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Robert P. Adams
Baylor University

Adam Boratynski

Magda Dou Dagher-Kharrat

Hagar V. Leschner
Hebrew University

Tuğrul Mataraci

See next page for additional authors

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Geographic variation in *Juniperus drupacea*: DNA sequencing and volatile leaf oils: Further evidence of putative Pleistocene genetic isolation between Europe and Asia

Robert P. Adams
Biology Department, Baylor University - Utah Lab, 2240 E. Cobalt Dr., Suite 25, St. George, UT. 84790, USA, robert_adams@baylor.edu

Adam Boratyński
Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035, Kórnik, Poland

Magda Dou Dagher-Kharrat
Laboratoire Caractérisation Genomique des Plantes, Université Saint-Joseph, Campus Sciences et Technologies, Mar Roukos, Mkalles, BP 1514, Riad el Solh, Beirut 1107 2050, Lebanon

Hagar V. Leschner
Collection Manager, The Herbarium (HUJ), The National Natural History Collections, The Hebrew University, Edmond J. Safra Campus, Giv'at Ram, Jerusalem 91904 Israel

Tuğrul Mataraci
Eskidji Müz. AŞ., Sanayi Cad. Vadi Sokak No:2, Tarabya, İstanbul, Turkey

and

Andrea E. Schwarzbach
Department of Health and Biomedical Sciences, University of Texas - Rio Grande Valley, Brownsville, TX 78520, USA.

ABSTRACT

Recently, Sobierajska et al. (2016), using nSSR and morphology, showed that *Juniperus drupacea* exhibited differentiation between Greece and Turkey/ Lebanon, suggestive of Pleistocene genetic isolation. Here, we report that leaf terpenoids and DNA sequence data support their hypothesis by confirming differentiation between Greece and Turkey/ Lebanon/ Israel. The leaf oils of the Turkey/ Lebanon plants contained one unique terpene (trans-verbenol, 0.1-1.4%) that was absent in the Greece plants. The Greece oil contained three terpenes not found in the Lebanon/ Turkey plants: (ar)-curcumene (2.2%), β-alaskene (0.3%) and α-alaskene (0.4%). Four other terpenes were in higher concentration in the Greece oils: camphene (0.4%), δ-3-carene (10.9%), p-mentha-1,5-dien-8-ol, isomer (0.3%) and 4-terpineol (0.3%). Three terpenes were higher in Turkey and Lebanon oils: α-pinene (10.5 - 32.9%), hexadecanoic acid (0.4 - 1.4%) and trans-totarol (0.3 - 1.2%). Only one SNP was found (in nrDNA) that separated Greece from Turkey-Lebanon-Israel. No informative SNPs were found in petN-psbM, trnS-trnG, trnD-trnT or trnL-trnF cp regions. Published on-line www.phytologia.org *Phytologia 99(2): 249-257 (Dec. 18, 2017).* ISSN 030319430.

KEY WORDS: *Juniperus drupacea,* Cupressaceae, geographic variation, terpenoids, nrDNA, cp DNA.

Recently, Sobierajska et al. (2016) reported on variation in nSSR and morphology for *Juniperus drupacea* Labill. from Greece, Turkey and Lebanon. They found morphology clearly separated Greece plants from those in Turkey and Lebanon (Fig. 1). In addition, they found some differentiation between plants from the Taurus (Turkey) and Lebanon mountains (Fig. 1).

Analysis using nSSR data gave a similar pattern, with some differentiation within Greece, and within Turkey populations.

Sobierajska et al. (2016) discovered a lower level of diversity in nSSR data in the Greece populations versus more diversity in Turkey and Lebanon populations. They reasoned that genetic isolation during the Pleistocene had led to the differentiation between the European and Asia populations.

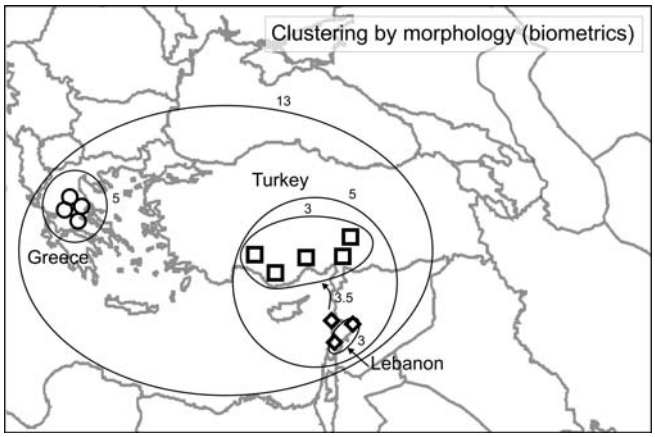

Figure 1. Clustering of *J. drupacea* populations by morphological data (adapted from Sobierajska et al. (2016)).

Juniperus drupacea is ancestral in the phylogeny of *Juniperus* (Adams, 2014, Adams and Schwarzbach, 2013). Adams (1997) reported the composition of the volatile leaf oil of *J. drupacea* was low in α-pinene in Greece (3.5-5.1%) but high in Turkey (14.3%) and the Crimea (cultivated) (22.1%) and high in limonene (+β-phellandrene) in Greece (46.7 - 48.4 %), Turkey (55.6%) and Crimea (50.3%). *Juniperus drupacea* oil was high, but variable in δ-3-carene (7.0 - 22.3%). The analysis by Adams (1997) based on a few samples from Greece and literature reports from Turkey and Crimea.

The purpose of the present study was to present a more complete report on the composition of the volatile leaf oil of *J. drupacea* from Greece, Turkey and Lebanon, utilizing additional samples, all of which were analyzed in the same lab. In addition, nrDNA and cp DNA sequencing was performed to determine if the differentiation reported by Sobierajska et al. (2016) is discernable in DNA sequencing (and in leaf volatile oils data).

METHODS

Specimens for DNA and volatile leaf oils were collected of *J. drupacea* from:
Greece:

2-3m tall, strong central axis, common with *Quercus coccifera.* Female cones falling off. Pollination in Sept? On highway, 18 km E of Tripolis at Achladokampos Pass, 700m, Greece, Prov. Arkadia. 7 Aug. 1987, Coll: *Robert P. Adams 5651,5652,*

small trees to 3 m, female common in the Achladokampos Pass but all trees young. 18 km E. of Tripolis. Prov. Arkadia. Lat. 37° 31.44'N, Long. 22° 37.92'E., 560m, Greece, 29 Aug. 1999, Coll: *Robert P. Adams 8795*,

large trees to 10m x 0.5 m dbh, male, abundant, 7.3 km E of Kosmos. Parnonas Mts., Mt. Parnonas Ski lift, 37° 08.00'N, 22° 45.63'E, 870m, Greece, 29 Aug. 1999, Coll: *Robert P. Adams 8796*,

pyramidal trees to 3-4m tall, strong central axis, common with *Quercus coccifera*. female cones falling off. Pollination in Sept? some trees monecious. On highway 7, 18km E of Tripoli at Achladokampos Pass, Country: Greece, Prov. Arkadia, 37° 31' 25" N, 22° 38' 19" E., 600m, 21 May 2017, Coll. *Robert P. Adams 15227-15236.*

Israel:

Mt Hermon, 33.31° N, 35.77° E, 1700m, June 2015, Israel, Coll. *Hagar V. Leschner sn*, Lab Acc. *Robert P. Adams 14580, 14581*

Lebanon:

Ehmej, on the road up the mountain. 34° 07' 58" N, 35° 49' 04" E, 1350 m, 22 Sept 2015, Lebanon, Coll. *Madga Bou Dagher-Kharrat, Ehmej 1-5* (=LEB-2), Lab Acc: *Robert P. Adams 14675-14679;*
Mrebbine, Sir Danniyeh, 34° 24' 29" N, 36° 05' 13" E, 1400 m, 20 Sept 2015, Lebanon, Coll. Madga Bou Dagher-Kharrat, Mrebbine 1-5 (=LEB-3, ~=Dufram), Lab Acc: *Robert P. Adams 14680-14684.*
Mchati Keserwan, 34° 02' 02" N, 35° 45' 53" E, 1180 m, 22 Sept 2015, Lebanon, Coll. *Madga Bou Dagher-Kharrat, Mchati Keserwan 1-5* (=LEB-1), Lab Acc: *Robert P. Adams 14685-14689*

Turkey:

Camliyayla (Namrun), above Kole village, vicinity of Hotel Dag, 37° 10' 6.41" N, 34° 36' 3.8" W., 1250 m, Jan. 2017; Turkey, Coll. *S. Tugrul Koruklu sn,* Lab Acc. *Robert P. Adams 15220*;
Alanya, Derekoy, Gedevet Pasture, Park Orman. 36° 39' N, 32° 10' E, elev. ca 1200 m, 19 Sept 2015, Prov. Antalya: Turkey, Coll. *Tugrul Mataraci & Sezai Vzun 2015-31,* Lab Acc: *Robert P. Adams 14693*;
Gedevet Oastyrem Derince, on road to Sapacictlik, in the river bed. 36° 38' 23.27" N, 32° 03' 38.44" E., 1036 m, 13 Sep. 2017, Prov. Antalya, Turkey, Coll. *T. Mataraci, O. Demir & B. Cingay 8332,* Lab Acc. *Robert P. Adams 15307*
Gedevet Oastyrem Derince, on road between Antalya and Askeki county,30 km, 37° 03' 32.85" N, 31° 44' 47.34" E, 1132m, 12 Sep. 2017, Prov. Antalya, Turkey, Coll. *T. Mataraci, O. Demir & B. Cingay BCNG 8328A,* Lab Acc. *Robert P. Adams ,15308*

Voucher specimens are deposited in the herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters, available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

Fresh 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 -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

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 the Adams 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.

RESULTS

The composition of the leaf essential oils from Greece (avg. 10 plants), Turkey (3 plants), and three locations in Lebanon: Leb-Mb (avg. 5 plants), Leb-Mc (avg. 5 plants), and Leb-Eh (avg. 5 plants) are given in Table 1. The leaf oils of the Turkey/ Lebanon plants contained one unique terpene (trans-verbenol, 0.1-1.4%) that was absent in the Greece plants (Table 1). The Greece oil contained three terpenes not found in the Lebanon/ Turkey plants: (ar)-curcumene (2.2%), β-alaskene (0.3%) and α-alaskene (0.4%) (Table 1). Four other terpenes were in higher concentration in the Greece oils: camphene (0.4%), δ-3-carene (10.9%), p-mentha-1,5-dien-8-ol, isomer (0.3%) and 4-terpineol (0.3%). Three terpenes were higher (Table 1) in Turkey and Lebanon oils: α-pinene (10.5 - 32.9%), hexadecanoic acid (0.4 - 1.4%) and trans-totarol (0.3 - 1.2%).

Factoring the similarity matrix (based on quantitative comparisons of sixteen major compounds), resulting in three eigenroots that accounted for 84% of the variance among OTUs. The eigenroots appeared to asymptote after the third eigenroot. Principle Coordinate Ordination (PCO) analysis of the similarity matrix revealed the major trend (coordinate 1, 40%, Fig. 2) was the differentiation of the Greece oil from the oils of Turkey and Lebanon (Fig. 2).

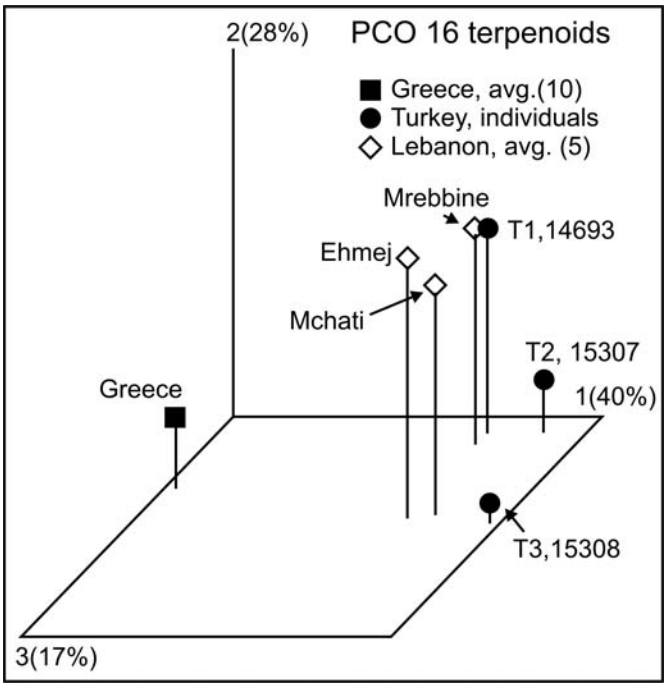

Figure 2. PCO of Greece population (avg. 10), three Lebanon populations (5 each, Ehmej, Mchati, Mrebbine) and three Turkey plants (T1, T2, T3).

The second coordinate (28%) separated Lebanon (Ehmej, Mchati, Mrebbine) and T1 (Turkey, 14693) from two other Turkey individuals (T2, T3, Fig. 2).

The third coordinate (17%) separated T2 from T3 and (Eh, Mc) from (Mb, T1, Fig. 2).

Plotting contoured similarity clustering on a geographic map gives one the spatial aspect of the variation (Fig. 3). Notice that all the Turkey-Lebanon oils cluster at a 0.75 similarity (Fig. 3), whereas the Greece oil is only admitted into the final cluster at a similarity of 0.52. This shows the magnitude (considerable) of differentiation between Greece and Turkey-Lebanon oils. In general, the clustering is similar to that of the morphology (cf. Fig. 1 vs. Fig. 3).

It is also interesting that two of the Lebanon populations (Eh, Mc) cluster at 0.93, as shown by their nearly identical percent concentrations of components in Table 1. One Lebanon population (Mb) clusters with a Turkey plant (T1) at 0.92 (also notice the similarity in their oils in Table 1).

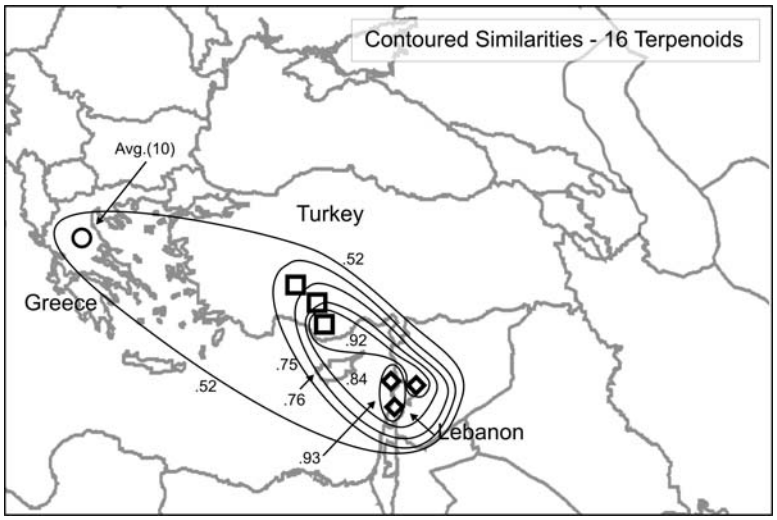

Figure 3. Contoured clustering based on 16 major terpenoids. See text for discussion.

It is informative to compare the morphological clustering (Sobierajska et al. 2016) versus clustering using 16 terpenoids (Fig. 4). Both morphology and terpenoids show major differentiation between Greece (Europe) and Asia plants. Both data show an individual from Lebanon clustering with a plant from Turkey (Morphology: Leb-2, TAT-1; Terpenoids: T1, LEB3-Mrebbine, Fig. 4).

Among the Turkey plants, morphology displays less diversity than is seen in terpenoids (Fig. 4). Otherwise, the clustering is very similar between the morphology and terpenoids data sets.

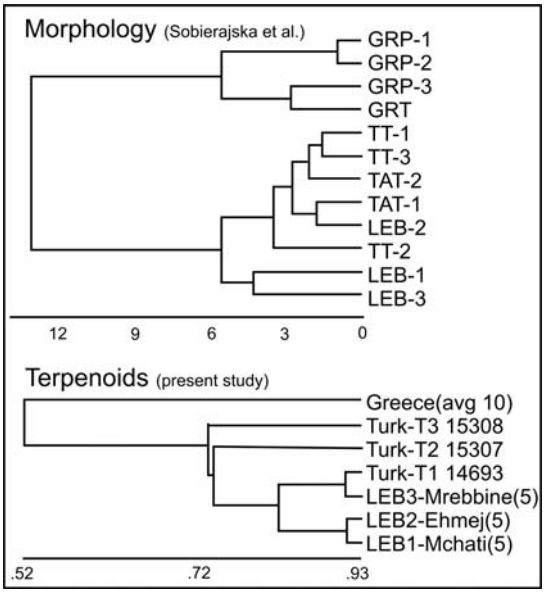

Figure 4. Comparison of clustering based on morphology (Sobierajska et al. 2016) and terpenoids (this study).

In addition to the analysis of terpenoids, we also sequenced nrDNA (ITS) and four cp regions: petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF. These gene regions produced 3,423 bp of data. Only one SNP was found (in nrDNA) that separated Greece from Turkey-Lebanon-Israel. No informative SNPs were found in petN-psbM, trnS-trnG, trnD-trnT or trnL-trnF cp regions.

A Bayesian tree shows (Fig. 5) *J. drupacea* (monotypic in sect. Caryocedrus) well resolved from *Juniperus* sect *Juniperus* as previously shown (Adams, 2014, Adams and Schwarzbach, 2013). All the Greece plants group together and that clade is separated by one SNP in the nrDNA region. One SNP (in nrDNA) resolved the clade containing: Israel, 14580; Mc Leb, 14685 and Mc Leb, 14686. Eh Leb, 14686 contained a unique SNP, but otherwise there was no variation found. Although the divergence of the Greece plants was based on only one SNP, it was consistent among the seven plants and supports the morphology, nSSR, and terpenoids in showing differentiation between Greece (Europe) and Asia plants.

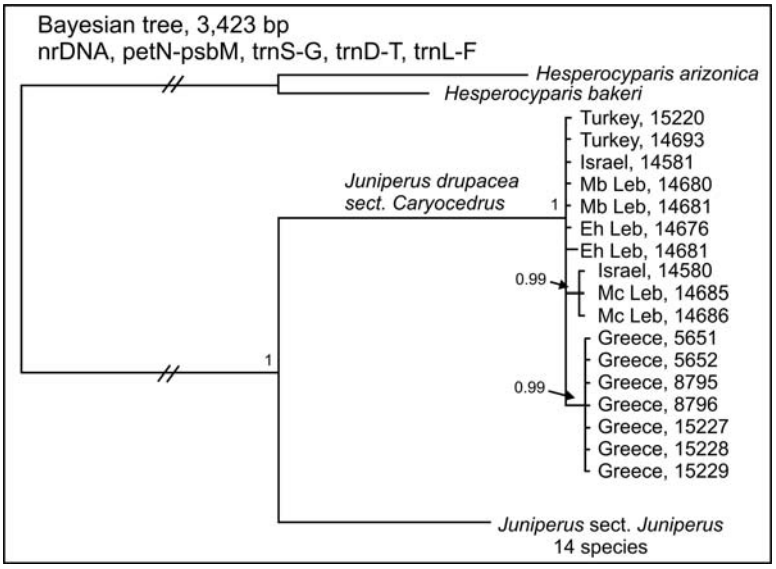

Figure 5. Bayesian tree based on 3,423 bp, nrDNA, petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF. Numbers at the branch points are posterior probabilities.

This study reconfirms the work of Sobierajska et al. (2016) that differentiation has occurred between Europe (Greece) and Asia (Turkey and Lebanon) in *J. drupacea*. Although the differences do not support the recognition of a new variety or subspecies, they present significant evidence that supports the thesis that the Aegean Sea presented a significant barrier during the Pleistocene to gene flow between Europe and Asia populations of *J. drupacea* and has contributed to the differentiation documented in this study and that of Sobierajska et al. (2016).

A hypothetical ancestor of the species, *Juniperus drupacea-pliocenica* Rer., probably diverged prior to 30–35 Mya (Mao et al., 2010) and evolved on the plate of the European continent (Palamarev, 1989; Kvaček, 2002; Palamarev, Bozukov, Uzunova, Petkova, Kitanov 2005). The colonization of terrains of the contemporary Peloponnese, Anatolia and the north-western part of the Arabian plateau might have occurred during Oligocene and the early Miocene (Burdigalian, 20.44–15.97 Mya), when a solid land connection supporting plant migrations was formed between these regions (Rögl, 1999; Meulenkamp & Sissingh, 2003; Popov et al., 2006). Subsequently, the separation of the Peloponnese microplate from Anatolia (Tortonian 11.60–27.24 Mya) created the Aegean Sea. This presented a barrier, triggering their differentiation, afterward magnified during Messinian salinity crisis. The geographic range of *J. drupacea* was reduced during Pliocene climate cooling and drying (Thompson, 2005). These climate changes probably caused altitudinal migration, resulting in spatial isolation between populations inhabiting particular mountain massifs and, consequently, in subsequent genetic divergence (Sobierajska et al. 2016). The very dynamic climate fluctuations during Pleistocene, with cold (glacial) and hot (interglacial) periods, additionally modified by humid and/or dry phases (Roberts et al. 2011; Dean et al. 2015) influenced geographic distribution of organisms, including *J. drupacea*. These factors seem likely responsible for the current geographic range and at least partly for genetic, biochemical and morphological differentiation in populations of *J. drupacea*.

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for *Juniperus drupacea* from Greece, Turkey and Lebanon.

921	Tricyclene	t	t	t	t	t	t	t
924	α-thujene	t	t	t	t	t	t	t
932	**α-pinene**	**5.0**	**10.5**	**16.6**	**12.5**	**12.3**	**32.9**	**32.8**
945	α-fenchene	t	t	t	0.1	t	t	t
946	**Camphene**	**0.4**	t	t	**0.1**	**t**	**0.2**	**0.2**
969	Sabinene	0.2	t	t	0.2	0.2	0.2	0.2
974	β-pinene	0.3	0.9	0.7	1.0	0.8	1.8	1.5
988	Myrcene	2.3	3.2	2.7	3.1	2.6	3.2	3.0
1002	α-phellandrene	t	0.2	t	0.1	t	t	t
1008	**δ-3-carene**	**10.9**	**-**	**1.2**	**0.7**	**0.4**	**t**	**t**
1014	α-terpinene	t	t	t	t	t	t	t
1020	p-cymene	0.2	t	0.5	0.1	t	t	t
1024	**Limonene**	31.3	26.2	17.4	34.6	32.0	21.5	21.3
1025	**β-phellandrene**	28.1	26.2	17.4	34.7	31.2	21.5	21.3
1054	γ-terpinene	0.1	t	t	0.1	t	t	t
1086	Terpinolene	1.1	0.3	0.6	0.4	0.4	0.7	0.5
1099	Linalool	t	0.2	0.8	0.1	0.1	0.2	0.2
1100	n-nonanone	-	t	t	t	t	t	t
1118	cis-p-mentha-2-en-1-ol	t	t	t	t	t	-	-
1123	α-camphenal	t	0.1	0.5	-	0.2	0.4	0.5
1132	cis-limonene oxide	0.1	-	-	-	-	-	-
1135	trans-pinocarveol	-	t	0.9	-	0.2	0.6	0.7
1136	trans-p-mentha-2-en-1-ol	-	0.1	t	0.1	-	-	-
1139	trans-limonene oxide	-	-	-	-	-	-	-
1140	**trans-verbenol**	**-**	**0.2**	**1.4**	**0.1**	**0.4**	**0.7**	**0.8**
1141	Camphor	t	-	-	-	-	-	-
1156	**p-mentha-1,5-dien-8-ol, isomer**	**0.3**	**t**	**t**	**t**	**t**	**0.1**	**t**
1166	p-mentha-1,5-dien-8-ol	0.2	t	t	t	0.1	0.2	0.2
1174	**terpinen-4-ol**	**0.3**	**0.1**	**t**	**0.1**	**0.1**	**0.1**	**0.2**
1176	m-cymen-8-ol	0.1	t	t	t	t	t	t
1179	p-cymen-8-ol	0.1	t	t	t	t	t	t
1186	**α-terpineol**	**0.4**	**0.3**	**0.5**	**0.2**	**0.1**	**0.1**	**0.1**
1191	cis-dihydrocarvone	t	-	-	-	-	-	-
1195	Myrtenol	t	t	0.2	t	t	0.1	0.2
1195	cis-piperitol	t	-	-	-	-	-	-
1204	trans-dihydrocarvone	-	-	-	-	-	-	-
1204	Verbenone	0.1	t	0.6	t	0.1	0.2	0.2
1215	trans-carveol	0.1	t	0.3	t	0.3	0.2	0.3
1223	Citronellol	t	-	-	-	-	-	-
1226	cis-carveol	t	-	-	-	-	-	-
1239	Carvone	0.1	0.2	0.3	0.1	0.1	t	t
1241	carvacrol, methyl ether	-	t	t	t	0.1	t	t
1244	car-3-en-2-one	t	-	-	-	-	-	-
1249	Piperitone	t	-	-	-	-	-	-
1287	bornyl acetate	t	t	t	t	t	t	t
1345	α-cubebene	0.5	t	0.4	0.1	0.2	0.2	0.2
1374	α-copaene	0.5	t	0.7	0.1	0.4	0.4	0.5
1387	β-bourbonene	t	t	t	t	t	t	t
1417	(E)-caryophyllene	0.4	0.6	1.2	0.2	0.9	0.9	0.9
1429	cis-thujopsene	t	-	-	-	-	-	-
1448	cis-muurola-3,5-diene	t	t	t	t	-	-	-
1452	α-humulene	t	0.5	0.7	0.2	0.4	0.4	0.4
1465	cis-muurola-4(14),5-diene	0.3	0.5	0.5	0.2	t	t	t
1478	γ-muurolene	t	0.7	1.4	0.1	0.3	0.3	0.3
1480	germacrene D	1.5	2.9	3.5	1.5	1.9	1.9	2.1
1480	**(ar-)curcumene**	**2.2**	**-**	**-**	**-**	**-**	**-**	**-**
1496	Valencene	-	0.3	0.4	t	0.1	t	0.1
1498	**β-alaskene**	**0.3**	**-**	**-**	**-**	**-**	**-**	**-**
1500	α-muurolene	0.2	t	0.4	t	0.2	0.2	0.2
1505	(E,E)-α-farnesene	-	-	-	-	0.2	t	t
1512	α-alaskene	**0.4**	-	-	-	-	-	-
1513	γ-cadinene	0.6	1.3	1.8	t	1.1	0.6	0.8
1521	trans-calamenene	0.4	t	0.7	0.1	0.1	0.1	0.2
1522	δ-cadinene	t	t	0.7	0.1	0.1	0.2	0.3

KI	Compound	Greece avg.	Turk-T2 15307	Turk-T3 15308	Turk-T1 14693	Leb-Mb 14691	Leb-Mc 14692	Leb-Eh 14690
1533	10-epi-cubenol	0.2	0.4	0.5	0.1	0.2	0.1	0.1
1533	trans-cadina-1,4-diene	0.3	0.4	0.5	0.1	0.2	0.1	0.2
1561	(E)-nerolidol	-	0.1	0.4	0.2	0.1	-	t
1570	Dendrolasin	-	0.1	0.1	t	-	-	-
1582	(ar-) tumerol	t	-	-	-	-	-	-
1582	caryophyllene oxide	0.3	1.0	1.5	t	0.7	0.6	0.6
1586	germacra-4(15),5,10(14)-trien-1-al, isomer I	t	0.5	0.5	t	0.3	0.2	0.2
1594	salvial-4(14)-en-1-one	t	0.5	0.7	t	0.3	0.2	0.2
1608	humulene epoxide II	0.4	0.5	0.8	t	0.3	0.2	0.3
1610	**salvial-4(14)-en-1-one, isomer I**	**0.6**	**0.9**	**1.2**	**0.1**	**0.3**	**0.2**	**0.2**
1638	epi-α-cadinol	0.2	0.3	0.4	0.1	0.3	0.2	0.3
1640	epi-α-muurolol	0.2	0.3	0.3	0.1	0.3	0.2	0.3
1652	α-cadinol	1.1	1.8	2.1	0.7	0.8	0.6	0.7
1685	germacra-4(15),5,10(14)-trien-1-al	0.3	0.7	0.5	0.2	0.4	0.3	0.3
1687	eudesma-4(15),7-dien-1-β-ol	0.4	0.7	0.6	0.3	0.4	0.3	0.3
1711	Pentadecanal	t	t	0.4	t	t	t	t
1840	hexahydrofarnesyl acetone	-	t	t	t	t	t	t
1959	**hexadecanoic acid**	**t**	**1.4**	**1.3**	**0.7**	**0.4**	**0.5**	**0.4**
1987	manoyl oxide	1.3	2.5	4.5	0.3	4.0	1.9	0.8
2055	Abietatriene	t	0.5	0.5	0.2	0.1	0.3	t
2105	**Isoabienol**	**0.3**	**1.3**	**2.1**	**0.6**	**0.7**	**0.8**	**0.5**
2112	**phytol, isomer I**	**0.2**	**1.3**	**0.8**	**0.4**	**0.4**	**0.6**	**0.3**
2231	methyl communate, isomer I	-	t	t	t	0.1	0.3	0.2
2314	**trans-totarol**	**t**	**0.3**	**1.2**	**0.3**	**0.3**	**0.5**	**0.3**
2331	**trans-ferruginol**	**t**	**0.5**	**0.6**	**0.2**	**t**	**0.2**	**t**

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.