1.0	0.5	-	-	0.8	0.6
1640	epi-α-muurolol	1.3	0.4	-	-	-	-
1649	β-eudesmol	t	t	0.1	0.7	t	t
1652	α-eudesmol	4.0	1.6	-	t	-	-
1653	α-cadinol	-	-	0.3	4.0	0.4	0.7
1670	(Z)-6-pentadecen-2-one	-	-	-	-	12.4	11.5
1686	germacra-4(15),5,10,(14)-trien-1-al	-	-	-	-	0.2	0.5
1688	3-hydro farnesol	-	-	-	-	0.4	0.6
1689	*cis*-14-nor-muurol-5-en-4-one	-	-	0.2	0.5	-	-
1698	2-pentadecanone	-	-	-	-	0.2	2.0
1718	(Z,Z)-farnesol	-	-	-	-	1.4	0.4
1733	oplopenone	3.2	1.0	-	-	-	-
1746	(E,Z)-farnesol	-	-	-	-	0.3	t
2010	13-epi-manoyl oxide	-	-	1.0	1.3	13.2	12.5
2057	abietatriene	-	-	-	-	2.3	2.4

RI = Retention Index on BP-5 column; FL = fresh leaves; DL = air-dried leaves. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

The water distillation of *J. oxycedrus* from Tensift Al Haouz area yielded clear and colorless oils. The oil yield of fresh and dried leaves was very low (0.01%) compared to the oil yield of *J. oxycedrus* ssp. *badia* (0.27%). The study of the leaf oil showed the presence of 65 known components accounting for approximately 80% of the oil. This sample was clearly dominated by 13-epi-manoyl oxide (12.5–13.2%); (Z)-6-pentadecen-2-one (11.5–12.2%) and α-pinene (8.5–17.1%) were the second major components, followed by others such as 2-tridecanone (3.7–6.6%) and γ-cadinene (2.6–3.2%). The mean chemical composition determined for *J. oxycedrus* from Amassine-Ourika has been found to have some qualitative differences with those reported for oils of *J. oxycedrus* ssp. *badia* from Spain. They found a higher amount of α-pinene (39.8–65.1%) and manoyl oxide (10.2%) and lower amounts of 2-tridecanone and 13-epi-manyol oxide and the absence of (Z)-6-pentadecen-2-one.

Finally, the oil yield of *J. phoenicea* and *J. thurifera* varied with the fresh and air dried leaves, which increased for dried leaves and decreased for fresh leaves, because the leaves might have some terpenoids stored as glycosides. These glycosides may have been hydrolyzed during leaf drying, making free terpenoids available for isolation. There is a report of increased oil yield from dried leaves in *Eucalyptus camaldulensis* Dehn (22), in contrast to the general case of the oils being lost upon drying.

The leaf oil compositions from fresh and air-dried leaves (16 days) from three species are show in Table I. The major component for *J. thurifera* var. *africana* from Atlas Mountains was sabinene. The Moroccan populations were clearly much different in their quantities of sabinene, δ-2-carene, limonene, linalyl acetate, and manoyl acetate compared to Spain (and other European populations) (11).

The leaf oil of *J. phoenicea* from Amassine-Ourika (Atlas Mountains, Marrakech) was dominated by α-pinene, which is very similar to the reference samples of *J. phoenicea* oil from the others population of Spain, Corsica and Portugal.

The qualitative composition of leaf oil of *J. oxycedrus* from Amassine-Ourika (Atlas Mountains, Marrakech) differed from the composition of terpenoids found in the other leaf oils of *J. oxycedrus* from Spain (16–19). However, the Moroccan population was dominated by 13-epi-manoyl oxide, but α-pinene was the main compound of the Spanish population(19).

A chemical variability in the leaf oil from *J. thurifera* var. *africana*, *J. phoenicea* and *J. oxycedrus* could be explained by geographical distribution. In general, there is a very good agreement between the oil composition from fresh and dried leaves. This seems to imply that comparisons between juniper species are probably valid when oil is from fresh or air-dried leaves.

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