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Effect of the Leaf Drying and Geographic Sources on the Essential Oil Composition of *Juniperus thurifera L.* var. *Africana* Maire from the Tensift – Al Haouz, Marrakech Region.

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Abstract

Essential oils of Moroccan *Juniperus thurifera* var. *africana* from three provenances (Forêt Islane, Ait Lkak and Plateau of Matat) in the Tensift-Al Haouz, Marrakech region, were analyzed by combined capillary gas chromatography / mass spectrometry. The oil yields from fresh leaves showed on differences among geographical sources. Air dried leaves appeared to yield more oil at the highest elevation (1.03%, Ait Lkak, 2900 m) than lower sites (0.67%, Plateau of Matat, 2200 m; 0.57%, Forêt Islane, 2000 m). The essential oils from each geographic site had very similar composition in fresh versus air dried leaves.

The essential oils from provenance Ait Lkak and Plateau of Matat were very similar and characterized by a high sabinene content (21.2, 35.9%), in contrast to 10.% sabinene from the provenance Forêt Islane. The oil from Foret Islane had a high δ-cadinene content with 12.7%, whereas Aik Akak and Plateau of Matat contained only 0.6 and 0.8%. The Moroccan oils (*J. thurifera* var. *africana*) were compared to European *J. thurifera* var. *thurifera* (Spain) oil. Moroccan oils were found to be higher sabinene and limonene than the European oil.

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Key Word Index

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Introduction

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (1). They are mixtures of more than 200 compounds (2) that can be grouped basically into two fractions, a volatile fraction, that constitutes 90–95% of the whole oil and contains monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters, and a non volatile residue, that constitutes from 5–10% of the whole oil and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, psoralens and flavonoids.

The thuriferous juniper (*Juniperus thurifera* L.) is located on the western part of the Mediterranean basin (3,4). The species grows in France, Spain, Algeria and Morocco in stands ranging from 100 to 150 000 ha (5,6)

In Morocco, the thuriferous juniper grows in High and Middle Atlas Mountains (Fig. 1)(7,8,9). Holm oaks (*Quercus ilex*) and Atlas cedar (*Cedrus atlantica*) are the two arboreal species which can grow with the thuriferous juniper.

This hardy species is able to support extreme climatical conditions: in the high valleys of the Atlas Mountains, winters are cold and summers very warm and dry. *Juniperus thurifera* often represent the tree limit. These trees can have very impressive dimensions. Some of them reach 19m high and leave more than 700 to 800 years. The thuriferous, whose current area is estimated in Moroccan Atlas to 20,000ha, is considered as the forest species having most regressed in Morocco, with a recession of 90% as compared to it's potential distribution area (6).

Juniper when applied externally is useful in cases of rheumatism, sciatica and dermatitis and has been reported as having therapeutic effects in the treatment of neurasthenic neurosis when used as a bath. It is also reported as having antimicrobial properties.

Many papers on the biological activity of essential oils have been published. The data, however, show much discordance between the same essences. The reasons of this variability can be understood if we take into account all the factors influencing the chemical composition of the oils, namely, climatic, seasonal and geographic conditions, harvest period and distillation technique, among others (10).

A recent study (3) analyzed geographic variation in the volatile oils from different Moroccan populations thuriferous juniper (Tizi-n'Ait-Imi (M1, M2), Oukaimden (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 missing in M1 but 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. The population of Tizi n'Tichka (TM) was only about 100Km from the population of Tizi-n'Ait-Imi (M1, M2), but the oil was quite different in several major compounds (α-pinene, sabinene, γ-terpinene, cis-sabinene hydrate, terpinen-4-ol, elemol and cedrol).

This paper presents additional analyses of the oil yields and composition of *Juniperus thurifera* var. *africana* from different populations ranging in elevation from 2000 to 2900 m in Morocco. In addition, the oil compositions of fresh and air dried juniper leaves are compared.

Experimental

The plant materials were collected for P1: 2900 m, Ait Akak, Oukaimden, Atlas Mts, Morocco, N. Achak, A. Romane and M. Mahroug, 3 trees, ns, 12/12/2003; P2, 2200 m, Plateau of Matat, Atlas Mts, N. Achak, A. Romane and M. Mahroug, 3 trees, ns, 18/03/2003; P3: 2000 m, Forêt Islane, Oukaimden, Atlas Mts, N. Achak, A. Romane and M. Mahroug, 3 trees, ns,12/12/2003. Voucher specimens are deposited at the Laboratory of Applied Organic Chemistry, Faculty of Science Semlalia, Cadi Ayyad University.

A portion of the leaves from each of the three trees (per population) were air dried for 16 days at room temperature (ca. 22 C) to produce the dried leaf samples. A portion of the fresh leaves from three trees (at each population) were combined to produce 1 kg of leaves that were steam distilled for 4h. The plant material is extracted by direct steam, produced in the still and the oil samples were stored at -20°C until analyzed. The extracted leaves were oven dried (4h, 105°C) for determination of oils yields.

The oils were analysed by gas chromatography using a Varian 3800 gas chromatograph fitted with a fused silica capillary column J & W DB5, 0,25mm id, 25m, 0.25μm coating thickness directly coupled to a Saturn 2000 MSD mass spectrometer. The GC/MSD 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 1.0 ml/min; injection 0.1μL (10% solution); split 1:10, 500ng on column. Identifications were made using combined MS and RI data from authentic compounds (11).

Results and discussion

The oil yields and percent of sabinene and δ-cadinene of the leaf essential oils of *Juniperus thurifera* obtained from different provenances are given in Table 1. There were essentially no differences between populational sources for the yields from fresh leaves (0.73, 0.73, 0.68%, Table I). In contrast, yields from air dried leaves (16 days) seem to vary some by populational source (Table I) with highest yield from the highest (elevation) population. One would expect to lose the more volatile terpenoids during storage but that does not seem to be the case. Sabinene decreased in dried leaves in P 1 and P3 (Table II), but increased in P2 and α-pinene increased in dried leaves in P1 and P2, and decreased in P3. The oil of *J. thurifera* var. *africana* is stored in

Table I. Yield and % of sabinene and δ-cadinene of the leaf essential oils of *Juniperus thurifera L.*

Altitude (m)	Population	Yield (%)		Percent of sabinene (%)		Percent of δ-cadinene (%)	
		Fresh leaves	Dried leaves	Fresh leaves	Dried leaves	Fresh leaves	Dried leaves
2900	P1: AIT Lkak (Oukaimden)	0.73	1.03	1.06	1.15	0.03	0.08
2200	P2: Plateau of Matat	0.73	0.67	1.6	1.7	0.08	0.22
2000	P3: Forêt Islane (Oukaimden)	0.68	0.57	0.66	0.4	0.4	0.48

glands, imbedded in the leaves, covered with a waxy cuticle. This may have prevented the loss of oil by evaporation. Alternatively, the plants from Ait Lkak (2900m) that appear to have a higher yield (Table I), might have some terpenoids stored as glycosides. These glycosides may have been hydrolyzed during leaf drying, making free terpenoids available for extraction. There is a report of increased oil yield from dried leaves in *Eucalyptus camaldulensis* (12), in contrast to the general case of the essential oils in plants being lost upon drying. Additional research is being conducted on *J. thurifera* to clarify this situation.

The leaf oil compositions from fresh and air dried leaves (16 days) from three sites are show in Table II. The major components in each case are sabinene (8.0–37.1%), α-pinene (1.2–9.1%), γ-terpinene (1.5–11.5%) and δ-cadinene (0.6–12.7%). 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. However, comparisons of oils between populations should utilize either fresh or air dried leaves but not samples of fresh and samples of dried leaves.

The fresh leaf essential oil of *J. thurifera* var. *africana* from P1 and P2 is clearly dominated by sabinene 21.1 and 35.9%, respectively, but much less in P3 (10.1%). Population 3 has a large amount of δ-cadinene (12.4%) compared to P1 (0.6) and P2 (0.8) (Table 2). Population 2 has a moderate amount of terpinen-4-ol (4.5%) compared to lower amounts in P1 (1.4) and P3 (1.3). Thus, each of the populations examined seem to have a profile that is differentiated from other populations.

Quantitative amounts were calculated for sabinene (terpene) and δ-cadinene (sesquiterpene) for each of the three populations. Figure 3 shows that the yields (mg/g dry wt) of sabinene and δ-cadinene were very similar from either fresh or dried leaves from most populations. The percent of each component from fresh or dried leaves appeared to vary mostly by provenance (Table II), rather than between fresh and dried leaf sources (Table II).

A comparison with the oil from the Consuegra, Spain, CS population of Adams et al (4) is included in Table II. As in the previous study (4), the Moroccan populations are clearly much different in their quantities of sabinene, δ-2-carene, limonene, linalyl acetate, and manoyl acetate compared to Spain (and other European populations of Adams, et al. (4).

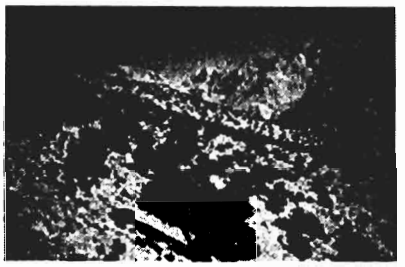

Figure 1. *Juniperus thurifera* var. *africana*, Tensift Al Haouz – Marrakech, Morocco.

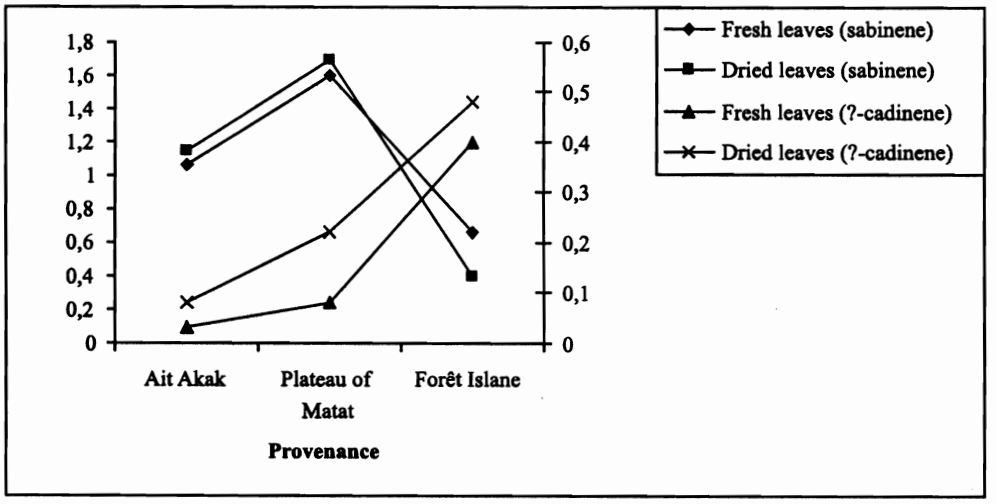

Figure 2. Variation in the yields of sabinene and δ-cadinene in the oils of *Juniperus thurifera* var. *africana*.

Table II. Comparison of leaf oil constituents from *Juniperus thurifera* from different populations.

	P1 2900 m		P2 2200 m		P3 2000m		Spain(4)
KI Compound	FL	DL	FL	DL	FL	DL	FL
926 tricyclene	0.4	0.8	0.2	0.1	0.5	t	-
931 α-thujene	1.4	2.6	2.3	1.6	2.1	0.2	0.1
939 α-pinene	**7.6**	**9.1**	**4.6**	**4.9**	**5.5**	**1.2**	**2.0**
953 α-fenchene	1.1	1.2	0.1	0.4	1.0	0.6	-
953 camphene	0.8	t	t	t	0.7	t	t
976 sabinene	**21.2**	**16.5**	**35.9**	**37.1**	**10.1**	**8.0**	**1.5**
980 β-pinene	0.2	0.3	12	0 .4	0.6	t	0.1
991 myrcene	0.5	1.1	3.5	3.3	1.4	0.4	2.5
1001 δ-2-carene	-	-	-	-	-	-	**2.0**
1005 α-phellandrene	t	t	2.6	4.1	0.1	t	t
1011 δ-3-carene	0.9	1.0	4.4	5.1	t	t	0.5
1018 α-terpinene	**2.2**	**1.5**	**2.4**	**8.5**	**1.4**	**1.4**	**0.1**
1026 p-cymene	0.7	0.3	1.1	2.4	0.8	0.8	t
1031 limonene	**0.7**	**0.7**	**1.1**	**1.0**	**0.5**	**t**	**52.8**
1031 β-phellandrene	t	0.4	t	t	t	t	t
1037 (Z)-β-ocimene	t	0.5	-	-	t	-	t
1050 (E)-β-ocimene	0.2	0.3	0.1	0.5	t	-	0.2
1062 γ-terpinene	**9.3**	**11.5**	**1.5**	**3.1**	**6.7**	**2.6**	**0.2**
1068 *cis*-sabinene hydrate	0.1	0.2	0.6	0.6	0.3	0.1	0.1
1088 terpinolene	t	0.1	1.7	2.4	0.2	t	0.8
1097 *trans*-sabinene hydrate	1.2	1.6	2.7	2.2	1.2	0.3	-
1098 linalool	-	-	-	-	-	-	**5.7**
1102 *cis*-thujone (=β-thujone)	0.4	0.4	t	0.3	0.8	0.2	-
1114 *trans*-thujone (=α-thujone)	0.1	0.1	0.8	1.2	t	0.2	t
1121 *cis*-p-menth-2-en-1-ol	1.5	1.0	0.4	0.4	0.6	0.5	0.2
1125 α-comphlenal	t	t	t	-	t	-	
1139 *trans*-pinocarveol	t	t	-	-	-	t	-
1140 *trans*-p-menth-2-en-1-ol	t	1.2	0.3	0.5	1.0	t	-
1143 camphor	-	-	t	0.1	-	-	t
1144 E-tagetone	-	-	0.3	0.2	-	-	-
1156 sabina ketone	-	t	0.2	0.2	-	t	-
1163 pinocarvone	t	t	t	t	t	t	-
1165 borneol	-	t	t	t	t	t	-
1171 umbellulone	0.3	0.3	-	-	0.2	0.8	-
1177 terpinen-4-ol	1.4	2.3	4.5	6.9	1.3	3.4	0.5
1183 p-cymen-8-ol	0.5	0.8	0.2	0.4	0.6	0.1	t
1189 α-terpineol	0.4	0.6	0.6	0.9	t	t	0.6
1191 myrtenol	-	-	t	t	-	-	-
1193 4Z-decenal	0.3	-	-	-	-	-	-
1205 *trans*-piperitol	-	-	-	t	-	-	-
1217 *trans*-carveol	0.1	-	0.2	0.4	0.5	-	-
1228 citronellol	-	t	-	t	-	t	0.4
1252 piperitone	-	-	-	-	-	-	3.5
1252 *trans*-sabinene hydrate	-	-	t	0.2	-	-	-
1257 linalyl acetate	**0.8**	**1.0**	**2.4**	**2.2**	**2.1**	**1.2**	**13.7**
1269 p-menth-2-ene-1,4-diol	0.6	0.9	-	0.1	0.8	-	-
1277 pregeijerene B	0.3	0.4	-	-	0.3	-	-
1285 bornyl acetate	0.7	1.1	1.6	0.9	0.5	0.6	0.3
1300 terpinen-4-ol acetate	0.4	0.3	0.3	0.4	0.3	0.3	
1312 unknown(43,57,95,68,152)	1.0	-	-	-	0.7	-	1.0
1339 δ-elemene	-	-	t	t	t	t	-
1350 α-terpinyl acetate	**0.3**	**0.7**	**0.3**	**0.6**	**0.3**	**0.8**	**3.6**
1362 muurol acetate	0.1	0.2	-	-	t	-	-
1376 α-copaene	0.3	0.4	t	0.3	t	0.6	-
1383 β-bourbonene	t	t	0.1	0.2	t	t	-
1391 β-elemene	t	t	t	0.1	t	t	-
1415 β-funebrene	-	-	0.2	0.6	-	-	-
1418 (E)-caryophyllene	0.1	0.1	t	0.2	0.2	0.3	-
1454 α-humulene	-	-	t	t	-	t	-
1477 γ-muurolene	0.2	0.2	0.1	0.3	0.2	t	-
1480 germacrene D	0.5	0.9	0.1	0.5	1.4	1.4	0.2
1499 α-muurolene	2.3	1.8	0.1	0.8	3.0	3.3	-
1513 γ-cadinene	2.0	2.6	0.3	1.2	3.4	4.6	0.1
1524 δ-cadinene	**0.6**	**1.2**	**0.8**	**1.5**	**12.4**	**12.7**	**0.4**

Table II. Continued

		P1 2900 m		P2 2200 m		P3 2000m		Spain(4)
KI	Compound	FL	DL	FL	DL	FL	DL	FL
1538	α-cadinene	0.4	0.5	t	0.5	0.7	1.2	-
1549	elemol	4.0	1.8	1.1	0.9	2.4	3.8	0.3
1556	germacrene B	0.1	0.2	0.1	0.3	0.2	0.2	0.2
1574	germacrene D-4-ol	-	-	-	-	-	-	0.4
1581	caryophyllene oxide	t	t	t	t	-	t	-
1596	cedrol	0.6	0.7	0.5	0.4	0.4	1.1	0.2
1608	β-oplopenone	0.7	0.8	-	-	1.0	1.0	0.5
1611	epi-cedrol	-	-	-	t	-	-	-
1627	1-epi-cubenol	-	-	-	-	-	-	t
1630	γ-eudesmol	t	t	0.1	t	t	t	0.1
1640	epi-α-cadinol	1.0	0.5	t	0.1	1.4	2.3	0.1
1640	epi-α-muurolol	1.3	0.4	t	0.1	1.1	1.5	0.1
1645	α-muurolol	-	t	-	t	t	t	t
1649	β-eudesmol	t	t	0.5	0.4	t	t	0.4
1652	α-eudesmol	4.0	1.6	-	0.4	3.4	4.7	0.5
1653	α-cadinol	-	-	-	t	-	-	0.4
1733	**oplopenone**	**3.2**	**1.0**	**0.1**	**0.2**	**1.0**	**1.5**	-
1961	sandaracopimara-8(14),15-diene	-	-	-	t	t	-	
1989	manoyl oxide	-	-	-	-	-	-	1.1
2080	abietatdiene	-	-	-	-	-	-	t

P1 = Ait Lkak, Tensift Al Haouz – Marrakech; P2 = Plateau of Matat, Tensift Al Haouz – Marrakech; P3 = Forêt Islane, Tensift Al Haouz – Marrakech. FL= fresh Leaves, DL= air dried Leaves. KI = Kovat's Index on DB-5 (=SE54) column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

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