

## *Juniperus procera* of East Africa: Volatile Leaf Oil Composition and Putative Relationship to *J. excelsa*

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**Key Word Index**—*Juniperus procera*; *J. excelsa*; Cupressaceae; terpenes; volatile leaf oil; taxonomy.

**Abstract**—The composition of the volatile leaf oils of *Juniperus procera* from Ethiopia and Kenya are reported and compared with the leaf oil of *Juniperus excelsa* from Greece. The leaf oil of *J. procera* is dominated by  $\alpha$ -pinene (22.3%), 3-carene (18.7%), *trans*-totarol (8.9%) and abietadiene (7.8%) as well as moderate levels (2–4%) of elemol,  $\alpha$ -eudesmol, myrcene,  $\beta$ -phellandrene,  $\beta$ -pinene and terpinolene. Particularly unusual for *Juniperus* is the large yield of 12 diterpenoids. In contrast, the oil of *J. excelsa* from Greece is dominated by cedrol (28.1%),  $\alpha$ -pinene (22.5%) and limonene (22.7%), with moderate amounts of 3-carene and myrcene as well as numerous sesquiterpenes in small concentrations. The divergence of *J. procera* from *J. excelsa* as early as the Mio-Pliocene has resulted in numerous differences in their leaf volatile oils and also a few morphological changes. The proposal that *J. procera* is conspecific with *J. excelsa* is rejected.

### Introduction

*Juniperus procera* Hochst. ex Endl. is the only species of the genus that grows naturally in the southern hemisphere. The taxon was described by Hoshstetter and published by Endlicher in 1847 [1] from material obtained in Ethiopia. Melville [2] lists it as the only juniper in East Africa, where it occurs in the mountainous regions from 1500–2500 m [3] in Ethiopia, Kenya, Tanzania, Uganda, Malawi, Zaire and Zimbabwe. The Zimbabwe site is the southernmost native population of any known juniper [4]. *Juniperus procera* has been considered as a variant (not formally treated as a variety) of *J. excelsa* M.-Bieb. from Europe [5] and more recently treated as conspecific with *J. excelsa* by Kerfoot [6, 7]. Kerfoot [7] postulated that *J. procera* originated from *J. excelsa* in Asia Minor in the Mio-Pliocene times as *J. excelsa* expanded southward along the western mountains of the Arabian Peninsula, thence across the strait to Ethiopia and southward along the East African rift mountains as far south as Zimbabwe. If the origin of *J. procera* was by migration of *J. excelsa* with concomitant or subsequent differentiation, then one would expect to detect this differentiation in the leaf volatile oils.

The volatile leaf oil of *J. excelsa* from Greece has been recently examined [8]. Although the wood oil of *J. procera* has been used as a source of cedarwood oil [9] and has been reported to contain mostly  $\alpha$ -cedrene and cedrol [10], the leaf oil has been little analysed. Rovesti [11] reported on the wood and leaf oils of *J. procera* from Ethiopia and noted that the leaf oil contains "pinene and limonene, with a small quantity of borneol". The volatile oil from the fruit of putative *J. procera* from the El-Taif region of Saudi Arabia has been found to contain alkanes and a monoterpene alcohol or lactone [12] but the leaf oil was not examined.

The purposes of this paper are to report on the leaf oils of *J. procera* (*sensu stricto*) from Ethiopia and Kenya and compare the oils with those of *J. excelsa* from northern Greece.

### Results and Discussion

The yields of steam volatile leaf oils from *J. procera* were 0.58% (2 h) and 1.12% (24 h). This compares with yields of 0.78 and 1.85% from *J. excelsa* [8]. The volatile leaf oil of *J. procera* is complex but dominated by  $\alpha$ -pinene, 3-carene and diterpenes such as *cis*- and *trans*-totarol, abietadiene and *cis*-abietal. Moderate amounts of the eudesmol are present, as well as elemol, along with lesser quantities of  $\beta$ -pinene, myrcene,  $\beta$ -phellandrene and terpinolene (Table

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TABLE 1. VOLATILE LEAF OIL COMPOSITIONS OF *JUNIPERUS PROCERA* FROM ETHIOPIA AND TWO LOCATIONS IN KENYA COMPARED TO *J. EXCELSA* LEAF OIL FROM NORTHERN GREECE [8]

Compound*	Percentage total oil			
	<i>J. procera</i>			<i>J. excelsa</i>
	Ethiopia Addis Ababa	Kenya Nairobi	Kijabe	Greece
2-Hexenal	0.2	0.1	0.2	—
Tricyclene	t	t	t	0.1
$\alpha$ -Thujene	t	t	t	t
$\alpha$ -Pinene	28.1	12.5	26.3	22.5
$\alpha$ -Fenchene	1.5	0.1	1.1	0.2
Camphene	t	0.1	t	0.5
Sabinene	0.4	t	t	t
1-Octen-3-ol	0.5	0.3	t	—
$\beta$ -Pinene	4.35	1.2	4.8	0.6
Myrcene	4.70	1.2	3.8	1.9
$\alpha$ -Phellandrene	t	—	—	0.1
Car-3-ene	29.6	6.1	20.5	2.3
$\alpha$ -Terpinene	0.1	t	t	0.1
<i>o</i> -Cymene	t	t	—	—
<i>p</i> -Cymene	t	t	t	0.4
Sylvestrene	0.4	0.1	0.3	—
Limonene	0.7	0.2	t	22.7
$\beta$ -Phellandrene	2.1	0.8	3.0	t
1,8-Cineole	—	t	t	—
<i>trans</i> -Ocimene	t	t	t	t
$\Gamma$ -Terpinene	0.2	t	0.2	0.6
Terpinolene	4.1	1.1	2.9	0.9
Linalool	1.0	0.5	0.5	—
<i>endo</i> -Fenchol	—	—	—	0.2
1,3,8- <i>p</i> -Menthatriene	0.1	t	t	—
<i>cis-p</i> -Menth-2-en-1-ol	t	t	—	—
<i>cis</i> -Pinene hydrate	—	—	—	t
$\alpha$ -Campholenal	t	t	—	0.1
<i>trans</i> -Pinocarveol	—	0.1	t	0.2
Camphor	0.4	0.2	0.1	0.5
Borneol	0.4	0.2	0.2	t
Nonanol	t	t	t	—
4-Terpineol	0.2	0.1	t	0.2
Naphthalene	—	—	—	t
<i>p</i> -Cymen-8-ol	0.1	0.1	t	t
$\alpha$ -Terpineol	0.4	0.5	0.2	t
Myrtenol	—	—	—	t
Verbenone	—	—	—	0.1
<i>trans</i> -Carveol	—	—	—	0.1
<i>endo</i> -Fenchyl acetate	—	—	—	0.3
Piperitone	—	—	—	t
RT1068	0.5	0.3	0.2	—
Bornyl acetate	1.2	0.4	0.3	0.4
Carvacrol	—	—	—	t
RT1172	—	—	—	3.3
$\beta$ -Cubebene	—	—	—	0.1
$\alpha$ -Cedrene	—	—	—	t
RT1424	—	—	—	1.7
$\beta$ -Cedrene	—	—	—	0.9
Caryophyllene	1.0	0.5	0.5	—
Thujopsene	—	—	—	0.4
$\alpha$ -Cadinene	—	—	—	0.2
$\alpha$ -Humulene	1.3	0.7	0.8	0.2
<i>cis</i> - $\beta$ -Farnesene	—	—	—	0.2

TABLE 1. CONTINUED

Compound*	Percentage total oil			
	Ethiopia Addis Ababa	Kenya Nairobi	Kijabe	<i>J. excelsa</i> Greece
$\beta$ -Acoradiene	—	—	—	0.1
$\beta$ -Cadinene	—	—	—	0.4
$\Gamma$ -Muuroolene	—	—	—	t
Germacrene D	0.7	0.3	0.4	0.9
Valencene	—	—	—	0.5
$\alpha$ -Muuroolene	—	—	—	0.2
$\alpha$ -Alaskene	—	—	—	0.3
$\Gamma$ -Cadinene	—	—	—	0.8
1 <i>S</i> , <i>cis</i> -Calamenene	—	—	—	t
$\delta$ -Cadinene	—	—	—	0.7
<i>trans</i> - $\Gamma$ -Bisabolene	—	—	—	0.2
Elemol	1.8	4.3	2.2	—
Caryophyllene oxide	0.4	0.5	0.3	—
RT1854	—	—	—	2.0
Cedrol	—	—	t	28.1
Cubenol	—	—	—	0.6
$\Gamma$ -Eudesmol	0.4	1.4	0.3	—
$\tau$ -Cadinol	—	—	—	t
$\tau$ -Muurolol	—	—	—	t
Torreyol	—	—	—	t
$\beta$ -Eudesmol	0.6	2.3	0.6	—
$\alpha$ -Eudesmol	1.2	3.8	1.3	—
$\alpha$ -Cadinol	—	—	—	t
RT2033	—	—	—	0.6
(Elemol acetate)	0.6	1.3	0.6	—
8- $\alpha$ -Acetoxyelemol	0.6	3.5	0.8	—
<i>epi</i> -13-Manool	—	0.2	0.2	—
Manoyloxide	2.1	0.5	1.9	—
Abietatriene	0.1	1.3	0.6	—
Abietadiene	1.9	15.4	6.2	—
RT2937	—	2.6	9.0	—
RT3080	—	0.8	0.3	—
<i>cis</i> -Totarol	—	0.6	t	—
<i>cis</i> -Abietal	0.3	1.7	0.9	0.1
RT3282	—	1.0	0.3	—
<i>trans</i> -Totarol	0.8	21.4	4.6	—
(Ferruginol)	t	2.4	0.9	—
Total	95.6	93.4	98.1	98.5

\*Compounds are listed in order of their elution from a DB5 (=SE54) column. Compounds in parenthesis are tentatively identified. Compositional values less than 0.1% are denoted as traces (t) and counted as 0.05% in computing the totals. Unidentified constituents smaller than 0.5% are not reported.

1). Numerous compounds are present in small concentrations (Table 1). Cedrol, the major component of the wood oil [9–11], is either missing or present as a trace (in the Kijabe population) in *J. procera* (Table 1). It is possible that the trace might have come from twigs in the branchlets used in steam distillation. The most unusual

aspect of the *J. procera* leaf oils are the large yields of the diterpenes (particularly in the Kenyan populations). The second surprising result, in light of the contention that *J. procera* is conspecific with *J. excelsa* [6, 7], was that the oils are very different from the Grecian *J. excelsa*. It is noteworthy that *J. excelsa* has a great diversity of oxygenated terpenes and sesquiterpene hydrocarbons; these are absent in every population of *J. procera*. Cedrol, the major component of the leaf oil of *J. excelsa*, is missing or found in only trace amounts in *J. procera*. Limonene, another major component in *J. excelsa*, is also quite low in *J. procera* (Table 1). A total of 91 components are reported in Table 1, of which 28 are unique to *J. procera* and 34 are unique to *J. excelsa*, with only 29 shared between the two taxa. Previous evolutionary studies on *Juniperus* leaf oils in the western hemisphere [13–15] have shown the remarkable utility of these chemical components in detecting hybridization, introgression and, particularly, defining the relationships among sibling species (e.g. in *J. procera* and *J. excelsa*). Indeed, the differences in the oils of *J. procera* and *J. excelsa* are much larger than found between the three sibling species, *J. horizontalis*, *J. scopulorum* and *J. virginiana* [16, 17].

In spite of the fact that *J. procera* clearly differs in the number of seeds per cone (1–3 [2] or 1–4 [5]) from *J. excelsa* (5–6 [18]), Kerfoot [7] and Kerfoot and Lavranos [6] suggest submerging *J. procera* under *J. excelsa*. By the criteria established for species recognition of *Juniperus* in the western hemisphere [14, 15], *J. excelsa* and *J. procera* should be treated as distinct species. The long period of isolation of *J. procera* from *J. excelsa* since the Mio-Pliocene [7] has clearly led to the evolution of numerous chemical differences but only a few morphological changes. Of particular interest will be the analysis (Adams, in progress) of the volatile oils of *J. excelsa* from Turkey, western Arabia (putative *J. excelsa* [7]) and East African *J. procera* plants to determine if the western Arabian populations are intermediate.

In conclusion, it appears that *J. excelsa* and *J. procera* are sibling species. Their divergence and continued separation has led to numerous, presumably genetically-mediated, chemical differences and *J. procera* appears to have a unique common gene pool that it is maintaining in the

three separate populations examined. Therefore, the recommendation [6, 7] to merge the taxa is not acceptable. *Juniperus procera* should be maintained at the specific rank.

## Experimental

Specimens were collected from Addis Ababa, Ethiopia, 2500 m, 15 Oct. 1985, Adams 5292–5293; approx. 38 km N.E. of Nairobi, Kenya, 2170 m, 21 Oct. 1985, Adams 5333–5335; approx. 6 km S.W. of Kijabe, Kenya, 1500 m, 9 Jan. 1989, Adams 6007–6009. Voucher specimens are deposited at BAYLU herbarium. The volatile leaf oil was isolated by steam distillation of approx. 200 g of foliage suspended in a chamber above a boiling flask for 2 and 24 h to determine yields [19]. It is important with many species to not boil the leaves in water, due to free plant acids that can cause rearrangements. Yields were calculated as: oil weight/ [the oven dry weight (48 h, 100°C) of the extracted leaves plus oil weight]. The oil samples were concentrated (ether trap removed) under nitrogen and stored in Teflon capped vials at –20°C until analysed. Mass spectra were recorded with a Finnigan Ion Trap mass spectrometer (ITMS), model 800, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB5(=SE54), 0.26 mm i.d. × 30 m, 0.25 µm coating thickness, fused silica capillary column. The GC-ITMS was operated under the following conditions: injector temperature: 220°C; transfer line: 240°C; oven temperature programmed: 60–240°C at 3°C min<sup>-1</sup>; carrier gas: He at 31.9 cm s<sup>-1</sup> or 1.017 ml min<sup>-1</sup> (at 210°C); injection: 0.1 µl (10% soln), split 1:20, 500 ng/on column. Tuning values for the ITMS 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. Identifications were made by library searches of our volatile oil library [20], LIBR(TP), using the Finnigan library search routines and standardized retention times [20, 21].

Four components with values greater than 0.5% in one of the populations remain unidentified and are under further study (Adams, in progress). Their mass spectra are: RT1068, MW204?, *m/z* (% int.) 41(45), 53(24), 67(17), 79(100), 91(58), 105(31), 119(13), 133(7), 147(17), 161(3), sesquiterpene?; RT2937, MW272?, *m/z* (% int.) 41(100), 55(45), 67(46), 79(42), 95(40), 109(23), 121(18), 135(14), 149(11), 161(7), 177(10), 191(33), 204(7), 257(12), diterpene; RT3080, *m/z* (% int.) 41(100), 55(50), 67(48), 79(72), 91(82), 105(62), 123(44), 133(42), 147(21), 161(17), 175(8), 187(4), 201(5), 215(5), 243(7), 258(13), 271(30), 286(13), diterpene alcohol; RT3282, *m/z* (% int.) 41(100), 55(23), 69(28), 77(22), 91(32), 107(40), 115(19), 131(48), 143(40), 157(14), 173(20), 187(18), 199(15), 211(6), 227(12), 245(43), 255(12), 270(12), 284(3), diterpene alcohol; spectra for the unknowns from *J. excelsa* (see Table 1) have been previously published [8].

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