The volatile leaf oils of three *Juniperus communis* varieties from Bulgaria

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ABSTRACT

The compositions of the leaf essential oils of *Juniperus communis, J. c.* var. *sibirica* (*J. communis* var. *saxatilis* Pall.) and *J. c.* var. *pygmaea* (*J. c.* var. *saxatilis*) from Bulgaria are reported and compared with *J. communis* (Sweden) and *J. c.* var. *saxatillis* (Switzerland). The leaf volatile essential oils of *J. communis, J. pygmaea and J. sibirica* from Bulgaria are high in α-pinene (21.4 - 38.4%), sabinene (10.5 - 19.6%), limonene (1.8 - 5.5%), β-phellandrene (2.7 - 8.3%) and terpinen-4-ol (3.2 - 7.5%). PCO revealed some clustering of the *J. sibirica* samples, but most of the samples were interspersed. It seems likely that hybridization is occurring and, if so, could explain these results. At the present time, *J. pygmaea* appears to be conspecific with *J. c.* var. *saxatilis* and *J. sibirica* (of Flora Bulgaria) seems to be a distinct, shrubby form of *J. communis* with very short leaves. Published on-line **www.phytologia.org** *Phytologia 95(4): 302-307 (Nov. 1, 2013)*. ISSN 030319430

KEY WORDS: *Juniperus communis, J. c.* var. *sibirica, J. c.* var. *pygmaea,* Bulgaria, leaf terpenes.

The Flora of Bulgaria (Dimitrov, 2002) lists 6 native *Juniperus* species in Bulgaria: *J. communis, J. excelsa, J. oxycedrus, J. pygmaea, J. sabina and J. sibirica.* Adams and Tashev (2012) reported that *J. oxycedrus* from Bulgaria is actually *J. deltoides* that grows from Italy eastward through Turkey. Of interest to the present work, are the resolution and taxonomy of *J. communis, J. pygmaea* and *J. sibirica* (the latter two taxa treated as *J. c.* var. *saxatilis* by Adams, 2011 and Farjon, 2005, 2010). Of these 3 taxa, *J. communis* var. *communis*, grows as a small tree, whereas *J. pygmaea* and *J. sibirica* are small to spreading shrubs. They differ in their leaf morphology (Fig. 1), with *J. pygmaea* leaves being very

Figure 1. *J. communis, J. pygmaea* and *J. sibirica* specimens from Bulgaria.

similar to *J. communis*. *J. sibirica* leaves differ by being shorter, curved and more appressed to the stem (Fig. 1). In fact, the Bulgarian *J. sibirica* leaves are quite similar to *J. communis* var. *saxatilis* (Fig. 2, Mongolia), but more appressed to the stem than in the specimen from Mongolia (Fig. 2).

The leaf oils of *J. communis* have been extensively studied; see Adams et al. (2010) and Adams (2013) for a review of the literature. Most recently, Lohani et al. (2013) reported the major components of *J. c.* var. *saxatilis* from alpine India to be α-pinene (31.8-49.5%), limonene (13.7 - 19.5%) and δ-3-carene (9,7 - 14. 7%).

The purpose of the present study was to compare the leaf volatile essential oils from *J. communis, J. pygmaea and J. sibirica* from Bulgaria to determine if their oils differ. Note that both *J. pygmaea* and *J. sibirica* as treated as synonyms of *J. saxatilis* by Farjon (2005), but the usage as per the Flora of Bulgaria (Dimitrov, D., ed. 2002) is used for this paper to avoid confusion.

Fig. 2. Specimen of *J. c.* var. *saxatilis* from Mongolia.

Plant material - Bulgaria, *J. communis* var. *communis*, *Adams 13730-31, 14058-60, Alex Tashev, 2012-JC1-5*, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca,* 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m.

J. pygmaea K. Koch (cf. *J. communis* var. *saxatilis* Pall.), *Adams 13734-35, 14064-66, Alex Tashev, 2012-JP1-5*, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies,* 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.

Juniperus sibirica Burgsd. (cf. *J. communis* var. *saxatilis*), *Adams 13732-33, 14061-63, Alex Tashev, 2012-JSI1-5,* Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus, V. uliginosum, Ribes petraeum, Rubus idaeus, Calamagrostis arundinaceae, Festuca valida (Bulgarian endemic),* 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.

Exemplar specimens: *J. communis* var. *communis,* Stockholm, Sweden, *Adams 8167 (7846-7848); J. communis* var. *saxatilis,* Switzerland, *Adams 11164 (7618-7621)*. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20º C until analyzed. The extracted leaves were oven dried (48h, 100º C) for the 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 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). Principal components analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

The leaf volatile essential oils (Table 1) of *J. communis, J. pygmaea and J. sibirica* from Bulgaria are high in α-pinene (21.4 - 38.4%), sabinene (10.5 - 19.6%), limonene (1.8 - 5.5%), β-phellandrene (2.7 - 8.3%) and terpinen-4-ol (3.2 - 7.5%). They are noticeably different from *J. communis* (Sweden) and *J. c.* var. *saxatilis* (Switzerland) in α-pinene and sabinene. However, α-pinene and sabinene are known to vary considerable geographically (Filipowicz et al., 2006, Adams et al., 2010). Filipowicz et al. (2006) reported low-sabinene chemotypes with 0.0 - 2.12% sabinene and high-sabinene chemotypes with 25.6 - 55.3% sabinene were interspersed between *J. communis* from lowlands and *J. nana* (= *J. communis* var. *saxatilis*) from high elevation. About 85% of the low-(to medium) sabinene plants were *J. communis* and about 50% of the *J. nana* plants had amounts of high-sabinene. Interestingly, *J. communis* (low elevation, Sweden) had 0.7% sabinene and *J. c. var. saxatilis* (high elevation, Switzerland) had 32.8% sabinene (Table 1). However, the Bulgarian samples do not follow this trend, but have the lowest sabinene (10.5%) from the highest elevation (1848m, Table 1) and the highest sabinene (19.6%) from the lowest elevation (1200m, Table 1).

To view the over-all trend among samples, similarities were computed among samples and the exemplars, *J. communis*, Sweden and *J. c.* var. *saxatilis*, Switzerland using 26 terpenoids. PCO of the similarity matrix resulted in six eigenroots before they began to asymptote. These six eigenroots accounted for 25.5, 14.1, 10.0, 7.7 and 6.6% of the variance among samples (OTUs). Ordination shows that most of the samples are not grouped (Fig. 3). There is some clustering of the *pygmaea* samples on the lower left quadrant (Fig. 3). Samples of *J. communis* and *J. sibirica* (*J. c.* var. *saxatilis*) are interspersed (Fig. 3).

It is very likely that hybridization is occurring between all three taxa. If so, this could lead to an ordination as seen in Fig. 3.

At the present time, *J. pygmaea* appears to be conspecific with *J. c.* var. *saxatilis* and *J. sibirica* (of Flora Bulgaria) seems to be a distinct, shrubby form of *J. communis* with very short leaves. Additional research will be needed to clarify the situation.

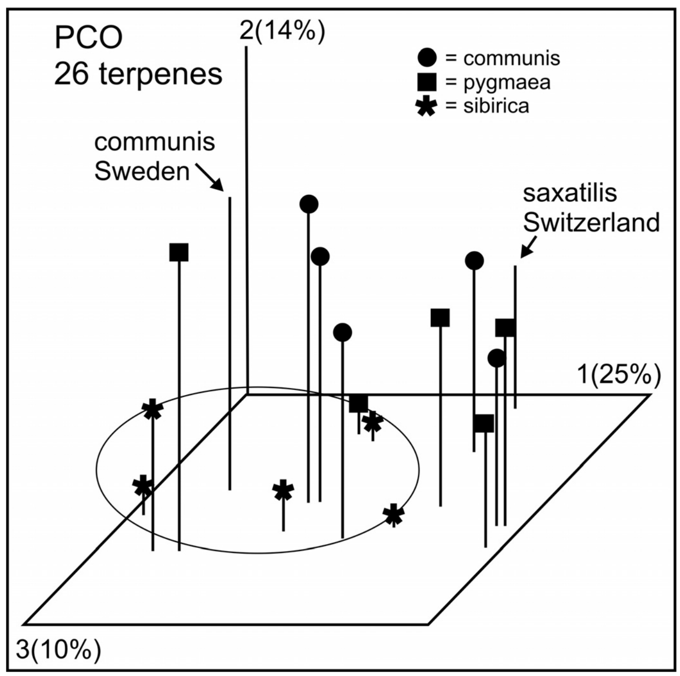

Figure 3. PCO based on 26 terpenes. see text for discussion.

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Table 1. Comparison of the leaf oils of *J. communis* from Bulgaria. comm Bulg - *J. communis* var. *communis*, Bulgaria; com sib - *J. sibirica*, Bulgaria; com pyg = *J. pygmaea*, Bulgaria; saxit = *J. c.* var. *saxatilis*, Switzerland; comm Swed = *J. c.* var. *communis*, Stockholm, Sweden. saxit Switz and comm Swed data from Adams et al. (2010). Components in boldface were used in numerical analyses.

KI	Compound	com Bulg 1200m	pyg Bulg 1756m	sib Bulg 1848m	saxit Switz	comm Swed
846	(E)-2-hexenal	0.5	0.7	0.5	1.2	0.7
921	tricyclene	t	t	t	t	0.3
924	α-thujene	1.9	1.9	1.0	4.1	0.1
932	**α-pinene**	**26.7**	**21.4**	**38.4**	**14.1**	**56.8**
945	α-fenchene	t	t	t	0.1	0.3
946	camphene	0.2	0.2	0.3	0.2	0.6
961	verbenene	-	t	0.4		
969	**sabinene**	**19.6**	**16.3**	**10.5**	**32.8**	**0.7**
974	**β-pinene**	**2.0**	**1.4**	**2.1**	**1.9**	**4.4**
988	**myrcene**	**3.5**	**3.4**	**3.1**	**5.0**	**5.2**
1001	δ-2-carene	0.1	0.2	t	0.4	0.2
1002	**α-phellandrene**	**1.0**	**1.5**	**0.4**	**0.5**	**2.1**
1008	δ-3-carene	0.8	0.9	0.9	0.5	4.7
1014	**α-terpinene**	**1.6**	**1.9**	**0.9**	**1.9**	**t**
1020	**p-cymene**	**1.4**	**2.1**	**0.5**	**0.3**	**0.3**
1024	**limonene**	**5.5**	**4.3**	**1.8**	**6.7**	**5.1**
1025	**β-phellandrene**	**8.3**	**6.5**	**2.7**	**0.6**	**8.9**
1044	(E)-β-ocimene	0.2	0.3	t	0.1	0.1
1049	pentyl isobutyrate	-	-	-	-	0.2
1054	**γ-terpinene**	**2.8**	**3.4**	**1.7**	**3.4**	**t**
1065	**cis-sabinene hydrate**	**0.8**	**1.3**	**0.4**	**1.8**	**t**
1086	**terpinolene**	**2.0**	**2.2**	**1.4**	**3.0**	**1.1**
1095	linalool	0.5	0.8	0.3	-	0.1
1098	trans-sabinene hydrate	0.5	0.9	0.4	1.3	-
1100	n-nonanal	t	-	-	-	-
1103	isoamyl-isovalerate	-	-	-	t	0.1
1112	3-me-3-butenyl-isovalerate	-	-	-	-	t
1112	trans-thujone (= β-thujone)	0.2	0.2	0.1	0.6	-
1118	cis-p-menth-2-en-1-ol	0.4	0.6	0.2	-	t
1122	α-campholenal	0.3	0.5	0.1	-	t
1135	trans-pinocarveol	0.7	0.9	0.2	-	-
1140	trans-verbenol	0.3	0.9	0.2	-	-
1147	3-me-2-butenyl-isovalerate	-	-	-	-	t
1154	sabina ketone	0.3	0.3	t	-	-
1165	borneol	0.4	0.6	0.6	t	0.2
1166	p-mentha-1,5-dien-8-ol	-	-	-	-	t
1174	**terpinen-4-ol**	**5.2**	**7.5**	**3.2**	**7.3**	**0.2**
1179	p-cymen-8-ol	0.5	0.7	0.1	t	t
1179	naphthalene	-	-	-	0.3	t
1186	**α-terpineol**	**0.8**	**2.5**	**0.5**	**0.4**	**0.2**
1194	**myrtenol**	**0.2**	**0.5**	**0.8**	**-**	**-**
1204	verbenone	0.2	0.2	0.1	-	t
1207	trans-piperitol	0.1	0.2	t	-	-
1215	trans-carveol	0.3	0.3	t	-	-

1223	citronellol	0.3	0.5	0.2	-	-
KI	Compound	com Bulg	pyg Bulg	sib Bulg	*saxit* Switz	*com*m Swed
1232	thymol, methyl ether	0.2	t	0.4	0.1	-
1249	piperitone	-	-	-	-	t
1257	methyl citronellate	-	-	-	-	t
1267	**(E)-cinnamaldehyde**	**0.5**	**1.3**	**0.3**	**-**	**-**
1283	**α-terpinen-7-al**	**0.3**	**0.6**	**0.2**	**-**	**-**
1285	bornyl acetate	0.3	0.3	0.6	0.2	0.9
1324	**myrtenyl acetate**	**0.2**	**0.1**	**0.7**	**-**	**t**
1346	α-terpinyl acetate	0.1	t	0.4	0.5	-
1350	citronellyl acetate	-	-	-	-	t
1374	α-copaene	0.2	0.5	0.3	-	-
1385	trans-myrtanyl acetate	-	-	-	t	-
1389	β-elemene	0.2	0.1	0.5	t	0.2
1417	(E)-caryophyllene	0.5	0.4	0.4	t	0.7
1452	α-humulene	0.4	0.4	0.3	t	0.5
1478	γ-muurolene	t	t	0.3	t	t
1480	**germacrene D**	**0.6**	**1.4**	**0.7**	**0.4**	**0.7**
1492	cis-β-guaiene	0.2	0.1	0.5	-	-
1493	epi-cubebol	-	-	-	-	t
1500	α-muurolene	0.3	0.1	0.5	0.2	0.2
1508	germacrene A	0.1	t	0.3	0.2	0.1
1513	γ-cadinene	0.5	0.8	0.9	0.4	0.2
1522	**δ-cadinene**	**0.8**	**0.9**	**2.1**	**0.8**	**0.5**
1537	α-cadinene	t	t	0.3	t	t
1548	elemol	t	t	0.3	-	t
1559	germacrene B	t	0.4	0.6	0.3	0.3
1561	(E)-nerolidol	0.5	0.1	0.3	-	-
1574	**germacrene D-4-ol**	**0.7**	**0.2**	**1.2**	**1.8**	**0.8**
1577	spathulenol	0.1	0.6	0.2	-	t
1582	**caryophyllene oxide**	**0.6**	**0.8**	**0.1**	**-**	**t**
1606	humulene epoxide II	0.4	0.5	0.3	-	t
1627	1-epi-cubenol	0.1	0.1	0.3	t	t
1638	epi-α-cadinol	0.3	0.3	0.6	0.5	t
1639	epi-α-muurolol	0.3	0.4	0.6	0.5	0.4
1644	α-muurolol	0.1	t	0.2	0.1	t
1652	**α-cadinol**	**0.7**	**0.5**	**1.4**	**1.3**	**0.5**
1685	germacra-4(15),5,10(14)-trien-1-al	t	0.2	t	-	t
1688	shyobunol	0.1	0.1	t	-	0.7
1722	(2E,6Z)-farnesol	t	0.3	t	-	-
1987	manoyl oxide	t	0.3	0.5	0.1	-
2055	abietatriene	t	0.4	0.9	0.2	-
2087	**abietadiene**	**t**	**0.3**	**1.4**	**0.4**	**-**
2105	**isoabienol**	**0.2**	**0.1**	**5.0**	**0.1**	**-**
2314	**trans-totarol**	**t**	**0.6**	**2.2**	**-**	**-**
2370	trans-ferruginol	t	0.1	0.3	-	-

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.