

**Geographic variation in the volatile leaf oils *J. phoenicea* var. *phoenicea* from throughout its range****Robert P. Adams**

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**ABSTRACT**

The compositions of the volatile leaf oils from 5 populations from throughout the range of *Juniperus phoenicea* var. *phoenicea* were analyzed. Two chemotypes were found: normal leaf oils and leaf oils containing cedarwood oil components. Except for the chemotypes (hi cedrol), the leaf oils of *J. phoenicea* are high in  $\alpha$ -pinene (41.2 - 51.9%) and manoyl oxide (14.0 - 28.0%) with moderate amounts of  $\alpha$ -pinene, myrcene,  $\beta$ -phellandrene and (E)-caryophyllene. Little geographic variation was found in the major components from Narbonne to Andorra, Zaragoza thence to El Peñón. The oil from the high cedrol plants at Grazalema seems quite different due to the presence of cedarwood oil components, but it is actually not very different, if one removes the heartwood terpenoids and re-normalizes the remaining terpenoids. Trees with high cedarwood oil had 16.4 - 31.9% cedrol and moderate amounts of other cedarwood oil components (eg.,  $\alpha$ - &  $\beta$ -cedrene, 2-epi-funebrene, cis-thujopsene,  $\alpha$ - &  $\beta$ -alaskene, (E)- $\beta$ -bisabolene, liguloxide, allo-cedrol). Published on-line [www.phytologia.org](http://www.phytologia.org) *Phytologia* 96(2): 110-116 (April 1, 2014).

**KEY WORDS:** *Juniperus phoenicea* var. *phoenicea*, *Cupressaceae*, terpenes, leaf essential oil, geographic variation.

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Recently, Adams et al. (2013) analyzed nrDNA and petN sequences for *J. phoenicea* L. (*sensu stricto*) from throughout the Mediterranean region (Fig. 1). They found *J. phoenicea* var. (or subsp.) *phoenicea* was restricted to Spain and France, whereas *J. phoenicea* var. *turbinata* (Guss.) Parl. (*J. turbinata* Guss.) were widely distributed from the Canary Islands to the Sinai (Fig. 4).

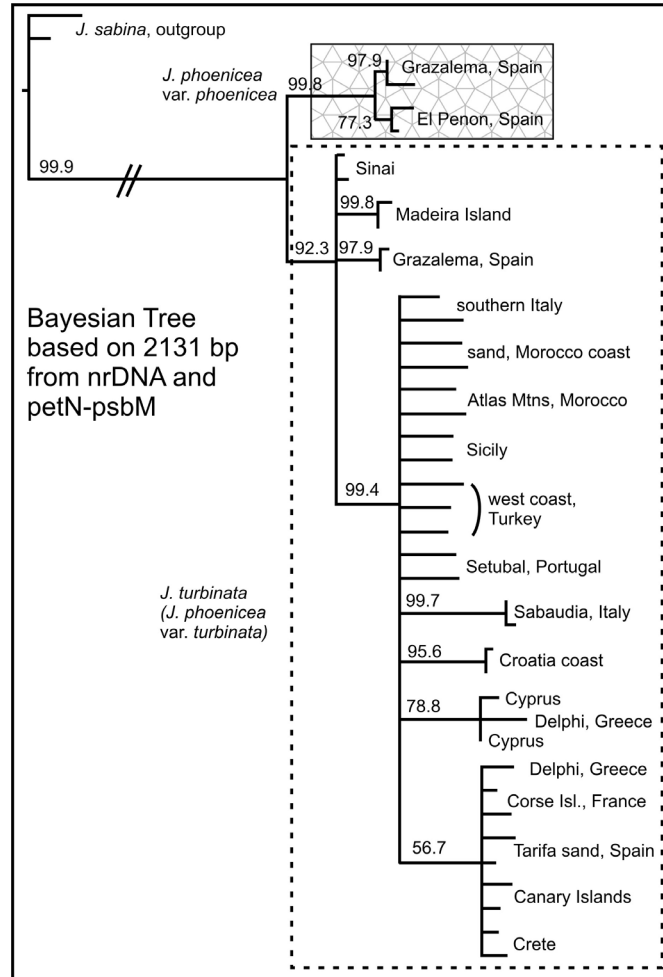
No differentiation was found between the typical Mediterranean and Canary Island populations, offering no support for the recognition of *J. phoenicea* subsp. *canariensis* (Guyot) Rivas-Martínez (Fig. 1). *Juniperus turbinata* appears to be widespread from Madeira - Canary Islands to the Sinai with few DNA differences among most populations. However, some populations (Grazalema, Madeira, Sinai, central Italy) displayed (Fig. 1) moderate amounts of divergence (3-4 mutations).

In a broad phylogenetic study of *Juniperus*, Adams and Schwarzbach (2013) found that *J. phoenicea* was 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*. This lends support for the recognition of *J. turbinata* Guss., as proposed by Lebreton and Pérez 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 with var. *turbinata* widespread throughout the Mediterranean region. Lebreton and Pérez de Paz (2001) found a clear

separation between *J. phoenicea* (Spain and France) and all other populations examined (*J. turbinata*).

Several studies have been made on the leaf terpenoids of *J. phoenicea*. San Feliciano and workers examined acidic diterpenes (San Feliciano et al., 1988; 1993). Incomplete analyses have been published on the volatile leaf oils of *J. phoenicea* from Egypt (Afifi et al., 1992), Saudi Arabia (Dawidar et al., 1991) and France (Tabacik and La Porte, 1971). See Adams, Barrero and Lara (1996) for a review of the early literature.

Adams, Barrero and Lara (1996) presented the first comprehensive analyses of the volatile leaf oils of *J. phoenicea*, *J. p.* subsp. *eu-mediterranea* and *J. p.* var. *turbinata*; they concluded that *J. p.* subsp. *eu-mediterranea* and var. *turbinata* were conspecific as their oils were nearly identical. More recently, Adams et al. (2009) presented complete analysis of the leaf oils of *J. phoenicea* (var. *turbinata*) from the Canary Islands and Madeira and compared these with oils from Morocco and Spain.



The purpose of the present study is to present a detailed analyses of the volatile leaf oils from populations of *J. phoenicea* var. *phoenicea* from throughout its ranges. Figure 1. Bayesian tree of *J. phoenicea* and *J. turbinata* (*J. p.* var. *turbinata*) from throughout the species ranges. (from Adams and Schwarzbach, 2013).

## MATERIALS AND METHODS

Figure 2 shows the distributions of *J. phoenicea* var. *phoenicea* and populations sampled in this study.

Specimens used in this study: ***J. phoenicea* var. *phoenicea*:**

France, Narbonne, near St. Pierre sur Mere, 43° 10' 0.2" N, 3° 09' 57.6" E, 23 m, *J. Altarejos 1-5*, Baylor specs. Adams 14123-14127.

Andorra, Coll de Jou near Sant Julià de Lòria, 42° 26' 56.8" N, 1° 28' 04.6" E, 1426 m, *J. Altarejos 6-10*, Baylor specs. Adams 14128-14132.

Spain, Zaragoza, Montes de la Retuerta de Pina W of Bujaraloz, 41° 28' 59"N, 0° 19' 31.2"W, 317 m, *J. Altarejos 11-15*, Baylor specs. Adams 14133-14137.

Spain, El Peñón, 37° 35' 38" N, 3° 31' 22" W, 760 m, Adams 7077-7079,

Spain, Cádiz, Sierra de Grazalema, 36° 47' 51.5" N, 5°24' 43.7"W, 835 m; *M. Arista 1-5*, Baylor specs. Adams 13813-13817.

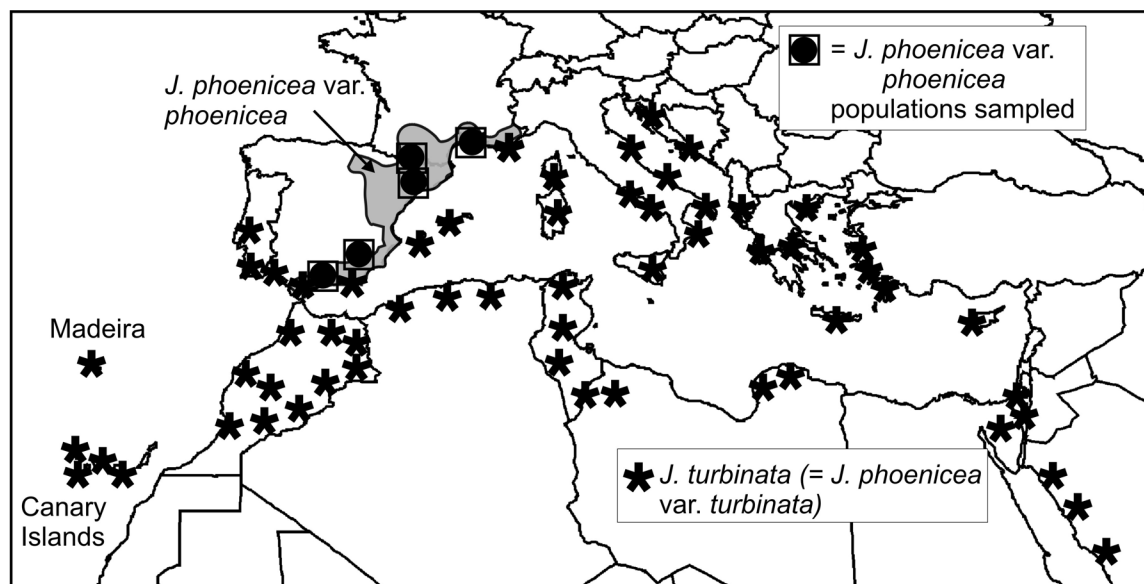


Figure 2. Distribution of *J. phoenicea* (adapted from Lebreton and Pérez de Paz, 2001, and Adams et al. 2010). Squares show the five populations of *J. phoenicea* sampled in the present terpene study.

Fresh, air dried leaves (50-100 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.

Oils from 4 - 5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 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 (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

## RESULTS AND DISCUSSION

The composition of the volatile leaf oils of four of the five populations varied very little except for a chemotype (one tree) in the Zaragoza population that was high in cedrol and other cedarwood terpenoids (Table 1). However, all five trees sampled in the Grazalema population had the cedarwood chemotype and were high in cedrol (Table 1). The activation of the cedarwood oil pathway (in the leaf glands) reduces the concentrations of the non-heartwood components, as the terpene pool is siphoned off to produce cedarwood components in the leaf oil. There appears to be a single gene ('cedarwood synthase') that is turned on in the heartwood (or some associated tissue) that activates the cedarwood oil pathway ( $\alpha$ - &  $\beta$ -cedrene, 2-epi-funebrene, cis-thujopsene,  $\alpha$ - &  $\beta$ -alaskene, (E)- $\beta$ -bisabolene, liguloxide, allo-cedrol, cedrol, widdrol, epi-cedrol, etc. (see Adams, 2014). Normally, this gene ('cedarwood synthase') is not active in *Juniperus* (and Cupressaceae) leaf oil glands. Most *Juniperus* species produce

two kinds of essential oils: leaf oils and heartwood oils and these oils have few components in common (Adams, 1991). *Juniperus phoenicea*, *J. excelsa*, *J. foetidissima*, *J. polycarpos*, and *J. seravschanica* have leaf oils that may contain significant amounts of the heartwood oil components (Adams and Hojjati, 2013). For example, Adams (1990) reported 4.4, 0.2, trace and 8.3% cedrol in the leaf oils from four trees of *J. foetidissima* from Greece. Whereas, Tunalier et al. (2004) reported 13.0 and 12.2% of cedrol and widdrol in the stem heartwood of *J. foetidissima* from Turkey. Ucar and Balaban (2002) analyzed the sapwood (white wood) of *J. excelsa*, Turkey, and reported the oil to contain 22.5% widdrol and 9.0% cedrol (these components are difficult to separate on non-polar columns and the mass spectra are nearly identical, so their identification is often problematic).

When *Juniperus* species contain heartwood components in the leaf oils, it is common to find chemical polymorphisms in cedrol (and associated heartwood terpenes) between trees. That is the case for trees from Zaragoza. Four trees had only the typical leaf oil components (Table 1) and their oil is very similar to nearby populations at Andorra and Narbonne, France (Table 1). However, one of 5 trees in the Zaragoza population had 31.9% cedrol and related compounds (Table 1) and thus, only 33.4%  $\alpha$ -pinene. The oil of this tree, is quite similar to the hi cedrol Grazalema population that has 16.4% cedrol and 29.7%  $\alpha$ -pinene (Table 1). It is interesting to compare cedrol + manoyl oxide for hi cedrol Zaragoza (31.9+13.3 = 45.2) vs. hi cedrol Grazalema (16.4+32.9 = 49.3%).

Except for the cedarwood oil chemotypes (hi cedrol), the leaf oils of *J. phoenicea* are high in  $\alpha$ -pinene and manoyl oxide with moderate amounts of  $\alpha$ -pinene, myrcene,  $\beta$ -phellandrene and (E)-caryophyllene. Little geographic variation was found in the major components from Narbonne, Andorra, Zaragoza thence to El Peñón. The oil from the high cedrol plants at Grazalema seems quite different due to the presence of cedarwood oil components, but the oil is actually not very different, if one removes the heartwood terpenoids and re-normalizes the remaining terpenoids (Table 1).

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Table 1. Composition of the leaf oils of *J. phoenicea* (var. *phoenicea*): Narbonne, France; Andorra; El Peñón, Spain; Zaragoza (lo and hi cedrol), Spain; and hi cedrol, Grazalema, Spain. Those compounds that appear to distinguish taxa are in boldface. Cedarwood oil components are in italics. Values in parenthesis ( ) for larger components of hi cedrol Zaragoza and hi cedrol Grazalema columns are corrected values, computed by correcting for cedarwood oil components.

AI	Compound	lo cedrol France	lo cedrol Andorra	lo cedrol El Peñón	lo cedrol Zaragoza	hi cedrol Zaragoza	hi cedrol Grazalema
921	tricyclene	0.1	0.1	0.1	0.1	0.1	t
<b>932</b>	<b><i>α</i>-pinene</b>	<b>42.4</b>	<b>42.9</b>	<b>41.2</b>	<b>51.9</b>	<b>33.4(45.7)</b>	<b>29.7(35.4)</b>
945	<i>α</i> -fenchene	0.2	0.1	0.1	0.1	0.1	t
946	camphene	0.3	0.3	0.1	0.4	0.3	0.3
953	thuja-2,4-diene	t	0.1	0.1	0.1	0.1	t
961	verbenene	0.1	0.1	0.3	0.1	0.1	t
969	sabinene	t	t	0.1	t	t	t
974	<i>β</i> -pinene	1.7	1.7	2.1	2.0	1.2(1.6)	1.2(1.4)
988	myrcene	2.7	2.6	3.2	2.8	1.9(2.6)	2.3(2.7)
1001	<i>δ</i> -2-carene	t	t	0.1	t	t	t
1002	<i>α</i> -phellandrene	0.3	0.2	0.7	0.2	0.2	t
1008	<i>δ</i> -3-carene	2.0	1.3	1.5	t	t	t
1014	<i>α</i> -terpinene	t	t	0.1	t	t	t
1020	p-cymene	0.3	0.3	0.4	0.3	0.2	0.6
1024	limonene	0.6	0.6	0.6	0.5	0.4	0.4
<b>1025</b>	<b><i>β</i>-phellandrene</b>	<b>2.0</b>	<b>1.9</b>	<b>4.9</b>	<b>1.8</b>	<b>1.1(1.5)</b>	<b>0.6(0.7)</b>
1054	<i>γ</i> -terpinene	0.2	0.2	0.2	0.2	0.2	0.9
1069	cis-linalool oxide	0.2	0.2	t	0.1	t	t
1086	terpinolene	0.7	0.6	0.7	0.6	0.4	0.4
1095	linalool	0.7	0.3	1.0	0.6	0.5	0.2
1118	cis-p-menth-2-en-1-ol	t	t	0.2	t	t	-
1122	<i>α</i> -campholenal	0.1	0.2	0.2	0.2	0.1	t
1135	trans-pinocarveol	0.1	0.2	0.3	0.4	0.2	t
1139	C <sub>10</sub> OH, 41,55,81,95,152	-	-	1.4	-	-	-
1140	trans-verbenol	-	-	-	-	-	0.2
1141	camphor	0.4	0.5	-	0.5	0.3	-
1144	neo-isopulegol	0.3	0.4	t	0.4	0.3	t
1158	trans-pinocamphone	t	t	0.1	t	t	-
1165	borneol	0.2	0.3	0.6	0.3	0.1	-
1172	cis-pinocamphone	0.1	0.1	0.2	0.2	0.1	-
1174	terpinen-4-ol	t	t	0.1	t	t	t
1178	naphthalene	0.3	0.1	t	t	t	-
1179	p-cymen-8-ol	t	t	0.1	t	t	-
<b>1186</b>	<b><i>α</i>-terpineol</b>	<b>0.8</b>	<b>0.5</b>	<b>2.3</b>	<b>0.5</b>	<b>0.4</b>	<b>t</b>
1195	myrtenal/ myrtenol	t	0.1	0.1	0.2	0.1	-
1204	verbenone	t	0.2	0.2	0.2	0.1	-
1215	trans-carveol	0.1	0.1	0.1	0.2	t	-
1223	citronellol	0.2	0.2	0.5	0.4	0.1	-
1249	piperitone	t	t	0.2	t	t	-
1255	(4Z)-decenol	0.1	0.1	0.2	0.1	t	t
1315	(E,E)-2,4-decadienal	t	0.5	0.3	0.2	t	-
1335	<i>δ</i> -elemene	0.1	0.1	t	0.2	0.1	t
1387	<i>β</i> -bourbonene	0.1	t	-	0.1	t	-
1389	<i>β</i> -elemene	0.4	0.1	0.1	0.3	0.1	-
1400	<i>β</i> -longipinene	t	0.4	t	0.4	0.1	t
<i>1410</i>	<i>α</i> -cedrene	-	-	-	-	<i>0.9</i>	<i>1.0</i>

AI	Compound	lo cedrol France	lo cedrol Andorra	lo cedrol El Peñón	lo cedrol Zaragoza	hi cedrol Zaragoza	hi cedrol Grazalema
1411	2- <i>epi-funebrene</i>	-	-	-	<i>t</i>	0.9	-
<b>1417</b>	<b>(E)-caryophyllene</b>	<b>2.9</b>	<b>2.6</b>	<b>1.2</b>	<b>2.7</b>	-	<b>1.3(1.5)</b>
1429	<i>cis-thujopsene</i>	-	-	-	-	0.4	0.3
1434	$\gamma$ -elemene	0.1	<i>t</i>	<i>t</i>	0.1	-	-
1452	$\alpha$ -humulene	0.2	0.1	-	0.2	-	-
1454	(E)- $\beta$ -farnesene	-	-	-	-	0.3	-
1478	$\gamma$ -muurolene	<i>t</i>	<i>t</i>	-	0.1	0.6	-
1484	germacrene D	2.1	1.1	0.5	1.7	<i>t</i>	0.3
1484	allo-aromadendr-9-ene	<i>t</i>	<i>t</i>	-	0.1	-	-
1498	$\beta$ -alaskene	-	-	-	-	0.1	-
1500	$\beta$ -himachalene	-	-	-	-	0.1	-
1505	$\beta$ -bisabolene	-	-	-	-	-	0.4
1509	C <sub>15</sub> OH, <u>41,55,81,161,220</u>	-	-	0.3	-	-	-
1512	$\alpha$ -alaskene	-	-	-	-	0.6	0.4
1513	$\gamma$ -cadinene	0.1	0.1	0.1	0.1	<i>t</i>	<i>t</i>
1521	$\beta$ -sesquiphellandrene	-	-	-	-	0.3	-
1522	$\delta$ -cadinene	0.3	0.2	0.2	0.3	<i>t</i>	0.2
1529	(E)- $\gamma$ -bisabolene	-	-	-	-	0.2	-
1534	liguloxide	-	-	-	-	0.2	-
1535	C <sub>15</sub> OH, <u>41,69,105,161,204</u>	-	-	1.0	-	-	-
1541	C <sub>15</sub> OH, <u>43,95,207,222</u>	0.7	1.1	-	0.7	-	-
1548	elemol	0.5	0.9	1.8	1.8	0.5	0.5
1559	germacrene B	1.5	1.1	0.6	1.9	0.9	0.2
1561	(E)-nerolidol	0.1	0.1	<i>t</i>	0.1	<i>t</i>	-
1574	germacrene-D-4-ol	0.1	0.1	0.2	0.1	<i>t</i>	-
1582	caryophyllene oxide	0.7	1.1	1.0	1.2	0.5	-
1589	allo-cedrol	-	-	-	-	1.4	1.1
<b>1600</b>	<b>cedrol</b>	<b>0.2</b>	<b>0.1</b>	-	<b>0.7</b>	<b>31.9</b>	<b>16.4</b>
1625	C <sub>15</sub> OH, <u>43,119,161,220</u>	0.3	0.5	0.4	0.6	<i>t</i>	-
1630	$\gamma$ -eudesmol	<i>t</i>	<i>t</i>	0.2	<i>t</i>	<i>t</i>	<i>t</i>
1632	$\alpha$ -acoreneol	-	-	-	-	0.4	-
1638	epi- $\alpha$ -cadinol	<i>t</i>	<i>t</i>	0.2	<i>t</i>	<i>t</i>	<i>t</i>
1638	epi- $\alpha$ -muurolol	<i>t</i>	<i>t</i>	0.1	<i>t</i>	<i>t</i>	<i>t</i>
1649	$\beta$ -eudesmol	0.2	0.4	0.4	0.6	0.2	-
1652	$\alpha$ -eudesmol	0.4	0.6	0.3	0.7	0.3	<i>t</i>
1652	$\alpha$ -cadinol	-	-	0.3	-	-	<i>t</i>
1687	eudesma-4(15),7-dien-1- $\beta$ -ol	-	-	0.1	-	-	-
1688	shyobunol	0.8	1.4	1.5	1.1	0.5	0.5
1715	(2Z,6E)-farnesol	<i>t</i>	<i>t</i>	1.2	0.2	<i>t</i>	-
1968	sandaracopimara-8(14), 15-diene	<i>t</i>	<i>t</i>	0.1	<i>t</i>	<i>t</i>	0.2
<b>1978</b>	<b>manoyl oxide</b>	<b>28.0</b>	<b>25.4</b>	<b>22.0</b>	<b>14.0</b>	<b>13.3(18.2)</b>	<b>32.9(39.2)</b>
2009	epi-13-manoyl oxide	<i>t</i>	<i>t</i>	0.1	<i>t</i>	<i>t</i>	0.2
2055	abietatriene	0.3	0.3	0.1	0.2	0.2	0.4
2087	abietadiene	0.1	0.2	0.1	0.1	<i>t</i>	<i>t</i>
2298	4-epi-abietal	0.2	0.4	0.2	0.4	0.2	0.2
2314	trans-totarol	0.9	1.2	0.2	0.4	0.5	1.9
2331	trans-ferruginol	0.1	0.1	<i>t</i>	<i>t</i>	<i>t</i>	0.3
	total % cedarwood cpds.	0.2	0.1	0.0	0.7	36.7	19.2

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.