The Volatile Leaf Oils of *Juniperus semiglobosa* Regel from India Compared with *J. excelsa* M.-Bieb. from Greece

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ABSTRACT: Analyses of high- and low-cedrol volatile leaf oils of *Juniperus semiglobosa* from India showed the taxon to be rich in sabinene (12.3-29.2%), p-cymene (0.4-8.5%), terpinen-4-ol (trace-11.0%) with cedrol ranging from 34.1 to 1.5%. Contrary to some reports, comparisons of the volatile leaf oils of *Juniperus semiglobosa* from India with those from *J. excelsa* from Greece indicates that *J. semiglobosa* is not conspecific with *J. excelsa*.

KEY WORD INDEX: *Juniperus excelsa*, *Juniperus macropoda*, *Juniperus polycarpos*, *Juniperus semiglobosa*, Cupressaceae, essential oil composition, taxonomy, evolution.

INTRODUCTION: There has been and continues to be considerable confusion concerning the taxonomic status of the Asiatic *Juniperus excelsa* M.-Bieb., *J. macropoda* Boiss., *J. polycarpos* K. Koch and *J. semiglobosa* Regel. *Juniperus macropoda* has been treated as a synonym of *J. polycarpos* (based on precedence: *J. polycarpos* having been described in 1849 by Koch (1) and *J. macropoda* described by Boissier in 1884 (2). In Armenia, Takhtadzhyan (3) reduced the local *J. polycarpos* to a subspecies of *J. excelsa* [*J. excelsa* ssp. *polycarpos* (K. Koch) Takht.].

Recent examination of *Juniperus* specimens at Kew (K!), revealed that *J. excelsa* tends to have more slender foliage and more seeds per cone than *J. excelsa* ssp. *polycarpos* (4-6 vs. 2-4, respectively). In contrast, our specimens in the present study had 2-3 seeds, and the fruit (female cones) were usually bilobed or, occasionally, subglobose female cones that are smaller than those of *J. excelsa* or ssp. *polycarpos*. Our specimens match the description and illustration for *J. semiglobosa* by Farjon (4). Farjon (4) further notes the taxon generally called *J. macropoda* by Mehra (16) and others, is not *J. macropoda* Boiss. but *J. semiglobosa* Regel in India.

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Juniperus excelsa ssp. *polycarpos* appears to occur from the western Himalayas, eastward through Afghanistan, Iran and Iraq. *Juniperus excelsa* M.-Bieb. (Grecian juniper) occurs from Northern Greece, eastward through the Balkan peninsula, Crimea, Turkey (5), and at least into Iran at elevations of up to 3300 m (6). Kerfoot (7) proposed extending the southernmost range into central east Africa by equating *J. procera* and *J. excelsa*, but a recent examination of the leaf oils of these taxa (8) supports the distinctiveness of *J. procera* in Africa, with the southernmost limits of *J. excelsa* thought to be the Saudi Arabian peninsula (Adams, in progress).

The eastern limit of *J. excelsa* (*sensu stricto*) is in considerable dispute. For example, Ilahi (9) mentions only four juniper species on the Indo-Pakistan subcontinent: *J. communis* L., *J. polycarpos*, *J. recurva* Buch.-Ham. ex D. Don [incorrectly cited as Buch-Ham. in (9)] and *J. wallichiana* Hook f. & Thomson ex Brandis [incorrectly cited as *J. wallichiana* Hook f. ex Parl. in DC Prod 16(2) 482 (1868)]. Ilahi (9) reported that the average number of seeds/berry (female cone) of *J. polycarpos* from four localities in Pakistan varied from 2.0 to 4.0, with 3.88 seeds/female cone in trees from Ziarat. Interestingly, a number of reports concerning the forests of Ziarat (Baluchistan) have used *J. excelsa* for the name of the common juniper there (10-13).

Gulati et al. (14) mention five native juniper taxa in the Himalayan range: *J. communis*, *J. macropoda* (=*J. semiglobosa*?), *J. wallichiana*, *J. recurva* and *J. recurva* var. *squamata*. They list (14) *J. pseudosabina* (Fisch., Mey. & Ave-Lall.?) as a synonym of *J. wallichiana*. However, Fischer, Meyer and Ave-Lallemant described *J. pseudosabina* in 1842 (Index Octavus, p. 65, 1842) as a shrub, whereas *J. wallichiana* was described in 1874 (15) as a tree. Mehra (16) lists five junipers for the western Himalayas: *J. communis* (actually var. *montana* Ait.), *J. excelsa* var. *farreana* (no author given), *J. macropoda* (=*J. semiglobosa*), *J. pseudosabina* Fisch., Mey. & Ave-Lall., and *J. squamata* D. Don [often treated as *J. recurva* var. *squamata* (D. Don) Parl.]. The description that Mehra (16) gives for *J. excelsa* var. *farreana*, fits closely *J. excelsa* var. *polycarpos*.

In a recent report on the major volatile leaf oil components of *J. excelsa*, Thappa et al. (17) listed four juniper species for the Himalayan region: *J. communis*, *J. excelsa*, *J. recurva* and *J. wallichiana* (*J. macropoda* was treated as a synonym of *J. excelsa*). Re-examination of the specimens, indicates that the juniper analyzed (17) was *J. semiglobosa*, with bilobed female cones and long terminal branchlets (4).

The major leaf oil components of *J. semiglobosa* (i.e., *J. excelsa*) were reported (17) as: sabinene (36.1%); cedrol (26.8); limonene (7.3); terpinen-4-ol (4.6); p-menth-1-ene (4.5) and β-cedrene (3.0).

As a part of a larger study on the junipers of the Himalayas, we report in this paper on the identification of the minor components of *J. semiglobosa* from two locations, and also compare the volatile leaf oil compositions with *J. excelsa* from Greece (18).

EXPERIMENTAL: *Plant materials*—Specimens and leaves of *J. semiglobosa* were collected from Jalma Therat in Lahul (Himachal Pradesh) and Khurboo in Ladakh (Jammu and Kashmir), 3100 to 3250 m elevation, June 1985 and September 1986 (BKK-12782, -15851). Voucher specimens are deposited in the RRL herbarium, Jammu.

The specimens of *J. excelsa* were collected from a native stand in northern Greece, approximately 7 km West of Lemos, NW of Mikri Prespa (lake), 1100 m elevation October 5, 1988 (Adams 5983-5985, 5986-5987). Voucher specimens of the Grecian plants are deposited at BAYLU Herbarium.

The foliage was kept cool and frozen two days after collection. The volatile leaf oils of the Grecian samples were distilled (200 g foliage, FW) using a circulatory Clevenger apparatus (19) for 2 and 24 h to determine yields.

The high-cedrol oil (#5901) from *J. semiglobosa* was obtained as previously described (17) (15 h with steam in a large stainless steel still). The low-cedrol oil (#6006) was also obtained from plants by distillation for 4 and 18 h in a Clevenger-type glass distillation unit.

The oil samples were concentrated (ether trap removed when collected in a Clevenger trap) with nitrogen and stored at –20°C until analyzed. Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 700, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB5, 0.26 mm id x 30 m, 0.25 micron coating thickness, fused silica capillary column. The GC/ITD was operated under the following conditions: injector temperature: 220°C; transfer line: 240°C; oven temperature programmed: 60°C-240°C at 3°C/min; carrier gas: He at 31.9 cm/sec or 1.017 ml/min (at 210°C); injection: 0.1 uL (10% soln.), split 1:20, 500 ng/on column. Tuning values for the ITD were 100, 100, 100, 100 using cedrol as a tuning standard. Internal standards (n-octane and n-eicosane) were added to each sample to aid in the standardization of retention times. Indentifications were made by library searches of our volatile oil library, LIBR(TP) (20) using the Finnigan library search routines based on fit. Additional searches were made of the EPA/NIH mass spectral data base (21).

Mass spectra for unidentified constituents [ITMS, m/z (rel. int.)]: RT1046 ?[M]$^+$, 43(76), 55(18), 71(12), 81(47), 95(12), 109(100), 127(27), 135(6), 153(7); RT1091, cis/trans isomer to RT1046; 150 [M]$^+$, 41(34), 51(27), 63(12), 79(67), 91(47), 105(55), 119(29), 135(100), 150(34), thymol type compound; RT1333 204?[M]$^+$, 41(100), 55(27), 67(64), 79(83), 91(50), 109(39), 121(25), 139(13), 161(5), sesquiterpene; RT1360 168?[M], 43(100), 55(23), 71(12), 79(37), 97(37), 107(23), 115(10), 125(50), 135(73), 150(23), 168(10); RT1573 ?[M]$^+$, 43(100), 55(18), 71(16), 81(18), 99(12), 109(25), 117(10), 127(24), 135(3), 145(6), 153(5); RT1714 222 [M]$^+$, 41(100), 55(26), 67(22), 81(37), 91(46), 105(55), 120(21), 133(20), 147(9), 161(76), 189(6), 207(53), sesquiterpene alcohol; RT2071 222 [M]$^+$, 41(100), 55(23), 65(17), 79(34), 91(41), 107(31), 121(38), 131(17), 149(33), 164(77), 191(11), 207(34), sesquiterpene alcohol.

RESULTS AND DISCUSSION: Re-examination of the high-cedrol oil of *J. semiglobosa* [=*J. excelsa* of (17)], reconfirmed the major components previously reported. As in the previous study (17), we found the oil rich in sabinene (29.2%) and cedrol (34.1%), with moderate amounts of terpinen-4-ol (5.1%) and α-cedrene (2.3%). In addition, we now report on the occurrence of moderate amounts of α-pinene (4.8%) and p-cymene (4.8%). The previous report (17) of p-menth-1-ene was reconfirmed but at a much lower concentration. Lower amounts of limonene and β-phellandrene were found in this analysis (Table I). Associated with the high cedrol are other components typically found in the heartwood oil of cedarwood (*Juniperus* wood) [see (19) for a review of cedarwood oil]. Note, for example, the presence of α- and β-cedrene, thujopsene, ar-curcumene, cuparene, α-alaskene and 6-isocedrol (Table I). These compounds have seldom been found in *Juniperus* leaf oils (22) and are known in the juniper leaf oils of the western hemisphere, only as trace components (cuparene, cedrol and widdrol) in *J. barbadensis* (23). However, cedrol and associated compounds are found in abundance in *J. excelsa* from Greece (18) (see Table I) and also in small concentrations in *J. foetidissima* (24). Interestingly, *J. procera*, which Kerfoot wanted to treat as *J. excelsa* (7), produces almost none of the cedrol/cedarwood oil compounds in its leaf oil (8).

As might be expected, the low-cedrol (1.5%, Table I) *J. semiglobosa* has larger concentrations of other components (cis- and trans-sabinene hydrate, cis- and trans-p-menth-2-

Table I. Comparisons of volatile leaf oils of *Juniperus semiglobosa* with high cedrol (17), and low-cedrol from Northern India with *J. excelsa* from Northern Greece (18)

RT	Compound	Percent total oil		
		semiglobosa high-cedrol	low-cedrol	*excelsa* Greece
301	tricyclene	t	-	t
307	α-thujene	1.5	0.7	-
319	α-pinene	4.8	1.5	22.5
337	α-fenchene	0.1	-	t
340	camphene	t	t	0.5
379	sabinene	29.2	12.3	-
386	β-pinene	0.1	-	0.6
408	myrcene	0.9	0.3	1.9
435	α-phellandrene	t	-	t
444	δ-3-carene	1.7	0.2	2.3
457	α-terpinene	0.1	0.1	t
464	p-menth-1-ene	t	-	-
471	p-cymene	4.8	8.5	0.4
481	limonene	1.0	1.4	22.7
482	β-phellandrene	t	-	-
519	(E)-β-ocimene	t	-	-
545	γ-terpinene	0.7	0.3	0.6
560	trans-sabinene hydrate	0.1	4.7	-
574	cis-linalool oxide	-	t	-
608	terpinolene	0.3	0.1	0.9
609	p-cymenene	-	0.1	-
629	cis-sabinene hydrate	0.1	4.4	-
632	linalool	t	-	-
661	cis-rose oxide	-	t	-
664	endo-fenchol	-	-	t
667	β-thujone	t	t	-
682	cis-p-menth-2-en-1-ol	0.2	1.2	-
692	α-campholenal	-	t	t
724	trans-pinocarveol	-	0.3	t
725	trans-p-menth-2-en-1-ol	0.2	0.8	-
734	camphor	-	-	0.5
735	trans-verbenol	-	0.5	-
766	β-pinene oxide	t	1.1	-
789	borneol	t	t	-
820	terpinen-4-ol	5.1	11.0	t
826	naphthalene	-	t	-
837	p-cymen-8-ol	0.2	1.8	-
852	α-terpineol	0.3	3.9	-
865	(methyl phenylethanol, isomer)	-	1.3	-
865	cis-piperitol	t	-	-
894	verbenone	-	0.3	t
896	trans-piperitol	t	t	-
923	trans-carveol	-	t	t
930	endo-fenchyl acetate	-	-	0.3
950	citronellol	0.3	0.6	-

Table I (Cont.).

RT	Compound	Percent total oil		
		semiglobosa high-cedrol	low-cedrol	*excelsa* Greece
977	cuminyl aldehyde	t	t	-
984	carvone	-	t	-
1014	cis-sabinene hydrate acetate	-	t	-
1026	trans-myrtanol	t	t	-
1035	methyl citronellate	t	1.9	-
1046	unknown	t	1.6	-
1073	citronellyl formate	t	t	-
1091	cis-verbenyl acetate	-	0.5	-
1091	unknown, isomeric to 1046	0.2	1.0	-
1099	bornyl acetate	t	t	0.4
1102	(thymol type isomer)	t	0.6	-
1137	carvacrol	t	0.4	-
1172	terpene alcohol	-	-	3.3
1192	(2,6-octadienoic acid, 3,7-dimethyl, methyl ester, E/Z)	t	0.6	-
1236	δ-elemene	t	t	-
1333	sesquiterpene	t	0.6	-
1334	α-copaene	t	t	-
1360	(sesquiterpene)	-	0.7	-
1371	β-cubebene	-	-	t
1375	β-elemene	t	0.7	-
1424	sesquiterpene	-	-	1.7
1421	α-cedrene	2.3	-	-
1441	β-cedrene	0.5	-	0.9
1467	thujopsene	0.4	t	0.4
1519	α-cadinene	-	-	t
1494	α-humulene	t	-	t
1537	(Z)-β-farnesene	t	-	t
1558	β-acoradiene	-	-	t
1573	unknown	t	1.8	-
1577	β-cadinene	t	-	0.4
1586	γ-muurolene	0.1	0.4	-
1594	germacrene D	-	-	0.9
1602	ar-curcumene	0.1	-	-
1624	valencene	-	-	0.5
1643	α-muurolene	0.3	0.5	t
1652	cuparene	0.1	-	-
1667	β-bisabolene	0.4	t	-
1675	α-alaskene	t	-	0.3
1676	γ-cadinene	0.3	1.0	0.8
1681	endo-1-bourbonanol	t	0.7	-
1695	cis-calamenene	t	0.4	t
1700	δ-cadinene	1.0	t	0.7
1714	sesquiterpene alcohol	-	0.7	-
1721	(E)-γ-bisabolene	-	-	t
1759	elemol	0.4	5.0	-
1837	caryophyllene oxide	t	0.3	-

Table I (Cont.).

RT	Compound	Percent total oil *semiglobosa* high-cedrol	*semiglobosa* low-cedrol	*excelsa* Greece
1854	sesquiterpene alcohol	1.0	t	2.0
1876	cedrol	34.1	1.5	28.1
1898	β-oplopenone	0.2	1.1	t
1907	6-isocedrol	0.1	-	t
1912	1,10-di-epi-cubenol	t	0.6	-
1944	1-epi-cubenol	t	t	0.6
1951	γ-eudesmol	t	t	-
1973	epi-α-cadinol	0.3	0.9	-
1976	epi-α-muurolol	0.2	1.1	-
1984	torreyol (= δ-cadinol)	t	0.4	-
1993	β-eudesmol	t	0.6	-
2003	α-cadinol	0.6	4.1	-
2033	(sesquiterpene alcohol)	-	-	0.6
2071	sesquiterpene alcohol	t	1.1	-
2183	oplopanone	t	0.7	-
2306	8-α-acetoxyelemol	t	1.0	-
2717	manoyl oxide	t	0.1	t
2891	abietadiene	-	-	0.3
3296	cis-abietal	-	-	t

Compounds are listed in order of their elution from a DB5 column. Compounds in parenthesis are tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

en-1-ol, terpinen-4-ol, α-terpineol, elemol, α-cadinol, etc., see Table I). In addition to being very low in cedrol, this oil is lower in sabinene (12.3%) and α-pinene (1.5%). Examination of Table I reveals that the high- and low-cedrol oils do share numerous compounds that are missing (or too small to quantify) in *J. excelsa* from Greece. Also, although the leaf oil of *J. excelsa* (Greece, Table I) shares the high concentration of cedrol (28.1%), it differs markedly by the dominance of α-pinene (22.5%) and limonene (22.7%) and absence of sabinene, as well as numerous mismatches with *J. semiglobosa* from India (Table I).

A total of eighty components was present in amounts of a trace or larger in the high-cedrol oil, with seventy-eight in the low-cedrol *J. semiglobosa* and only forty-nine in the *J. excelsa* (Greece) oil. The two Indian oils contained twelve compounds that are tentatively identified or unknown and they share nine of these. In contrast, the *J. excelsa* (Greece) oil has only three unknowns and of these, only one is shared with the two Indian oils. Because our library [LIBR(TP)] has been developed from the juniper oils from the western hemisphere, the relatively large number of unidentified components in the Indian oils might be anticipated. Many of the mass spectra of the unidentified constituents are most unusual, and it is anticipated that structural elucidation will reveal new compounds, reflecting the divergent evolution of this taxon.

Overall, one is impressed that both high- and low-cedrol leaf oils of *J. semiglobosa* do share more components in common than either shares with *J. excelsa* oil from Greece. Additional field work and analyses are planned, but the lack of access to intermediate sites (Iraq, Iran, Afghanistan) presents a serious barrier at present.

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