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Comparisons of the Leaf Essential Oils of *Juniperus phoenicea*, *J. phoenicea* subsp. *eu-mediterranea* Lebr. & Thiv. and *J. phoenicea* var. *turbinata* (Guss.) Parl.

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ABSTRACT: The leaf essential oils of *Juniperus phoenicea* L. (Greece and Spain), *J. phoenicea* subsp. *eu-mediterranea* Lebr. & Thiv. and *J. phoenicea* var. *turbinata* (Guss.) have been analyzed by GC/MS. All of the oils were dominated by α-pinene. *Juniperus phoenicea* also contained moderate amounts of myrcene, limonene, β-phellandrene, α-terpinyl acetate and 1-epi-cubenol. The oils of *J. phoenicea* var. *turbinata* (coastal Spain) and *J. phoenicea* subsp. *eu-mediterranea* (coastal Portugal) are most similar of the four taxa and distinct from typical inland *J. phoenicea*. These taxa may be conspecific and, if so, then the name *J. phoenicea* var. *turbinata* would take precedence for the divergent taxon.

KEY WORD INDEX: *Juniperus phoenicea*, subsp. *eu-mediterranea*, var. *turbinata*, Cupressaceae, essential oil composition, α-pinene, β-phellandrene, α-terpinyl acetate, geographic variation.

INTRODUCTION: *Juniperus phoenicea* L. is a small tree that is native to the northern lands bordering the Mediterranean Sea from Portugal to Israel. It is also native to N. Africa in Algiers and Morocco as well as the Canary Islands (1). Gaussen (1) discussed several infraspecific taxa: var. *turbinata* (Guss.) Parl.(= var. *oophora* Kunze) with female cones elongated (turbinate) in littoral sites throughout the Mediterranean; var. *canariensis* Guyot on the Canary Islands; var. *lycia* (L.) Gaussen (*pro specie*) (= *J. phoenicea*), France littoral zone; var. *mollis* M & W., common in Morocco; and var. *megalocarpa* Maire, dunes near Mogador, Morocco. Most likely these varieties are not distinct taxa but reflect the lack of access to the type specimen by botanists who described local populations as new varieties of *J. phoenicea*. More recently, LeBreton and Thivend (2), on the basis of total proanthocyanidins and the ratio of procyanidine to prodelphinidine, recognized *J. phoenicea* subsp. *eu-mediterranea* Lebr. & Thiv. as occurring on the Mediterranean islands, North Africa and southwestern Portugal. Later, LeBreton (3) expanded his work to include more sample locations and showed all of the southwestern coastal populations of Portugal and Spain to

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Table I. Comparisons of the total oil (percentage) for leaf essential oils of *Juniperus phoenicea* from Greece and Spain, *J. phoenicea* var. *turbinata*, Spain and *J. phoenicea eu-mediterranea*, Portugal

		J. phoenicea		var. *turbinata*	ssp. *eu-mediterranea*
KI	Compound	Greece	Spain	Spain	Portugal
926	tricyclene	0.3	0.1	0.1	0.1
931	α-thujene	-	t	-	-
939	**α-pinene**	**41.8**	**53.5**	**28.3**	**34.1**
953	camphene	0.6	0.4	0.2	0.2
957	thuja-2,4(10)-diene	0.1	t	t	-
967	verbenene	-	0.4	t	-
976	sabinene	0.1	t	t	t
980	β-pinene	1.3	2.5	1.3	1.0
991	myrcene	4.5	4.0	7.2	5.4
1001	δ-2-carene	0.1	t	0.4	0.3
1005	**α-phellandrene**	**0.6**	**0.8**	**4.1**	**3.1**
1011	δ-3-carene	-	1.7	-	-
1018	α-terpinene	t	t	0.3	0.3
1026	p-cymene	0.8	0.4	1.1	0.9
1031	**limonene**	**4.7**	**t**	**-**	**-**
1031	**β-phellandrene**	**3.5**	**5.9**	**25.3**	**19.2**
1050	(E)-β-ocimene	0.1	-	-	-
1062	γ-terpinene	0.7	0.2	0.2	0.3
1074	cis-linalool oxide (furanoid)	t	-	-	-
1088	terpinolene	1.3	0.6	1.6	1.3
1095	α-pinene oxide	-	0.2	-	-
1098	linalool	0.7	1.3	0.2	0.2
1112	α-fenchol	-	t	-	-
1121	**cis-pinene hydrate**	**0.1**	**0.2**	**-**	**-**
1121	cis-p-menth-2-en-1-ol	-	-	0.6	0.9
1125	α-campholenal	0.6	0.2	0.1	0.1
1139	**trans-pinocarveol**	**0.6**	**0.3**	**-**	**-**
1140	trans-p-menth-2-en-1-ol	-	-	0.5	0.7
1143	camphor	0.4	-	-	-
1144	trans-verbenol	-	-	t	0.2
1146	isopulegol	t	1.6	-	-
1165	borneol	-	0.4	-	t
1166	p-mentha-1,5-dien-8-ol	0.6	-	0.1	t
1177	terpinen-4-ol	1.3	t	0.2	0.2
1179	naphthalene	-	t	-	0.1
1183	p-cymen-8-ol	0.2	t	t	0.2
1189	α-terpineol	0.8	2.5	0.4	0.4
1193	**cis-piperitol**	**-**	**-**	**0.2**	**0.2**
1194	myrtenol	0.1	t	-	-
1200	trans-dihydrocarvone	-	t	-	-
1204	verbenone	-	0.1	-	-
1205	**trans-piperitol**	**-**	**-**	**0.2**	**0.3**
1217	trans-carveol	t	-	-	-
1220	α-fenchyl acetate	-	-	-	0.2
1228	citronellol	0.6	0.6	0.7	0.5
1235	myrtenyl acetate	-	-	0.1	-
1252	piperitone	0.8	0.2	t	0.3
1257	linalyl acetate	2.0	-	-	-
1258	**trans-myrtanol**	**-**	**-**	**0.5**	**1.3**
1273	isopulegyl acetate	3.3	-	1.1	0.6
1281	iso(iso)pulegyl acetate	0.2	-	-	0.6
1285	**bornyl acetate**	**-**	**-**	**0.3**	**0.6**

Table I. (Cont.)

KI	Compound	*J. phoenicea* Greece	*J. phoenicea* Spain	var. *turbinata* Spain	ssp. *eu-mediterranea* Portugal
1285	trans-linalool oxide acetate (pyranoid)	0.5	-	0.2	0.2
1291	2,4-decadienal	-	-	-	0.1
1296	9-undecenal	-	0.3	0.2	0.7
1337	trans-carvyl acetate	0.3	-	-	-
1344	**aromatic terpene**	**-**	**-**	**1.1**	**0.2**
1350	α-terpinyl acetate	4.6	-	15.5	12.5
1383	geranyl acetate	t	-	-	-
1390	β-cubebene	t	-	-	-
1418	**β-caryophyllene**	**0.5**	**1.0**	**t**	**0.2**
1446	cis-muurola-3,5-diene	-	-	-	-
1454	α-humulene	0.3	-	-	0.1
1473	β-cadinene*	0.4	-	-	-
1477	γ-muurolene	t	-	-	-
1480	germacrene D	0.8	0.4	0.2	0.5
1489	phenylethyl 2-methylbutyrate	t	-	-	-
1490	cis-β-guaiene	0.9	-	-	-
1493	epi-cubebol	0.6	-	-	-
1499	α-muurolene	0.2	-	t	0.1
1513	γ-cadinene	-	0.2	t	0.1
1514	cubebol	0.8	-	-	-
1521	cis-calamenene	t	-	-	-
1524	δ-cadinene	1.3	t	0.3	0.4
1532	cadina-1,4-diene	t	-	-	-
1542	sesquiterpene alcohol	-	0.8	-	-
1549	elemol	-	1.2	0.5	0.7
1556	germacrene B	t	0.4	0.2	0.4
1574	germacrene D-4-ol	-	t	0.2	0.3
1581	caryophyllene oxide	-	0.7	-	0.2
1601	humulene epoxide II	t	-	-	t
1627	1-epi-cubenol	2.3	-	-	0.2
1630	γ-eudesmol	-	t	-	0.1
1640	epi-α-cadinol (=T-cadinol)	t	-	0.1	t
1641	epi-α-muurolol (=T-muurolol)	-	-	0.2	0.2
1642	cubenol	0.7	-	-	-
1645	α-muurolol (=torreyol)	0.2	-	t	-
1649	β-eudesmol	-	0.2	0.2	0.3
1652	α-eudesmol	-	0.2	t	0.4
1653	α-cadinol	0.7	t	0.7	0.4
1666	bulnesol	-	0.1	-	0.1
1684	**sesquiterpene alcohol**	**0.7**	**-**	**-**	**-**
1688	**sesquiterpene alcohol**	**-**	**1.0**	**0.9**	**1.7**
1961	13-epi-manool	-	-	t	-
1989	**manoyl oxide**	**-**	**14.4**	**0.6**	**0.6**
2054	**abietatriene**	**0.5**	**-**	**t**	**0.1**
2080	**abietadiene**	**0.6**	**-**	**t**	**t**
2200	n-docosane	-	-	-	0.1
2278	cis-totarol	-	-	-	0.2
2288	4-epi-abietal	t	t	-	0.2
2303	trans-totarol	1.0	-	t	2.8
2325	trans-ferruginol	0.3	-	-	0.3
2400	tetracosane	-	-	-	0.1

KI = Kovat's Index on DB-5(=SE54) column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.
Boldface implies key differences among taxa.

have high proanthrocyanidines (implying *J. phoenicea* ssp. *eu-mediterranea*). He did not have samples from the Gilbraltar (Tarifa) region of Spain and indicated uncertainty by use of a question mark (?) on his map (see Figure 1 in reference 3). Tarifa is an area where *J. phoenicea* var. *turbinata* occurs and LeBreton's population 70 (3), west of Setubal, is in the middle of his distribution for *J. phoenicea* ssp. *eu-mediterranea*. In order to compare our samples with his, we sampled from the area of his population 70, his pure *J. phoenicea* population 66-65 and *J. phoenicea* var. *turbinata* from Tarifa as well as a reference population of *J. phoenicea* in Greece.

There are a number of reports on the terpenes of *J. phoenicea* but none comparing the entire essential oils of any of the taxa. For example, San Feliciano, and workers examined acidic diterpenes (4,5) and neutral diterpenes, 3 lignans, and 4 sesquiterpenoids (6) from the leaves of *J. phoenicea* subsp. *turbinata*. The leaf essential oil of *J. phoenicea* has been reported in varying detail from Egypt (7), Saudi Arabia (8, sesquiterpenes and diterpenes), France (9,10) and cultivated- origin unknown (11). There are numerous analyses of the fruit essential oils (12-16). There have been no reports on the complete leaf essential oils of *J. phoenicea*, *J. phoenicea* subsp. *eu-mediterranea* and *J. phoenicea* var. *turbinata*. In this paper we report on the essential composition of these taxa and discuss their relationships.

EXPERIMENTAL: Foliage was collected [*J. phoenicea*: Epidavios, Greece, R. P. Adams, 5653-5654; and El Penon, Spain, R. P. Adams, 7077-7079; *J. phoenicea* subsp. *eu-mediterranea*: West of Setubal, Portugal, R. P. Adams, 7074-7076; *J. phoenicea* var. *turbinata*: w. of Tarifa, Spain, R. P. Adams, 7302-7204] and voucher specimens are deposited at BAYLU! The volatile leaf oils were isolated by steam distillation (200 g foliage, FW) using a circulatory Clevenger apparatus (17) for 2 h. The oil samples were concentrated (ether trap removed) with nitrogen and stored at –20°C until analyzed. Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a J&W 30 m x 0.26 mm (0.25 micron coating thickness) J&W DB-5 fused silica capillary column temperature programmed from 60°-240°C at 3°C min. Identifications were made by library searches of our volatile oil library, LIBR(TP) (18) using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds.

RESULTS AND DISCUSSION: Oil yields (2 h) were 0.21% (Greece), 0.66% (El Penon, Spain), 0.41 (subsp. *eu-mediterranea*, Portugal) and 0.30% (var. *turbinata*, Spain) (oil wt./ extracted, oven dried foliage weight). The oil composition for each of the taxa is shown in Table I. Each of the oils is dominated by α-pinene. In addition, *J. phoenicea*, Greece, contains moderate amounts of myrcene, limonene, β-phellandrene, α-terpinyl acetate and 1-epi-cubenol. *J. phoenicea*, interior of Spain, is generally similar to the oil from Greece, except limonene is only a very small trace, α-terpinyl acetate is missing and manoyl oxide is a major component (Table I). In addition, the unknown sesquiterpene alcohol at KI 1684, from the Greek sample is missing in the samples from Spain and Portugal and the unknown sesquiterpene at KI 1688 is missing from the Greek sample but present in the samples from Spain and Portugal.

The oils of *J. phoenicea* var. *turbinata* (coastal Spain) and *J. phoenicea* subsp. *eu-mediterranea* (coastal Portugal) are most similar of the four taxa and both oils are dominated by α-pinene, β-phellandrene, and α-terpinyl acetate with no limonene and moderate amounts of myrcene and α-phellandrene. Both taxa share small amounts of compounds not found in *J. phoenicea* (*sensu stricto*): cis- and trans-p-menth-2-en-1-ol, cis- and trans-piperitol, trans-myrtanol, bornyl acetate and the aromatic terpene at KI 1344.

In general, two groups are seen in the oils:

- *J. phoenicea* (Greece and inland Spain); and
- *J. phoenicea* var. *turbinata* (coastal Spain) and *J. phoenicea* subsp. *eu-mediterranea* (coastal Portugal).

Our results agree with the proanthocyanidine data (2,3) in that *J. phoenicea* subsp. *eu-mediterranea* is distinct in its leaf oil from *J. phoenicea* (*sensu stricto*). However, it appears *J. phoenicea* ssp. *eu-mediterranea* and *J. phoenicea* var. *turbinata* may be conspecific. Some of the fruit (female cones) from *J. phoenicea* subsp. *eu-mediterranea* individuals had elongated cones (turbinate) as in the plants of *J. phoenicea* var. *turbinata*. In that case, the name *J. phoenicea* var. *turbinata* would take precedence by the rules of earliest published name. Additional research is in progress using DNA fingerprints to help establish the relationships among these taxa.

Mass spectra for unidentified constituents: [ITMS, m/z (rel. int.): KI 1344, 43(100), 51(7), 59(16), 65(7), 77(11), 91(32), 105(16), 134(33), aromatic terpene acetate; KI 1542, 43(100), 55(34), 67(33), 79(27), 95(33), 107(20), 121(14), 135(10), 149(11), 164(10), 175(2), 189(6), 207(20), sesquiterpene alcohol?; KI 1684, 41(100), 55(31), 67(37), 79(46), 91(60), 209(38), 117(21), 131(22), 145(7), 159(38), 177(11), 187(4), 202(10), 220(2), sesquiterpene alcohol; KI 1688, 41(100), 55(53), 67(43), 84(50), 93(22), 109(22), 121(14), 137(10), 149(2), 161(10), 189(2), 207(3), 222(1), sesquiterpene alcohol.

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