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The Leaf Oil of *Juniperus martinezii* Perez de la Rosa and Taxonomic Status

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ABSTRACT: The essential leaf oil of *Juniperus martinezii*, from the type locality, is dominated by α-pinene, camphor, sabinene, terpinen-4-ol, β-phellandrene, myrcene, linalool, kaur-16-ene, and bornyl acetate. Both morphologically and chemically, it appears to be more closely related to *J. flaccida* than to other Mexican junipers, and hence is recognized as a variety, *J. flaccida* var. *martinezii* (Perez de la Rosa) J. Silba. A distribution map of the 5 known populations is presented.

KEY WORD INDEX: Cupressaceae, *Juniperus martinezii, J. flaccida, J. flaccida* var. *martinezii*, essential oil.

INTRODUCTION: In 1985, a new species, *Juniperus martinezii* Perez de la Rosa, was described from the state of Jalisco, Mexico (1). Although the habit and foliage are very similar to *J. flaccida* Schlecht. and *J. flaccida* var. *poblana* Martinez, the female cones usually have only one or two seeds. Martinez (2) considered the number of seeds/cone to be very significant in delimiting taxa and sections of *Juniperus* and this idea was largely followed by Zanoni and Adams (3).

Perez de la Rosa compared the morphology of *J. martinezii* to most of the one (or few) seeded junipers of Mexico and found the new taxon to be quite distinct. Subsequently, Silba (4) relegated the species to a variety *(J. flaccida* var. *martinezii)*, noting that it is "A scarcely distinct taxon named from Jalisco, very similar to typical *J. flaccida* Schl. in general characteristics." Because the volatile leaf oil of this new taxon has not been previously reported and in order to help ascertain the taxonomic status of this new taxon, we initiated the present study of the volatile leaf oil.

EXPERIMENTAL: Separate leaf samples were taken from four trees of *J. martinezii* at the type locality (Lat. 21°25'N; Long. 101°36'W) in Jalisco on April 26, 1988 and the fresh foliage kept cool on ice until frozen. The leaves were then kept at −20°C until steam distilled. The foliage was steam distilled in a modified Clevenger apparatus (5).

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It should be noted that von Rudloff (5) showed that the modified Clevenger apparatus that uses a floating diethyl ether trap and has the plant material suspended above the boiling water gave results most similar to single leaf injection (direct volatilization into the injector). Because the leaves are not boiled in the flask, the problem of plant acids leaching from the leaves, generating low pHs and subsequent degradation and rearrangements are minimized. Steam distillations were performed for 2 h and 24 h to determine yields. The oils were concentrated under nitrogen, tightly sealed in glass vials with teflon lined caps and stored at − 20°C until analyzed. Voucher specimens (Adams, Perez de la Rosa and Charzaro, 5950-5953) are deposited at BAYLU!

The leaf oils of *J. flaccida* var. *flaccida* and *J. f.* var. *poblana* from our previous study (6), were re-analyzed on the ion trap mass spectrometer and found to be little changed after being stored at − 20° C in diethyl ether for five years. We have observed the same phenomenon when re-analyzing other juniper oils stored in ether at − 20°C.

Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 800, 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 @ 3°C/min; carrier gas: He @ 31.9 cm/sec or 1.017 mL/min (@ 210°C); injection 0.1 µL (10% soln.), split 1:20, 500 ng/on column. Turning 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. Identification were made by library searches of our volatile oil library (7), LIBR(TP) using the Finnigan library search routines based on fit and standardized retention times.

RESULTS AND DISCUSSION: The yields of essential oils from *J. martinezii* were 0.83 (2 hr) and 1.69 (24 hr, % dry wt.). These are comparable to yields from *J. flaccida* (6). The composition of the oil of *J. martinezii* (2 hr distillation) is compared with *J. flaccida* var. *flaccida* and *J. f.* var. *poblana* in Table I. (Note: the *J. flaccida* oils from a previous study (6) were re-analyzed on a DB5 column and GC/ITD).

Several unidentified compounds from our previous study (6) of the leaf oils of *J. flaccida* have now been identified: unknown 1, RRT 0.324 = α-pinene oxide; unknown 2, RRT 0.339 = α-campholenal; unknown 3, RRT 0.715 = caryophyllene oxide; "β-terpineol isomer" = cis-p-menth-2-en-1-ol; and "β-bisabolene" is actually trans-nerolidol. In addition, β-pinene and sabinene are resolved in this analysis, whereas they were previously (6) quantitated together. There still remains one unknown (larger than a trace), Table I: RT 1196, MW 164; m/z(%) 149(100), 91(50), 43(24), 51(18), 77(14), 65(12), 105(12), 164(10), 121(2), 134(2), very similar to methyl thymol, or methyl carvacrol, but with a longer retention time. It is possibly a methyl ether of an aromatic terpene.

The oil of *J. martinezii* is not dominated by α-pinene as are the oils of *J. f.* var. *flaccida* and var. *poblana* (Table I). The other major differences are the relatively larger amounts of sabinene, camphor, terpinen-4-ol, bornyl acetate, and kaur-16-ene in *J. martinezii* (Table I). Conversely, *J. martinezii* has much less α-pinene, δ-3-carene, and trans-verbenol than the two varieties of *J. flaccida*. The oil of *J. martinezii* has several compounds (larger than traces) that were not

found in the varieties of *J. flaccida:* trans-p-menth-2-en-1-ol; β-pinene oxide; myrtenyl acetate; *cis*-myrtanol; linalyl acetate; *trans*-verbenyl acetate; α-cadinene; β-cadinene; and kaur-16-ene. Conversely, α-fenchene; α-fenchol; *cis*-pinene hydrate; *cis*-verbenol; camphene hydrate; pulegol, estragole; germacrene D; and trans-nerolidol were found (in larger than trace amounts) in the *J. flaccida* varieties, and were not detected in our samples of *J. martinezii.*

In comparing the oil of *J. martinezii* with the oils of the other Mexican junipers from our previous studies (8-14), we found that *J. martinezii* oil shares more components and is quantatively more similar to the oils of the *J. flaccida* varieties than the oil of any other Mexican juniper. It is very interesting that this one (or two) seeded juniper has appreciable amounts of camphor and bornyl acetate, compounds that are associated with some (but not all) of the one-seeded junipers such as *J. ashei* and *J. saltillensis* (9), *J. pinchotii* (8), and *J. erythrocarpa* (11). These two monoterpenes are present, but in much smaller amounts in the two *J. flaccida* varieties.

The populations of *J. martinezii* do not appear to be growing intermixed with *J. flaccida.* Because the number of seeds/cone is not an obvious characteristic, the full range of the taxon is known imprecisely at present. Our field collections and examination of specimens at the University of Guadalajara Herbarium indicate that the range of *J. martinezii* is divided into five populations in central Mexico (Figure 1).

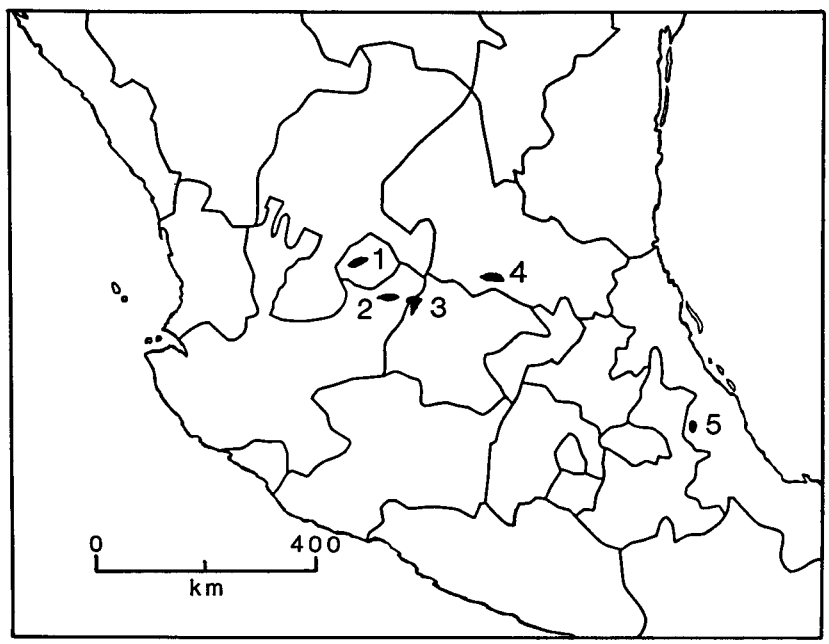

Figure 1. Distribution of *J. flaccida* var. *martinezii.* 1 = Sierra Fria, Aguascalientes; 2 = Sierra de Jala, Jalisco; 3 = Sierra Cuatralba, Jalisco-Guanajuato (type locality and used for essential oil in this paper); 4 = Sierra de San Miguelito, San Luis Potosi; 5 = Sierra de Mastaloyan, Veracruz. The distribution is based on field observations and examination of specimens at the Univ. of Guadalajara Herbarium.

Table I. Comparison of the volatile oil composition of leaves of *Juniperus martinezii* with the oils of *J. flaccida* var. *flaccida* and var. *poblana*.

Compound	*J. martinezii*	*J. flaccida* var. *flaccida*	*J. flaccida* var. *poblana*
Tricyclene	0.5*	T	T
α-Thujene	0.6	T	T
α-Pinene	13.5	34.8	58.8
α-Fenchene	—	0.2	T
Camphene	0.6	0.4	0.5
(6,6-Dimethyl-bicyclo(3.1) hepta-2(8),3-diene)	0.1	T	0.1
(Bicyclo(3,2,1)oct-2-ene, 3 methyl-4-methylene)	1.8	1.6	0.5
Sabinene	8.5	0.3	0.1
β-Pinene	1.1	4.0	4.4
Myrcene	4.0	4.4	5.0
δ-2-Carene	T	0.4	1.7
α-Phellandrene	0.9	T	0.1
δ-3-Carene	T	5.1	1.3
α-Terpinene	1.1	T	T
p-Cymene	1.2	0.7	T
Limonene	1.6	6.0	2.0
β-Phellandrene	5.0	3.5	4.2
1,8-Cineole	—	0.6	—
cis-Ocimene	T	T	T
trans-Ocimene	0.4	0.5	0.7
γ-Terpinene	2.0	0.1	T
(*cis*-p)-Menth-2-en-1-ol	0.5	0.2	—
(*cis*-)-Linalool oxide	T	T	—
(Eucarvone)	0.1	—	—
trans-Linalool oxide	—	T	—
Fenchone	—	T	T
Terpinolene	0.9	0.4	T
p-Cymenene	0.3	0.4	T
α-Pinene oxide	1.9	1.7	0.2
(*trans*-p)-Menth-2-en-1-ol	0.5	—	—
Linalool	3.0	3.2	1.4
n-Nonanal	—	T	—
α-Fenchol	—	0.1	0.2
β-Pinene oxide	0.2	—	—
cis-Pinene hydrate	—	0.25	0.2
α-Campholenal	0.4	0.9	0.7
cis-Limonene oxide	—	0.2	—
trans-Pinocarveol	0.7	0.9	0.8
cis-Verbenol	—	0.3	0.2
Camphor	11.4	0.5	T
trans-Verbenol	0.5	2.5	1.3
Isopulegol	—	0.5	T
Camphene hydrate	—	0.4	1.1
Citronellal	—	T	—
Pulegol	—	0.2	0.1
Pinocamphone	T	0.2	—
Pinocarvone	T	—	T
Borneol	0.9	1.7	0.8
Isopinocamphone	0.3	0.2	0.2
Terpinen-4-ol	8.2	1.1	0.2

Compounds are listed in order of their elution from a DB5 column.
Compounds in parenthesis are tentatively identified. T = less than 0.1% total oil.
*Data expressed as % total oil using total ion counts (TIC).

Table I (cont.). Comparison of the volatile oil composition of leaves of *Juniperus martinezii* with the oils of *J. flaccida* var. *flaccida* and var. *poblana*.

Compound	*J. martinezii*	*J. flaccida* var. *flaccida*	*J. flaccida* var. *poblana*
p-Cymen-8-ol	0.4	0.6	0.1
α-Terpineol	0.7	0.7	0.3
Myrtenal	0.2	0.2	0.2
Myrtenol	0.2	0.2	0.2
Estragole	—	0.3	0.3
Pinocamphone isomer, RT886	0.5	0.5	0.3
Verbenone	0.4	1.2	0.3
trans-Carveol	0.1	0.5	0.3
(endo-)-Fenchyl acetate	—	—	0.1
Methyl thymol	—	—	T
Myrtenyl acetate	0.5	—	T
Carvone	0.1	0.1	0.1
Methyl Carvacrol	—	T	—
cis-Myrtanol	0.4	—	—
Piperitone	0.4	0.6	0.5
trans-Myrtanol	—	0.1	T
Linalyl acetate	0.4	—	—
Methyl citronellate	—	T	—
Bornyl acetate	2.3	T	0.9
Thymol	T	T	T
trans-Verbenyl acetate	0.9	—	—
Carvacrol	0.1	T	—
RT1196,BP149,164	0.5	0.7	—
α-Terpinyl acetate	0.5	—	—
α-Cubebene	—	T	—
α-Copaene	T	—	—
β-Cubebene	T	—	—
Methyl eugenol	—	T	—
Caryophyllene	0.15	0.4	0.2
α-Cadinene	0.3	—	—
β-Cadinene	0.3	—	—
Germacrene D	—	0.3	0.2
α-Muurolene	T	T	—
γ-Cadinene	1.0	0.2	—
(1S,*cis*-)-Calamene	T	T	—
δ-Cadinene	0.9	0.2	—
Elemol	0.7	0.6	—
Elemicin	T	0.1	—
trans-Nerolidol	—	0.9	1.1
Caryophyllene oxide	0.2	0.7	0.2
Cubenol	1.0	0.3	—
γ-Eudesmol	0.2	0.3	—
τ-Cadinol	T	T	—
τ-Muurolol	0.2	T	—
Torreyol	T	T	—
β-eudesmol	0.3	0.6	—
α-eudesmol	0.3	0.7	—
(epi-13-)-Manool	0.9	0.1	—
Manoyl oxide	0.9	6.6	—
Abietatriene	0.3	0.6	—
Manool	0.3	T	—
Kaur-16-ene	2.5	—	—

Compounds are listed in order of their elution from a DB5 column.
Compounds in parenthesis are tentatively identified. T = less than 0.1% total oil.
*Data expressed as % total oil using total ion counts (TIC).

The question as to whether to recognize *J. martinezii* as a distinct species is difficult to answer. One can, at this point, only examine the evidence obtainable from the characters available. Clearly, the overall morphology of *J. martinezii* differs little from *J. flaccida* (mostly by the number of seeds/cone) and the chemical composition of the leaf oil shows strong affinity to *J. flaccida*. Thus, conspecific status of *J. martinezii* with *J. flaccida* seems reasonable. Yet, the fact that the oil differs qualitatively from the other *J. flaccida* varieties, indicates that this taxon has accumulated some gene differences. The maintenance of seemingly pure populations that occupy a different ecological niche than *J. flaccida* is also evidence that additional gene differences have accumulated in *J. martinezii*. Together these data justify the continued recognition of *J. martinezii* as a distinct taxon. It seems appropriate to use *J. flaccida* var. *martinezii* (Perez de la Rosa) J. Silba for the taxon until additional data, such as from DNA analyses, are available.

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