

**Geographic variation in the leaf essential oil of *Juniperus turbinata* from throughout its range in the Mediterranean**

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**ABSTRACT**

The volatile leaf oils from sixteen populations of *Juniperus turbinata* (= *J. phoenicea* subsp. *turbinata*) were analyzed. The variation in the major components,  $\alpha$ -pinene (17.7 - 67.9%) and  $\beta$ -phellandrene (0.5 - 31.5%), was extremely large. Overall, the oils are quite variable as one might expect from the great variation in habitats ranging from coastal (8 - 15 m) to high mountains (Algeria, 1451 m, Morocco, 940 m) and from desert (Sinai) to Mediterranean. Considerable geographical variation was found in the oils dividing the populations into five groups: Portugal - Spain; Mediterranean Basin - Madeira; Canary Islands; High Atlas mountains and Sinai. There appears to be more of a mosaic than continuous geographical pattern among the populations. The utilization of oil from 12 year old herbarium specimens was examined by comparing with fresh leaves from Crotone, Italy. It appears likely that both oxidation and free radical reactions have occurred in the 12 year old herbarium leaves. In this study, it was not possible to utilize oils from herbarium specimens. Published on-line [www.phytologia.org](http://www.phytologia.org) *Phytologia* 96(3): 149-158 (July 1, 2014). ISSN 030319430

**KEY WORDS:** *Juniperus turbinata*, *J. phoenicea*, var. *turbinata*, Cupressaceae, leaf essential oils geographic variation.

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The genus *Juniperus* is comprised of approx. 75 species in 3 sections (Adams, 2014) with serrate (denticulate) leaf-margined species found in both the eastern hemisphere (1 species) and western hemisphere (21 species). *Juniperus phoenicea* and *J. turbinata* are the only serrate-leaf juniper in the eastern hemisphere. They have been treated as *J. p.* var. *phoenicea* and var. *turbinata* (Adams, 2011) or as subsp. *turbinata* (Farjon, 2005). However, Adams and Schwarzbach (2013) have recently shown that *J. phoenicea* is not part of a clade of serrate-leaf junipers occurring in the western hemisphere, leading them to denote *J. phoenicea* as a 'pseudoserrate' juniper. In addition, they found *J. p.* var. *phoenicea* and var. *turbinata* to be as different in their DNA sequences as several other recognized species of *Juniperus*, lending support for the recognition of *J. turbinata* Guss. as proposed by Lebreton and Perez de Paz (2001) based largely on the concentration of prodelphinidin, a polymeric tannin. The prodelphinidin data suggested that *J. phoenicea* var. *phoenicea* was confined to the Iberian Peninsula and south France, with var. *turbinata* being widespread throughout the Mediterranean. Farjon (2005) considered subsp. *phoenicea* to be widespread in the Mediterranean and subsp. *turbinata* to be confined to littoral maritime habitats (sand and rocks). Adams (2011) followed the distributions of Farjon (2005), except for the Canary Islands and Madeira, which, based on DNA sequence data, have been shown (Adams et al. 2010) to be *J. turbinata* not *J. phoenicea* var. (subsp.) *phoenicea*.

The most comprehensive DNA sequence study to date (Adams et al. 2013) sampled 2 populations of *J. phoenicea* var. *phoenicea* and 19 populations of *J. phoenicea* var. *turbinata*. They found *J. phoenicea* var. *phoenicea* in a separate clade from *J. p.* var. *turbinata* (Fig. 1), with *J. phoenicea* var. *phoenicea* only found in the two Spain populations. *Juniperus turbinata* from the Canary Islands and Madeira, through the Mediterranean and eastward to the Sinai were all in a distinct clade (Fig. 1). It is this classification that was used for the basis of selecting *J. turbinata* populations for this study.

The volatile leaf oils of *J. phoenicea*, *sensu stricto*, have been recently examined (Adams, Altarejos and Arista, 2014) and the literature on the leaf oils of *J. phoenicea* and *J. p.* var. *turbinata* (*J. turbinata*) has been reviewed therein.

The purpose of this paper is to present new data on geographic variation in the volatile leaf oils of *J. turbinata* from throughout its range (Fig. 2).

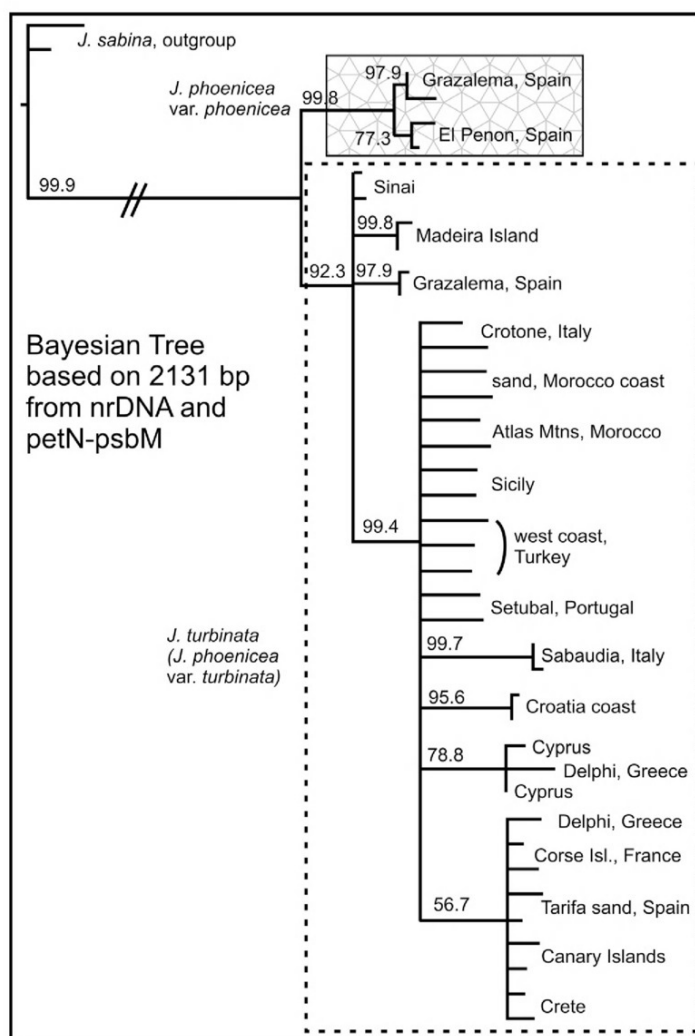


Figure 1. Bayesian tree showing *J. phoenicea* and *J. turbinata* (*J. p.* var. *phoenicea*) in well-supported clades. From Adams et al. 2013

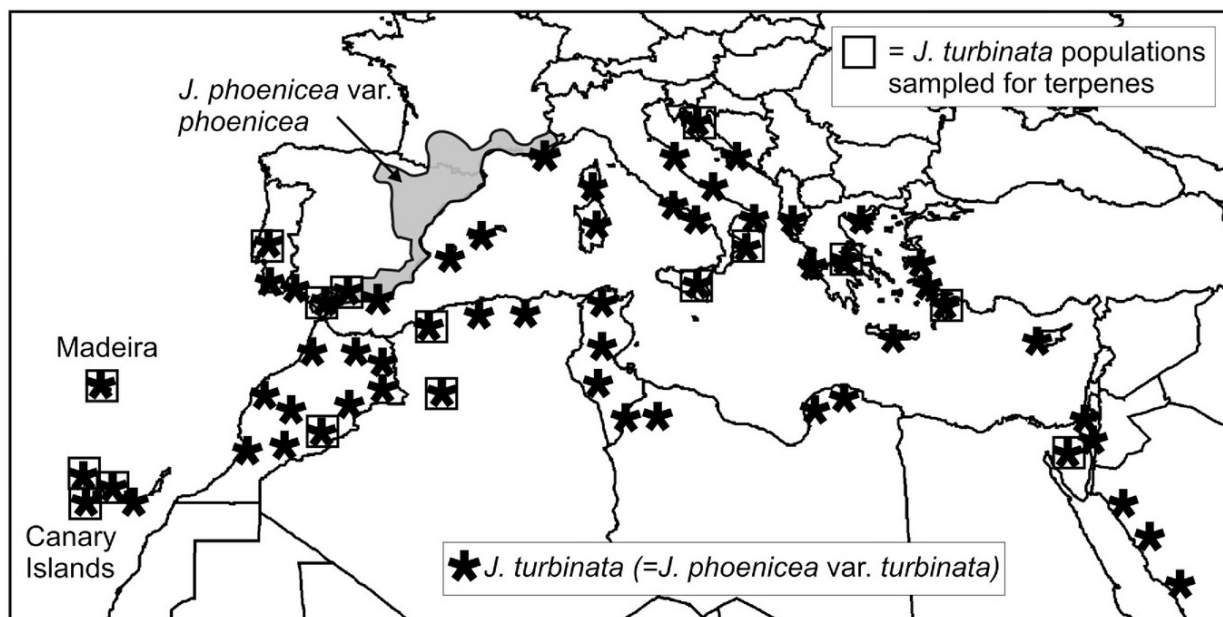


Figure 2. Distribution of *J. phoenicea* (adapted from Lebreton and Perez de Paz (2001) and Adams et al. (2010)). Squares show populations *J. turbinata* sampled in the present study.

## MATERIALS AND METHODS

Specimens used in this study, *J. turbinata*:

Algeria, inland Mountains (continental), 32° 41.332' N; 00° 29.003' E, 1451 m, *H. Houari*, 1-6, *Adams* 13984-13989, 23 April 2013 (fresh),

Algeria, coastal (littoral), 35° 47.374' N, 00° 29.074' E, 77 m, *H. Houari*, 7-12, *Adams* 13990-13995, (fresh),

Canary Islands, La Gomera, 28° 11.358' N; 17° 12.320' W, 370 m, *Adams* 11528-11530, (fresh),

Canary Islands, Tenerife, 0.5km S. of Tejina de Isora on rt. 822, 29° 10' 48" N, 16° 45' 53" W, ca. elev. 520m, *Adams* 8147-8149, (fresh),

Canary Islands, La Palma, 28° 44.250' N; 017° 44.198' W, 283 m, *Adams* 11514-11517, (fresh),

Croatia, Ugljan Island, 44° 05' 0.27" N, 15° 09' 39.29" E, elev. 20-32 m, *Zlato Liber* 1-5, *Baylor* specs. *Adams* 13589-13593, (fresh),

Greece, north of Nea Epidavos, 37° 41.091' N; 23° 07.686' E, 76 m, *Adams* 9439-9441, (fresh),

Italy, Crotone (south coast)

*Adam Boratynski* IT-2(1-5), *Baylor* specs. *Adams* 13341-13345, 38° 53' 36" N, 17° 05' 42" E, elev. 10 m, 9 Dec 2001 (12 yr old herbarium specimens),

*Pietro Minissale & Saverio Sciandrello* 1-6, *Adams* 14105-14110, Capo Rizzuto, near sea side. 38° 53' 36" N, 17° 05' 42" E, elev. 10 m, fresh, 7 Sep 2013, (same population as *Boratynski* above),

Madeira Island, Portugal, elev. ca. 20m, *Adams* 11502-11504, (fresh),

Morocco, rd to Oukaimeden, 31° 21.033' N, 07° 45.893' W, elev. 940m, *Adams* 9408-9410, (fresh),

Portugal, Setubal, Sa. da Arrabida Mountains, 12 km west of Setubal on N379-1, 225m. *Adams* 7074-7076, (fresh)

Sicily, near Piano Pirrera near Acate (Ragusa), 37° 01' 35.75" N; 14° 26' 07.86" E., 120 m, *Pietro*

*Minissale & Saverio Sciandrello* 1-5, *Baylor* specs. *Adams* 13778-13782, 18 Jan 2013, (fresh),

Sinai, 30°38'09"N, 33°26'53"E, elev. 700 m *Hagar Leschner* 1-5, *Baylor* specs. *Adams* 13495-13499, 15 Jun 2012 (fresh),

Spain, Sierra de Grazalema, 36° 48' 10.9"N, 5° 24' 21.2"W, elev. 829m, *M. Arista* 6-10, Baylor specs.

*Adams* 13818-13822 (fresh),

Spain, Tarifa sand dunes, elev. ca. 20m, *Adams* 7202-7204, (fresh),

Turkey, Mugla, main road between Marmaris - Datca, 36° 46' N; 27° 59' E, 308 m, *Tugrul Mataraci* 1-4,

*Adams* 13969-13972, (fresh),

Voucher specimens are deposited at BAYLU herbarium Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Additional steam distillation tests for up to 48 h revealed that the 2 h distillation removed about 23% (of the total oil for 48 h), providing a correction factor of 4.34 (x 2h = 48 h total). The yields appeared to asymptote at about 104% of the 48 h total oil.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (Gower, 1971; Adams, 1975). Principal Coordinate Ordination (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

The compositions of the leaf volatile oils for several populations are shown in table 1. The variation in the major components,  $\alpha$ -pinene (17.7 - 67.9%) and  $\beta$ -phellandrene (0.5 - 31.5%), is extremely large. Overall, the oils are highly variable as one might expect from the great variation in habitats ranging from coastal (8 - 15 m) to high mountains (Algeria, 1451 m, Morocco, 940 m) and from desert (Sinai) to Mediterranean.

To further examine geographic variation, PCO (Principal Coordinate Ordination) was performed on the oils from the 16 populations using 29 terpenoids (\* in table 1). Six eigenroots accounted for 24.5, 14.1, 10.1, 9.0, 6.2, and 5.9% of the variation (69.8% of total variance) before the eigenroots began to asymptote. The low amount of variance accounted for in the first few eigenroots suggests the variance cannot be explained for only a few trends among the populations. Ordination of the 16 *J. turbinata* populations (Fig. 3) shows four major groupings:

1. Portugal - Spain (GS, Grazalema, Spain; TS, Tarifa sands, Spain; PO, Setubal, Portugal);
2. Mediterranean: Italy, Greece, Sicily, Algeria (coastal), Croatia, Turkey and Madeira;
3. Canary Islands: TC, Tenerife; PC, La Palma; GC, La Gomera;
4. High Atlas mountains: Algeria, Morocco;
5. Sinai.

It is interesting that Madeira oil seems more associated with the central Mediterranean than with nearby Canary Islands oils (Fig. 3). However, it should be noted that only 45% of the variance among populations was accounted for in the PCO ordination (Fig. 3), so relationships between individual populations is distorted by ordination in only three dimensions.

To further examine geographical trends, similarity measures were calculated (using 29 terpenoids) and populations were clustered using a minimum spanning network. The clustering was then contoured mapped to present a geographical representation of trends (Fig. 4). The most similar populations were Tenerife and La Palma, Canary Islands, at 0.86 similarity (Fig. 4), followed by Spain (GR, Grazalema, TS, Tarifa sands, 0.82) and Greece - Turkey (0.82). In deference to the PCO (Fig. 3), Croatia and Sicily were quite similar (0.81, Fig. 4). The Atlas Mountains populations (Morocco, Algeria Mtns.) clustered at 0.80. But the Algerian coastal population clustered with Madeira, Canary Islands, Croatia - Sicily and Greece - Turkey populations that comprised the central Mediterranean group as seen in the PCO (Fig. 3). The final group entering the cluster was the Atlas Mountains group (Morocco, Algeria Mtns.) that are the most differentiated, joining with all the other populations of *J. turbinata* at a similarity of 0.75 (Fig. 4).

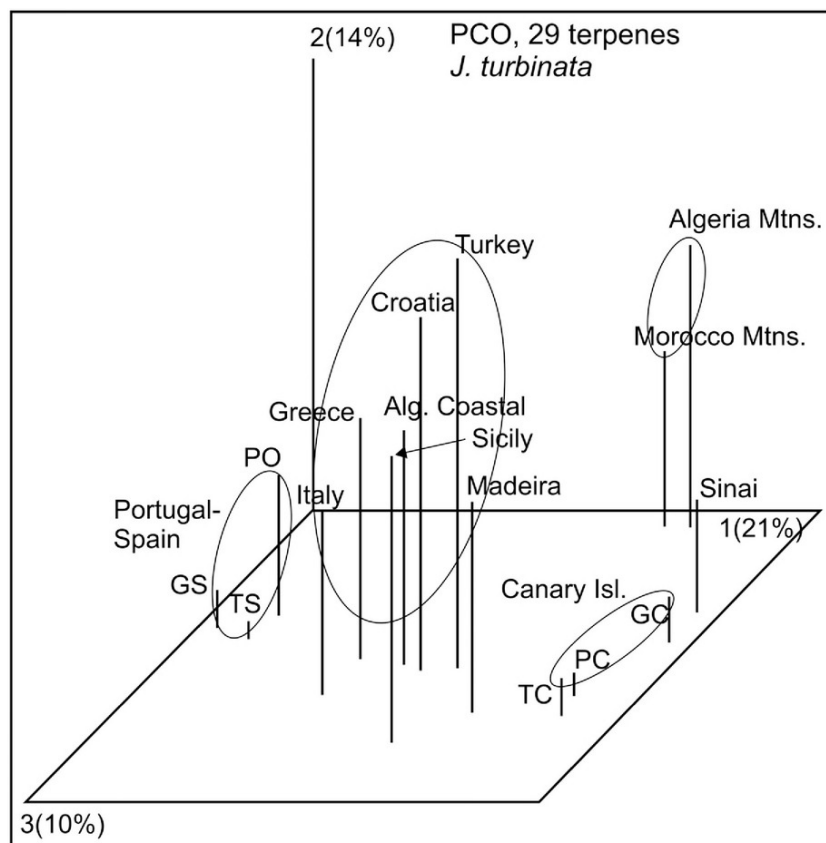


Figure 3. PCO of *J. turbinata* based on 29 terpenes.

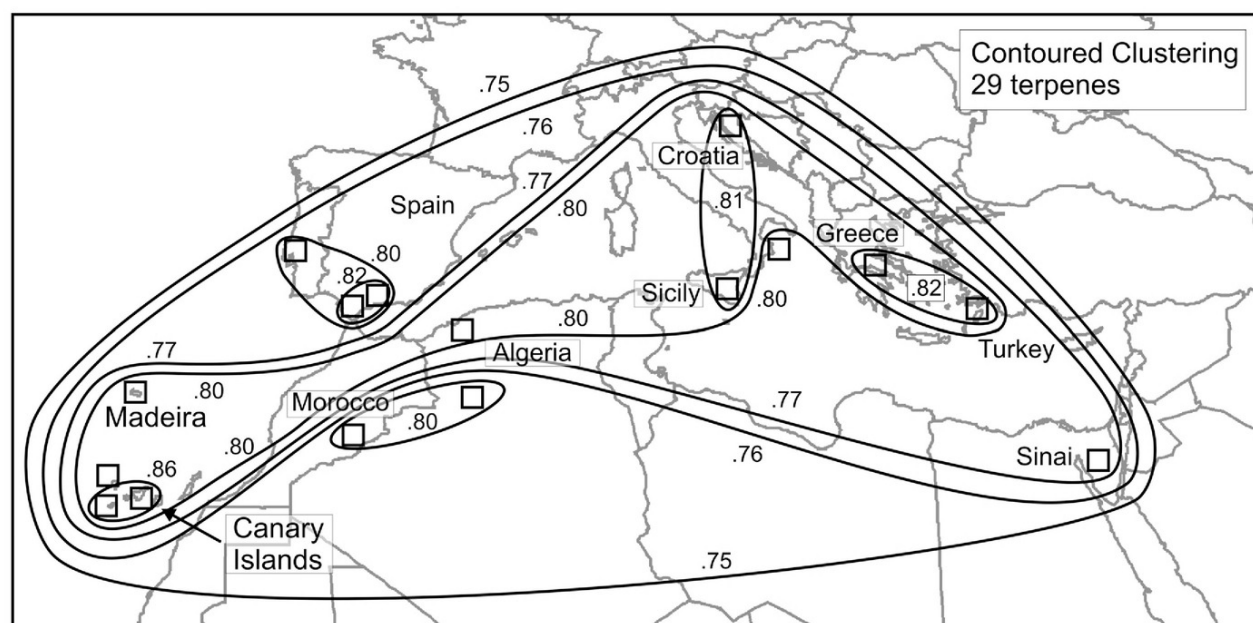


Figure 4. Contoured clustering with similarities based on 29 terpenes.



The pattern of differentiation appears mostly mosaic (Fig. 4), suggesting either a long period of isolation between populations or, perhaps, local selection for terpene profiles.

Initially, it was thought that this study could be done by utilizing oils obtained from herbarium specimens. Several studies (Achak et al. 2008; Adams, 2010; 2011; 2012a; 2012b; 2013a; 2013b; Shanjani et al. 2010) on the effects of leaf drying on volatile leaf oils have shown *Juniperus* terpenes to be quite stable (for up to 2-3 years at room temperature conditions). However, all of these studies utilized care in low temperature air drying of fresh leaves and mild storage conditions. Based on these studies, oils were obtained from herbarium specimens ranging in age from 3 to 12 yrs of storage. The Croton, Italy provides a good case study because the populations are very well identified by precise GPS coordinates and easy to re-locate and re-sample. Fresh material was collected and the oils from fresh and 12 year old herbarium leaves were analyzed. In contrast to previous studies of dried leaf oils, these oils were very different in composition (Table 2). Although one would expect lower concentration of the most volatile compounds in comparing fresh vs. 12 year old specimens, that was not the case for  $\alpha$ -pinene (17.7, 18.3%), but it was true for  $\beta$ -pinene (0.9, 0.4%), myrcene (5.3, 0.2%),  $\delta$ -2-carene (0.5, 0.0%),  $\alpha$ -phellandrene (3.3, 0.3%), and especially for  $\beta$ -phellandrene (24.6, 1.2%). But, some monoterpenes increased from fresh to 12 year old leaves: thuja-2,4-diene (trace, 0.7%),  $\delta$ -3-carene (0.0, 0.6%), and p-cymene (0.0, 1.1%).

Part of the changes in the oils may be due to oxidation to produce alcohols and ketones. Notice (Table 2) the differences in  $\alpha$ -campholenal (0.3, 1.8%), trans-pinocarveol (0.6, 5.4%), trans-verbenol (0.4, 5.4%), trans-pinocamphone (0.0, 0.7%), p-mentha-1,5-dien-8-ol (0.1, 1.7%), myrtenol (0.0, 2.0%), verbenone (0.0, 2.3%) and trans-carveol (0.1, 1.2%).

The large decrease in monoterpenes was correlated with an increase (Table 2) in some of the less volatile diterpenes: manoyl oxide (0.4, 11.9%), abietatriene (trace, 0.4%), trans-totarol (0.8, 2.2%) and trans-ferruginol (trace, 0.3%). However, some diterpenes decreased in the 12 year old specimens: abietadiene (0.2, trace), 4-epi-abietal (0.5%, trace). Several of the sesquiterpenes decreased and others increased between fresh and 12 year old leaves (Table 2)

It appears likely that both oxidation and free radical reactions have occurred in the 12 year old herbarium leaves. This same trend was seen in oils of *J. phoenicea* var. *phoenicea* between fresh and herbarium leaf oils (unpublished). So, in this study, it was not possible to utilize oils from herbarium specimens.

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Table 1. Composition of the leaf oils of *J. turbinata* (*J. phoenicea* var. *turbinata*): Canary Islands, Sinai, Morocco (940 m), Madeira, Turkey (sw coast), Sicily, Tarifa sand dunes, Crotone, Italy and *J. phoenicea* (var. *phoenicea*): phoen-El Pen (El Penon, Spain, low cedrol), phoen Graz (Grazalema, Spain, hi cedrol). Compounds that appear to distinguish taxa are in boldface. Cedarwood oil components are in italics.

KI	Compound	Canary Islands	Sinai	Morocco 940 m	Madeira	Turkey	Sicily	Tarifa sand	s Italy Crotone	phoen-El Pen	phoen-Graz
921	tricyclene	0.2	0.2	0.3	0.1	t	0.1	0.1	t	0.1	t
<b>932</b>	<b><math>\alpha</math>-pinene*</b>	<b>67.9</b>	<b>52.2</b>	<b>65.4</b>	<b>57.8</b>	<b>32.6</b>	<b>45.3</b>	<b>25.0</b>	<b>17.7</b>	<b>41.2</b>	<b>29.7</b>
945	$\alpha$ -fenchene	0.1	t	0.2	0.1	0.2	0.1	t	t	0.1	t
946	camphene	0.4	0.3	0.6	0.3	0.2	0.3	0.2	0.1	0.1	0.3
953	thuja-2,4-diene	0.1	0.1	0.5	0.2	t	-	-	t	0.1	t
961	verbenene	-	-	-	-	t	-	0.1	-	0.3	t
969	sabinene	0.4	0.1	0.2	0.2	t	t	t	t	0.1	t
<b>974</b>	<b><math>\beta</math>-pinene*</b>	<b>1.6</b>	<b>0.8</b>	<b>0.8</b>	<b>1.2</b>	<b>0.8</b>	<b>1.1</b>	<b>1.3</b>	<b>0.9</b>	<b>2.1</b>	<b>1.2</b>
<b>988</b>	<b>myrcene*</b>	<b>2.7</b>	<b>1.9</b>	<b>1.7</b>	<b>3.3</b>	<b>3.6</b>	<b>4.1</b>	<b>6.6</b>	<b>5.3</b>	<b>3.2</b>	<b>1.9</b>
1001	$\delta$ -2-carene	t	t	0.2	0.1	t	0.2	0.5	0.5	0.1	t
<b>1002</b>	<b><math>\alpha</math>-phellandrene*</b>	-	t	-	<b>1.1</b>	<b>1.2</b>	<b>1.6</b>	<b>4.4</b>	<b>3.3</b>	<b>0.7</b>	<b>0.2</b>
<b>1008</b>	<b><math>\delta</math>-3-carene*</b>	<b>0.3</b>	t	<b>2.3</b>	-	<b>4.6</b>	<b>2.0</b>	-	-	<b>1.5</b>	t
1014	$\alpha$ -terpinene	0.1	t	0.1	0.1	t	t	0.3	0.2	0.1	t
1020	p-cymene	0.1	0.3	0.6	0.5	1.1	0.9	1.3	-	0.4	0.2
1024	limonene	1.9	0.7	0.9	0.9	t	1.5	t	t	0.6	0.4
<b>1025</b>	<b><math>\beta</math>-phellandrene*</b>	<b>1.2</b>	<b>0.5</b>	<b>0.6</b>	<b>8.0</b>	<b>11.0</b>	<b>13.4</b>	<b>31.5</b>	<b>24.6</b>	<b>4.9</b>	<b>0.6</b>
1044	(E)- $\beta$ -ocimene	0.2	t	-	0.3	0.2	t	t	t	-	t
1054	$\gamma$ -terpinene*	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.2	0.2	0.9
1083	fenchone	t	-	1.0	t	-	-	-	-	-	-
1086	terpinolene*	0.6	0.5	-	1.0	1.1	0.7	1.8	0.9	0.7	0.4
1095	linalool*	0.4	0.3	0.3	1.0	0.7	0.5	0.1	0.2	1.0	0.2
1100	n-nonanal	0.1	-	-	t	-	-	-	-	-	-
1118	cis-p-menth-2-en-1-ol	-	-	-	-	t	0.2	0.6	0.6	0.2	-
1122	$\alpha$ -campholenal	0.1	0.4	0.5	0.7	0.7	0.2	0.1	0.3	0.2	t
<b>1135</b>	<b>trans-pinocarveol*</b>	<b>0.1</b>	<b>0.4</b>	<b>0.5</b>	<b>0.7</b>	<b>0.8</b>	<b>0.3</b>	<b>0.5</b>	<b>0.6</b>	<b>0.3</b>	t
1139	C <sub>10</sub> OH, 41,55,81,95,152	-	-	-	-	-	-	-	-	1.4	-
<b>1140</b>	<b>trans-verbenol*</b>	<b>0.1</b>	<b>0.4</b>	<b>0.6</b>	<b>0.1</b>	<b>0.5</b>	<b>0.3</b>	<b>0.1</b>	<b>0.4</b>	-	<b>0.2</b>
1141	camphor	-	0.5	1.3	-	-	-	-	-	-	-
1158	trans-pinocamphone	-	0.3	0.2	t	-	-	-	-	0.1	-
1160	pinocarvone	t	0.2	0.2	t	-	-	-	-	-	-
1165	borneol	-	-	-	-	-	-	-	-	0.6	-
1166	p-mentha-1,5-dien-8-ol*	0.1	0.2	0.3	0.4	0.5	0.2	0.1	0.1	-	-
1172	cis-pinocamphone	-	0.3	0.2	-	-	-	-	-	0.2	-
1174	terpinen-4-ol	0.2	0.2	0.3	t	0.2	t	0.2	0.1	0.1	t
1178	naphthalene	0.2	-	-	t	-	t	-	-	t	-
1179	p-cymen-8-ol	t	t	-	0.1	t	t	0.4	0.8	0.1	-
<b>1186</b>	<b><math>\alpha</math>-terpineol*</b>	<b>0.2</b>	<b>0.3</b>	-	<b>t</b>	<b>0.6</b>	<b>1.1</b>	<b>0.4</b>	<b>2.4</b>	<b>2.3</b>	t
1195	cis-piperitol	-	-	-	-	0.3	0.1	0.2	0.3	-	-
1195	myrtenol	-	0.3	t	0.1	-	t	-	-	0.1	t
1204	verbenone	0.2	0.2	0.3	0.3	-	t	-	-	0.2	-
1207	trans-piperitol	-	-	-	-	0.3	t	0.3	0.3	-	-
1215	trans-carveol	-	0.2	0.2	0.1	0.3	-	t	0.1	0.1	-
1223	citronellol*	t	0.3	1.4	0.1	1.0	0.2	0.6	0.9	0.5	-
1233	pulegone	-	0.2	-	-	-	-	-	-	0.1	-
1249	piperitone	-	-	-	-	1.0	0.2	0.3	1.9	0.2	-
1254	linalyl acetate	-	0.5	-	-	-	-	-	-	-	-
1255	(4Z)-decenol*	0.6	-	0.5	0.7	1.8	1.0	0.5	0.5	0.2	t
1257	methyl citronellate	-	0.2	-	-	-	-	-	-	-	-
1274	neo-isopulegyl acetate	t	0.3	0.1	0.2	1.2	-	0.8	0.5	-	-
1287	bornyl acetate	0.4	-	0.1	0.4	0.5	-	0.2	-	-	-
1287	trans-linalool oxide acetate (pyranoid)	-	t	-	-	-	-	0.2	-	-	-
1315	(E,E)-2,4-decadienal	0.2	t	t	-	t	0.7	0.3	-	0.3	-
<b>1346</b>	<b><math>\alpha</math>-terpinyl acetate*</b>	<b>0.2</b>	<b>0.2</b>	-	<b>5.0</b>	<b>8.1</b>	<b>4.1</b>	<b>13.1</b>	<b>14.6</b>	<b>0.1</b>	-
1346	$\alpha$ -cubebene	0.1	0.2	0.2	-	-	-	-	-	-	-
1374	$\alpha$ -copaene	-	0.5	0.1	-	-	-	-	0.2	-	-
1387	$\beta$ -bourbonene	-	0.3	0.1	-	-	-	-	-	-	-
1387	$\beta$ -cubebene	t	0.2	-	-	-	-	-	-	-	-



KI	Compound	Canary Islands	Sinai	Morocco 940 m	Madeira	Turkey	Sicily	Tarifa sand	s Italy Crotone	phoen-El Pen	phoen-Graz
1389	$\beta$ -elemene	-	0.2	-	-	-	-	-	-	0.1	
<b>1410</b>	<b><i><math>\alpha</math>-cedrene</i></b>	-	-	-	-	-	-	-	-	-	<b>1.0</b>
1417	(E)-caryophyllene*	0.6	1.8	0.8	0.9	0.8	1.0	0.1	1.5	1.2	1.3
<b>1429</b>	<b><i>cis-thujopsene</i></b>	<b>t</b>	-	<b>0.2</b>	<b>0.2</b>	-	-	-	-	-	<b>0.3</b>
1448	cis-muurolo-3,5-diene	0.5	0.8	0.3	t	-	-	-	-	-	
1452	$\alpha$ -humulene*	0.6	1.3	0.2	0.7	0.6	0.5	-	0.9	-	
1475	trans-cadina-1(6),4-diene	0.6	1.4	0.4	-	t	-	-	0.7	-	
1478	$\gamma$ -muurolene	-	0.4	0.5	0.1	t	-	-	0.2	-	
1484	germacrene D*	-	2.3	-	-	2.0	2.2	0.2	2.5	0.5	0.3
1493	trans-muurolo-4(14),5-diene	1.2	2.5	0.5	0.1	-	-	-	0.8	-	
1493	epi-cubebol*	0.6	1.2	0.4	0.2	0.5	-	-	0.6	-	
1495	$\gamma$ -amorphene	-	-	-	-	0.8	0.5	0.1	-	-	
1500	$\alpha$ -muurolene	0.3	0.7	0.3	0.2	0.4	0.2	0.1	0.4	-	
1505	$\beta$ -bisabolene	-	-	-	-	-	-	-	-	-	0.4
1509	C <sub>15</sub> OH,41,55,81,161,220	-	-	0.1	-	0.6	t	0.1	-	0.3	
1512	$\alpha$ -alaskene	-	-	-	-	-	-	-	-	-	0.4
1513	cubebol	1.2	3.2	0.4	0.3	-	-	-	-	-	
1513	$\gamma$ -cadinene*	1.6	t	-	0.5	0.9	0.3	0.1	1.2	0.1	t
1521	trans-calamenene	-	1.6	-	-	-	-	-	-	-	
1522	$\delta$ -cadinene	-	1.1	1.1	-	1.3	0.7	0.4	1.2	0.2	0.2
1528	zonarene	-	0.5	0.2	-	0.2	t	-	-	-	
1533	trans-cadina-1,4-diene	0.2	-	-	-	-	-	-	-	-	
<b>1535</b>	<b>C<sub>15</sub>OH,41,69,105,161,204</b>	-	-	-	-	-	t	<b>0.1</b>	-	<b>1.0</b>	
1544	$\alpha$ -calacorene	-	0.2	-	-	-	-	-	-	-	
<b>1548</b>	<b>elemol*</b>	<b>0.1</b>	<b>0.3</b>	<b>0.7</b>	<b>0.3</b>	<b>0.6</b>	<b>0.4</b>	<b>0.6</b>	<b>0.3</b>	<b>1.8</b>	<b>0.5</b>
1559	germacrene B	-	1.1	-	-	0.5	0.8	0.2	1.1	0.6	0.2
1561	(E)-nerolidol	-	t	0.9	-	-	-	-	-	t	
1574	germacrene-D-4-ol	0.2	-	0.1	0.5	0.3	0.2	0.2	0.1	0.2	
1582	caryophyllene oxide*	0.5	0.8	0.6	0.4	0.7	0.5	0.1	0.5	1.0	
<b>1589</b>	<b><i>allo-cedrol</i></b>										<b>1.1</b>
<b>1600</b>	<b><i>cedrol</i></b>	-	<b>0.3</b>	-	-	-	-	-	-	-	<b>16.4</b>
1608	humulene epoxide II	0.3	0.4	0.1	0.1	-	-	-	-	-	
1625	C <sub>15</sub> OH, 43,119,161,204,220	2.3	-	1.2	0.4	-	0.6	0.3	-	0.4	
<b>1627</b>	<b>1-epi-cubebol*</b>	-	<b>3.5</b>	-	-	<b>1.5</b>	-	-	<b>1.4</b>	-	
1630	$\gamma$ -eudesmol	-	t	-	-	t	t	0.1	t	0.2	t
1638	epi- $\alpha$ -cadinol	0.5	0.2	0.2	0.2	0.2	t	0.1	0.1	0.2	t
1638	epi- $\alpha$ -muurolol	0.5	0.2	0.2	0.3	0.2	t	0.2	0.1	0.1	t
1649	$\beta$ -eudesmol	-	t	0.2	0.2	0.2	0.3	0.2	0.1	0.4	
<b>1652</b>	<b><math>\alpha</math>-eudesmol*</b>	-	<b>0.2</b>	<b>0.2</b>	<b>0.9</b>	<b>0.4</b>	<b>0.4</b>	<b>0.2</b>	<b>0.4</b>	<b>0.3</b>	t
<b>1652</b>	<b><math>\alpha</math>-cadinol</b>	<b>1.0</b>	<b>0.2</b>	<b>0.2</b>	-	<b>0.4</b>	<b>0.3</b>	<b>0.3</b>	-	<b>0.3</b>	t
<b>1687</b>	<b>eudesma-4(15),7-dien-1-<math>\beta</math>-ol*</b>	<b>0.7</b>	<b>0.3</b>	<b>t</b>	<b>0.6</b>	<b>0.6</b>	<b>0.7</b>	-	<b>0.3</b>	<b>0.1</b>	
<b>1688</b>	<b><i>shyobunol*</i></b>	-	<b>0.6</b>	<b>0.5</b>	<b>1.0</b>	<b>1.0</b>	<b>1.0</b>	<b>0.8</b>	<b>0.6</b>	<b>1.5</b>	<b>0.5</b>
1715	(2Z,6E)-farnesol	-	-	0.1	-	-	-	-	-	1.2	
1968	sandaracopimara-8(14),15-diene	-	t	-	-	t	t	0.1	t	0.1	0.2
<b>1978</b>	<b>manoyl oxide*</b>	<b>1.1</b>	<b>2.6</b>	<b>2.6</b>	-	<b>0.9</b>	<b>1.2</b>	<b>0.4</b>	<b>0.4</b>	<b>22.0</b>	<b>32.9</b>
2009	epi-13-manoyl oxide	-	-	0.1	-	-	-	-	-	0.1	0.2
2055	abietatriene	0.1	t	-	0.3	0.3	0.4	t	t	0.1	0.4
2087	abietadiene	t	0.2	-	0.4	0.4	t	0.1	0.2	0.1	t
2298	4-epi-abietal	0.2	0.5	0.1	0.4	0.3	t	t	0.5	0.2	0.2
2314	trans-totarol*	0.4	0.2	0.1	2.1	1.0	t	0.2	0.8	0.2	1.9
2331	trans-ferruginol	-	t	-	0.2	0.2	t	t	t	-	0.3

KI = linear Kovats Index on DB-5 column. \*Used in numerical analyses. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of the oils from fresh leaves and 12 yr old herbarium leaves from Crotone, Italy. Compounds with large changes in concentration are in boldface.

KI	Compound	fresh leaves	12 yr old leaves
921	tricyclene	t	0.1
932	$\alpha$ -pinene	17.7	18.3
945	$\alpha$ -fenchene	t	0.1
946	camphene	0.1	0.2
<b>953</b>	<b>thuja-2,4-diene</b>	<b>t</b>	<b>0.7</b>
969	sabinene	t	0.3
<b>974</b>	<b><math>\beta</math>-pinene</b>	<b>0.9</b>	<b>0.4</b>
<b>988</b>	<b>myrcene</b>	<b>5.3</b>	<b>0.2</b>
<b>1001</b>	<b><math>\delta</math>-2-carene</b>	<b>0.5</b>	-
<b>1002</b>	<b><math>\alpha</math>-phellandrene</b>	<b>3.3</b>	<b>0.3</b>
<b>1008</b>	<b><math>\delta</math>-3-carene</b>	-	<b>0.6</b>
1014	$\alpha$ -terpinene	0.2	-
<b>1020</b>	<b>p-cymene</b>	-	<b>1.1</b>
1024	limonene	t	0.3
<b>1025</b>	<b><math>\beta</math>-phellandrene</b>	<b>24.6</b>	<b>1.2</b>
1044	(E)- $\beta$ -ocimene	t	-
1054	$\gamma$ -terpinene	0.2	t
1086	terpinolene	0.9	0.7
1095	linalool	0.2	0.3
<b>1118</b>	<b>cis-p-menth-2-en-1-ol</b>	<b>0.6</b>	<b>0.2</b>
<b>1122</b>	<b><math>\alpha</math>-campholenal</b>	<b>0.3</b>	<b>1.8</b>
<b>1135</b>	<b>trans-pinocarveol</b>	<b>0.6</b>	<b>5.4</b>
<b>1140</b>	<b>trans-verbenol</b>	<b>0.4</b>	<b>5.4</b>
<b>1158</b>	<b>trans-pinocamphone</b>	-	<b>0.7</b>
<b>1166</b>	<b>p-mentha-1,5-dien-8-ol</b>	<b>0.1</b>	<b>1.7</b>
1174	terpinen-4-ol	0.1	0.4
1178	naphthalene	-	0.4
1179	p-cymen-8-ol	0.8	1.1
<b>1186</b>	<b><math>\alpha</math>-terpineol</b>	<b>2.4</b>	<b>0.7</b>
1195	cis-piperitol	0.3	-
<b>1195</b>	<b>myrtenol</b>	-	<b>2.0</b>
<b>1204</b>	<b>verbenone</b>	-	<b>2.3</b>
1207	trans-piperitol	0.3	-
<b>1215</b>	<b>trans-carveol</b>	<b>0.1</b>	<b>1.2</b>
1223	citronellol	0.9	0.6
<b>1249</b>	<b>piperitone</b>	<b>1.9</b>	<b>0.6</b>
<b>1254</b>	<b>linalyl acetate</b>	-	<b>0.9</b>
<b>1255</b>	<b>(4Z)-decenol</b>	<b>0.5</b>	-
1274	neo-isopulegyl acetate	0.5	0.3
1287	trans-linalool oxide acetate(pyranoid)	-	0.1
1315	(E,E)-2,4-decadienal	-	0.1
<b>1346</b>	<b><math>\alpha</math>-terpinyl acetate</b>	<b>14.6</b>	<b>6.1</b>
1374	$\alpha$ -copaene	0.2	t
1387	$\beta$ -bourbonene	-	t

KI	Compound	fresh leaves	12 yr old leaves
1389	$\beta$ -elemene	-	0.2
1410	$\alpha$ -cedrene	-	t
<b>1417</b>	<b>(E)-caryophyllene</b>	<b>1.5</b>	<b>0.2</b>
1448	cis-muurolo-3,5-diene	-	0.2
<b>1452</b>	<b><math>\alpha</math>-humulene</b>	<b>0.9</b>	<b>0.1</b>
<b>1475</b>	<b>trans-cadina-1(6),4-diene</b>	<b>0.7</b>	<b>0.2</b>
<b>1478</b>	<b><math>\gamma</math>-muurolene</b>	<b>0.2</b>	<b>0.5</b>
<b>1484</b>	<b>germacrene D</b>	<b>2.5</b>	<b>t</b>
<b>1493</b>	<b>trans-muurolo-4(14),5-diene</b>	<b>0.8</b>	<b>0.4</b>
<b>1493</b>	<b>epi-cubebol</b>	<b>0.6</b>	-
1500	$\alpha$ -muurolene	0.4	0.4
<b>1513</b>	<b>cubebol</b>	-	<b>1.0</b>
<b>1513</b>	<b><math>\gamma</math>-cadinene</b>	<b>1.2</b>	-
<b>1521</b>	<b>trans-calamenene</b>	-	<b>2.0</b>
<b>1522</b>	<b><math>\delta</math>-cadinene</b>	<b>1.2</b>	-
1528	zonarene	-	0.1
1533	trans-cadina-1,4-diene	-	0.1
<b>1544</b>	<b><math>\alpha</math>-calacorene</b>	-	<b>0.6</b>
<b>1548</b>	<b>elemol</b>	<b>0.3</b>	<b>0.9</b>
<b>1559</b>	<b>germacrene B</b>	<b>1.1</b>	<b>t</b>
1574	germacrene-D-4-ol	0.1	-
<b>1582</b>	<b>caryophyllene oxide</b>	<b>0.5</b>	<b>4.7</b>
1600	cedrol	-	1.0
<b>1608</b>	<b>humulene epoxide II</b>	-	<b>2.8</b>
<b>1627</b>	<b>1-epi-cubenol</b>	<b>1.4</b>	<b>2.1</b>
<b>1630</b>	<b><math>\gamma</math>-eudesmol</b>	<b>t</b>	<b>0.6</b>
1638	epi- $\alpha$ -cadinol	0.1	t
1638	epi- $\alpha$ -muurolol	0.1	t
<b>1649</b>	<b><math>\beta</math>-eudesmol</b>	<b>0.1</b>	<b>0.7</b>
<b>1652</b>	<b><math>\alpha</math>-eudesmol</b>	<b>0.4</b>	<b>1.1</b>
<b>1687</b>	<b>eudesma-4(15),7-dien-1-<math>\beta</math>-ol</b>	<b>0.3</b>	<b>1.6</b>
<b>1688</b>	<b>shyobunol</b>	<b>0.6</b>	-
1968	sandaracopimara-8(14),15-diene	t	-
<b>1978</b>	<b>manoyl oxide</b>	<b>0.4</b>	<b>11.9</b>
<b>2055</b>	<b>abietatriene</b>	<b>t</b>	<b>0.4</b>
2087	abietadiene	0.2	t
<b>2298</b>	<b>4-epi-abietal</b>	<b>0.5</b>	<b>t</b>
<b>2314</b>	<b>trans-totarol</b>	<b>0.8</b>	<b>2.2</b>
2331	trans-ferruginol	t	0.3

KI = linear Kovats Index on DB-5 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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