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Enantiomeric Distribution of Terpenoids in *Juniperus* Essential Oils: Composition of *Juniperus horizontalis* and *Juniperus scopulorum* Leaf Essential Oils From Southwestern Idaho

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Abstract

The essential oils from the leaves and twigs of *Juniperus horizontalis* and *Juniperus scopulorum*, growing in southwestern Idaho, were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC(FID)). The major components in the essential oil of *J horizontalis* were α-pinene, 16.9%, predominantly (+)-α-pinene; sabinene, 37.1%, exclusively (+)-sabinene; myrcene, 5.0%; and terpinene-4-ol, predominantly (+)-terpinen-4-ol. The essential oil of *J scopulorum* was rich in sabinene, 29.8%, exclusively (+)-sabinene; terpinene-4-ol, 13.2%, nearly racemic; α-elemol, 9.7%; and 8α-acetoxyelemol, 6.4%. In addition, 12 commercial *Juniperus communis* fruit essential oils were analyzed by chiral GC-MS. α-Pinene showed considerable variation in enantiomeric distribution, depending on geographical location. The dominant enantiomer of sabinene in *J communis* essential oils was (+)-sabinene.

Keywords

essential oil, volatile phytochemistry, *Juniperus horizontalis*, *Juniperus scopulorum*, chiral, gas chromatography, Cupressaceae

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Introduction

Juniperus L. is the largest genus in terms of number of species in the Cupressaceae. World Flora Online currently lists 75 species of *Juniperus*.[1] Eighteen species of *Juniperus* are known to occur in North America.[2] Several species of *Juniperus* have commercial importance, including *Juniperus communis* L. (common juniper), used to flavor gin, *Juniperus oxycedrus* L. (cade juniper), the source of antiparasitic oil of Cade, and *Juniperus virginiana* L. (eastern red cedar), used as an insect repellent.[3] Many *Juniperus* species are used in local traditional medicine and several are important sources of essential oils.[4]

Juniperus horizontalis Moench (creeping juniper) ranges naturally across Canada and south into northern United States (eg Montana, Wyoming, North and South Dakota, Minnesota, Wisconsin, Michigan, New York Vermont, and Maine).[2] In Cheyenne traditional medicine, the plant was used as a remedy for colds, coughs, and fevers.[5] The leaf essential oil of *J horizontalis*, cultivated in Tehran, Iran, and rich in limonene and sabinene, showed good antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus anthracis*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Escherichia coli* with minimum inhibitory concentration (MIC) values ≤ 64 μg/mL.[6] Likewise, a *p*-cymene and linalool-rich *J horizontalis* leaf essential oil from Van, Turkey, showed good antibacterial activity against *Enterococcus faecalis* and *Salmonella typhimurium*.[7]

Juniperus scopulorum Sarg. (Rocky Mountain juniper) is found in western North America, including British Columbia, Washington, Oregon, Nevada, through Idaho, Montana, Wyoming, Utah Colorado, Arizona, and New Mexico, and east to North and South Dakota and western Nebraska.[2] Several Native North American tribes used *J scopulorum* in their traditional medicine to treat coughs, colds, and fevers (Cheyenne, Flathead, Kutenai, Navajo, Nez Perce, Sioux, Thompson), arthritis and rheumatism (Blackfoot, Montana, Navajo, Okanagan, Swinomish, Thompson).[5] The essential oil of *J scopulorum*, cultivated in Hoyo, Argentina, was dominated

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Table 1. Chemical Compositions of the Essential Oils From the Leaves and Twigs of *Juniperus horizontalis* and *Juniperus scopulorum* Growing in Southwestern Idaho.

RI_{calc}	RI_{db}	Compound	Percent Composition	
			J horizontalis	*J scopulorum*
922	923	Tricyclene	0.1	tr
925	925	α-Thujene	1.6	1.4
933	933	α-Pinene	16.9	3.7
942	943	1-Octanol	-	0.1
947	948	α-Fenchene	0.1	tr
949	950	Camphene	0.2	0.1
972	972	Sabinene	37.1	29.8
978	978	1-Octen-3-ol	2.1	-
979	978	β-Pinene	-	0.6
984	984	3-Octanone	-	tr
989	989	Myrcene	5.0	2.5
994	996	Butyl butyrate	0.1	-
1000	1000	δ-2-Carene	0.1	0.1
1007	1006	α-Phellandrene	tr	tr
1009	1008	δ-3-Carene	2.4	0.2
1017	1017	α-Terpinene	1.6	1.6
1019	1022	*m*-Cymene	tr	-
1024	1024	*p*-Cymene	0.3	2.0
1026	1026	2-Acetyl-3-methylfuran	tr	tr
1030	1030	Limonene	1.3	2.0
1031	1031	β-Phellandrene	0.3	0.2
1032	1032	1,8-Cineole	0.1	0.6
1035	1034	(*Z*)-β-Ocimene	0.1	0.1
1045	1045	(*E*)-β-Ocimene	0.1	tr
1055	1056	Isoamyl butyrate	tr	-
1058	1057	γ-Terpinene	2.5	2.9
1064	1064	Prenyl butyrate	tr	-
1070	1069	*cis*-Sabinene hydrate	1.0	1.3
1085	1086	Terpinolene	1.5	1.2
1101	1101	Linalool	1.5	3.3
1102	1101	*trans*-Sabinene hydrate	0.6	0.3
1118	-	*trans*-4-Methoxythujane	0.2	-
1124	1124	*cis-p*-Menth-2-en-1-ol	0.5	0.9
1142	1142	*trans-p*-Menth-2-en-1-ol	0.3	0.6
1172	1170	Borneol	0.1	-
1181	1180	Terpinen-4-ol	7.4	13.2
1185	1187	(3*Z*)-Hexenyl butyrate	tr	-
1186	1186	*p*-Cymen-8-ol	tr	0.1
1188	1188	(*E*)-β-Ocimenol	0.1	-
1191	1193	Butyl caproate	tr	-
1191	1194	*p*-Mentha-1,5-dien-7-ol	tr	-
1195	1195	α-Terpineol	0.4	0.6
1196	1196	*cis*-Piperitol	0.1	0.2
1206	1205	Verbenone	tr	-
1208	1208	*trans*-Piperitol	0.1	0.3
1227	1227	Citronellol	0.4	1.3
1253	1254	Piperitone	0.1	0.1
1257	1258	(4*Z*)-Decen-1-ol	0.1	0.3
1279	1278	Pregeijerene B	-	1.3
1283	1282	Bornyl acetate	0.7	0.6
1313	1324	(2*E*,4*E*)-Decadien-1-ol	-	0.1
1320	1319	Methyl geranate	-	0.1
1336	1335	δ-Elemene	tr	-
1349	1349	Citronellyl acetate	tr	0.1

(Continued)

Table 1. Continued

RI_{calc}	RI_{db}	Compound	Percent Composition	
			J horizontalis	*J scopulorum*
1376	1375	α-Copaene	tr	-
1384	1382	β-Bourbonene	tr	-
1390	1390	*trans*-β-Elemene	0.1	-
1418	1417	(*E*)-β-Caryophyllene	0.1	0.1
1429	1427	γ-Elemene	0.1	-
1439	1437	2-Phenylethyl butyrate	0.1	-
1446	1446	*cis*-Muurola-3,5-diene	tr	-
1449	1450	*trans*-Muurola-3,5-diene	tr	-
1454	1454	α-Humulene	0.1	0.1
1462	1463	*cis*-Cadina-1(6),4-diene	tr	-
1472	1472	*trans*-Cadina-1(6),4-diene	0.1	-
1475	1475	γ-Muurolene	0.1	tr
1480	1480	Germacrene D	0.3	0.1
1491	1490	γ-Amorphene	0.1	tr
1496	1497	*epi*-Cubebol	0.2	0.1
1499	1500	α-Muurolene	0.3	0.1
1513	1512	γ-Cadinene	0.3	0.2
1515	1515	Cubebol	0.1	tr
1518	1514	δ-Amorphene	-	0.2
1519	1518	δ-Cadinene	1.8	0.9
1523	1521	Zonarene	tr	-
1530	1533	10-*epi*-Cubenol	tr	-
1532	1533	*trans*-Cadina-1,4-diene	tr	-
1535	1536	α-Copaen-11-ol	-	0.1
1537	1538	α-Cadinene	0.1	0.1
1550	1549	α-Elemol	2.0	9.7
1558	1557	Germacrene B	0.1	-
1563	1560	(*E*)-Nerolidol	-	0.1
1572	1571	(3*Z*)-Hexenyl benzoate	-	0.1
1577	1574	Germacra-1(10),5-dien-4β-ol	1.9	0.4
1604	1607	β-Oplopenone	0.9	0.8
1607	1610	5-*epi*-7-*epi*-α-Eudesmol	-	tr
1615	1614	1,10-di-*epi*-Cubenol	0.1	tr
1623	1624	*epi*-γ-Eudesmol	tr	0.2
1628	1628	1-*epi*-Cubenol	0.1	0.1
1632	1632	γ-Eudesmol	0.1	0.9
1643	1643	τ-Cadinol	0.6	0.4
1645	1645	τ-Muurolol	0.7	0.5
1646	1644	α-Muurolol (=δ-Cadinol)	0.2	0.1
1656	1655	α-Cadinol	2.1	2.8
1665	1665	Intermedeol	0.1	0.1
1684	1686	Botrydiol	-	0.1
1689	1686	Shyobunol	0.1	tr
1736	1735	Oplopanone	0.1	0.1
1741	1740	8α,11-Elemodiol	-	0.1
1785	1793	8α-Acetoxyelemol	0.1	6.4
2089	2086	Abietadiene	tr	-
		Monoterpene hydrocarbons	71.1	48.3
		Oxygenated monoterpenoids	13.8	23.5
		Sesquiterpene hydrocarbons	3.5	1.7
		Oxygenated sesquiterpenoids	9.2	22.8
		Diterpenoids	tr	0.0
		Others	2.3	1.9
		Total identified	100.0	98.2

Note: RI_{calc} = Calculated retention index with respect to a homologous series of *n*-alkanes on a ZB-5 ms column. RI_{db} = Reference retention index from the databases.[10–13] tr = trace (< .05%).

by sabinene (86.5%) and demonstrated antifungal effectiveness against *Fusarium verticillioides*, *Aspergillus flavus*, *Aspergillus parasiticus*, and *Rhodotorula* sp.[8]

The purpose of this work was to examine the essential oil compositions of *J horizontalis* and *J scopulorum* growing in southwestern Idaho, with particular emphasis on the enantiomeric distribution of monoterpenoid components. A survey of enantiomeric monoterpenoid distributions in *Juniperus* is also presented for comparison.

Results and Discussion

J horizontalis and *J scopulorum* Essential Oil Compositions

The essential oils from the leaves and twigs of *J horizontalis* and *J scopulorum* were obtained in 0.318% and 0.543% yield, respectively, as colorless essential oils. The chemical compositions of the essential oils are compiled in Table 1. The compositions are qualitatively similar to those previously reported for *J horizontalis* and *J scopulorum* from Wyoming.[9] Key differences in the compositions of *J horizontalis* are the large concentration of α-pinene in this work (16.9%) compared to those found in the Wyoming collection (2.4%-6.6%) and the lower concentration of sabinene (37.1%) found in the Idaho sample compared to those found in the Wyoming collection (56.6%-61.0%). Pregeijerene B was found in 2.3% to 4.4% in the Wyoming samples, but was not detected in the Idaho sample.

The Idaho *J scopulorum* essential oil had higher concentrations of terpinen-4-ol (13.2%), α-elemol (9.7%), and 8α-acetoxyelemol (6.4%) compared to the essential oils from Wyoming (3.3%-5.9%, 0.0%-5.6%, and 0.0%-4.3%, respectively). The limonene concentration was lower in the Idaho sample (2.0%) than the samples from Wyoming (2.5%-22.0%).

Monoterpenoid Enantiomeric Distributions

A chiral gas chromatography–mass spectrometry (GC-MS) analysis was carried out on *J horizontalis* and *J scopulorum* essential oils to assess the enantiomeric distributions of monoterpenoids (Table 2). Additionally, 2 commercial *J communis* fruit essential oils from Nepal and 10 samples from Albania were analyzed for comparison. Enantiomeric distributions of monoterpenoids in *J communis*, *J oxycedrus*, and *Juniperus phoenicea* reported in the literature[14-16] are also included in Table 2 for comparison.

The monoterpene hydrocarbons α-thujene, sabinene, β-pinene, and δ-3-carene were found to occur in both *J horizontalis* and *J scopulorum* as their pure (+) enantiomers. (+)-α-Thujene dominated most of the *J communis* fruit essential oils examined with the exception of 2 samples from Albania (Table 2, samples #2 and #3). Pure (+)-sabinene and (+)-δ-3-carene were found in *J communis* leaf essential oils from Poland.[17] (+)-Sabinene dominated the fruit essential oils of commercial *J communis* (Table 2), but was the minor enantiomer in *J oxycedrus* and *J phoenicea* leaf essential oils.[16] (+)-δ-3-Carene also dominates the essential oils of *J oxycedrus*, *J phoenicea*, and *J communis* fruit essential oils. In contrast to *J horizontalis* and *J scopulorum* essential oils, pure (–)-β-pinene was found in *J communis* leaf oil,[17] which is consistent with *J communis* fruit essential oils (Table 2).

The major α-pinene enantiomer in *J horizontalis* and *J scopulorum* essential oils was (+)-α-pinene, consistent with that observed in *J communis* leaf essential oil[15,17,18] *J oxycedrus*,[14] and *J phoenicea*[16] leaf essential oils. Interestingly, commercial *J communis* fruit essential oils from Nepal showed (+)-α-pinene predominating, but those from Albania had (–)-α-pinene as the major enantiomer.

(+)-Limonene generally seems to predominate in *Juniperus* essential oils, but there are some exceptions in *J communis* leaf[17] and fruit (Albania samples #2 and #3) essential oils. (+)-Linalool predominated in the leaf essential oils of *J horizontalis*, *J scopulorum*, and *J phoenicea*, whereas (–)-linalool predominated in *J oxycedrus* leaf oil and the fruit essential oils of *J communis*. There was a slight excess of (+)-terpinen-4-ol in *J horizontalis* and *J scopulorum* essential oils, whereas a slight excess of (–)-terpinen-4-ol was observed in *J communis*, *J oxycedrus*, and *J phoenicea* leaf essential oils. Commercial *J communis* fruit essential oils also showed a slight excess of (+)-terpinen-4-ol with the exception of two samples from Albania (#2 and #3).

Conclusions

The essential oil compositions of *J horizontalis* and *J scopulorum* growing in Idaho showed qualitative similarities to those *Juniperus* essential oils growing in Wyoming, but with some quantitative differences. The enantiomeric distributions of the major components, α-pinene, sabinene, and terpinene-4-ol, were comparable to the enantiomeric distributions observed in commercial *J communis* fruit essential oils.

Materials and Methods

Plant Material

Samples of *J horizontalis* and *J scopulorum* were obtained from the Idaho Botanical Garden (43°36′04″N, 116°09′35″W, 862 m elevation) on July 29, 2021. The plants were identified by Daniel Murphy, Collections Curator, Idaho Botanical Garden. Voucher specimens (WNS22192758 and WNS22114504) have been deposited in the herbarium of the University of Alabama in Huntsville (HALA). For *J horizontalis*, fresh leaves and twigs (167.80 g) were hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane for 3 h to give a colorless essential oil (533.8 mg). Fresh leaves and twigs of *J scopulorum* (93.90 g) were hydrodistilled as above to give a colorless essential oil (510.1 mg).

Commercial Essential Oils from APRC

Commercial samples of *J communis* fruit essential oils from Nepal and from Albania were obtained from the Aromatic

Table 2. Enantiomeric Distribution [%(+):%(–)] of Monoterpenoids in *Juniperus* Essential Oils.

Compound	*Juniperus horizontalis*[a]	*Juniperus scopulorum*[a]	*Juniperus communis* [15]	*Juniperus oxycedrus* [14]	*Juniperus phoenicea* [16]	*Juniperus communis*, Nepal[b] #1	*Juniperus communis*, Nepal[b] #2
α-Thujene	100:0	100:0	-	-	-	82.0:18.0	100:0
α-Pinene	81.4:18.6	91.7:8.3	57.0:43.0	88.6:11.4	89.1:10.9	62.2:37.8	80.5:19.5
Camphene	62.7:37.3	52.0:48.0	38.3:61.7	79.1:20.9	80.1:19.9	-	65.9:34.1
Sabinene	100:0	100:0	-	40.1:50.9[c]	39.7:60.3	89.5:10.5	100:0
β-Pinene	100:0	100:0	45.7:54.3	56.4:43.6	51.7:48.3	15.3:84.7	30.9:69.1
δ-3-Carene	100:0	100:0	-	74.3:25.7	98.6:1.4	100:0	100:0
α-Phellandrene	-	-	-	-	-	-	-
α-Terpinene	-	-	-	-	-	-	-
Limonene	82.8:17.2	90.1:9.9	77.9:22.1	95.1:4.9	81.6:18.4	97.5:2.5	97.4:2.6
β-Phellandrene	30.9:69.1	46.9:53.1	-	-	-	-	29.3:70.7
cis-Sabinene hydrate	97.7:2.3	95.9:4.1	-	-	-	-	-
Linalool	61.2:38.8	87.6:12.4	-	36.6:63.4	73.1:26.9	-	-
trans-Sabinene hydrate	97.8:2.2	-	-	-	-	-	-
Borneol	0:100	-	8.6:91.4	68.9:31.1	49.7:50.3	-	-
Terpinen-4-ol	66.6:33.4	53.0:47.0	32.8:67.2	22.5:77.5	31.5:68.5	70.6:29.4	71.9:28.1
α-Terpineol	50.6:49.4	54.0:46.0	-	-	-	-	-
Bornyl acetate	0:100	0:100	-	94.5:5.5	91.5:8.5	0:100	0:100
α-Terpinyl acetate	-	-	-	-	-	-	-

Compound	*Juniperus communis*, Albania[b] #1	#2	#3	#4	#5	#6	#7	#8	#9	#10
α-Thujene	99.0:1.0	16.0:84.0	17.2:82.8	98.8:1.2	98.6:1.4	98.7:1.3	100:0	100:0	99.2:0.8	98.8:1.2
α-Pinene	28.7:71.3	46.9:53.1	40.5:59.5	33.5:66.5	29.3:70.7	35.4:64.6	31.7:68.3	34.2:65.8	48.3:51.7	49.7:50.3
Camphene	100:0	100:0	100:0	100:0	100:0	100:0	23.8:76.2	25.7:74.3	100:0	100:0
Sabinene	99.7:0.3	100:0	100:0	100:0	95.8:4.2	95.5:4.5	100:0	100:0	99.8:0.2	99.8:0.2
β-Pinene	10.5:89.5	6.8:93.2	6.8:93.2	6.5:93.5	8.3:91.7	10.2:89.8	11.3:88.7	7.6:92.4	24.7:75.3	25.9:74.1
δ-3-Carene	100:0	100:0	100:0	100:0	100:0	100:0	-	-	100:0	100:0
α-Phellandrene	-	100:0	100:0	36.6:63.4	55.9:44.1	56.1:43.9	53.9:46.1	60.4:39.6	14.5:85.5	14.6:85.7
α-Terpinene	-	-	-	-	100:0	100:0	-	-	-	-
Limonene	89.3:10.7	32.2:67.8	30.9:69.1	84.4:15.6	81.8:18.2	79.9:20.1	88.0:12.0	78.2:21.8	90.3:9.7	89.2:10.8
β-Phellandrene	0:100	-	-	0:100	0:100	0:100	22.7:77.3	28.6:71.4	0:100	0:100
cis-Sabinene hydrate	-	-	-	-	-	-	-	-	-	-
Linalool	-	-	-	39.5:60.5	40.0:60.0	42.1:57.9	-	41.3:58.7	-	-
trans-Sabinene hydrate	-	-	-	-	-	-	-	-	-	-
Borneol	-	-	-	-	-	-	-	-	-	-
Terpinen-4-ol	78.6:21.4	30.2:69.8	30.5:69.5	78.0:22.0	77.5:22.5	75.7:24.3	79.8:20.2	76.6:23.4	-	78.3:21.7
α-Terpineol	44.5:55.5	-	-	39.1:60.9	41.8:58.2	41.4:58.6	43.1:56.9	-	47.1:52.9	45.3:54.7
Bornyl acetate	0:100	-	0:100	0:100	0:100	0:100	0:100	0:100	0:100	0:100
α-Terpinyl acetate	0:100	-	-	-	-	-	-	-	0:100	0:100

Note: [a]*Juniperus* essential oil from Idaho.
[b]Commercial *Juniperus communis* fruit essential oils from the Aromatic Plant Research Center (APRC) collection.
[c]The reported percentages to not add up to 100%.

Plant Research Center (APRC) collection and analyzed as received.

GC-MS

The essential oils of *J horizontalis* and *J scopulorum* were analyzed by GC-MS as reported previously[19]: Shimadzu GCMS-QP2010 Ultra with a ZB-5 ms GC column (60 m length, 0.25 mm diameter, 0.25 μm film thickness), injector and detector temperatures 260 °C, He carrier gas with column head pressure of 208 kPa and flow rate of 2.00 mL/min, GC oven temperature program 50 °C to 260 °C (2 °C/min), and 1.0 μL injection of 5% solution of each essential oil in dichloromethane (split mode, 24:1). The retention indices (RIs) were determined using a series of reference *n*-alkanes. The compounds listed in Table 1 were identified by comparing the mass spectrum fragmentation data and calculated RIs with those in the databases.[10-13]

GC(FID)

The essential oils of *J horizontalis* and *J scopulorum* were analyzed by gas chromatography–flame ionization detection (GC(FID)) as reported previously[20]: Shimadzu GC 2010 with FID detector, ZB-5 GC column, same operating conditions as above for GC-MS. The percent compositions were determined from raw peak areas without standardization.

Chiral GC-MS

The *J horizontalis*, *J scopulorum*, and *J communis* essential oils were analyzed by chiral GC-MS as previously reported[20]: Shimadzu GCMS-QP2010S instrument, Restek B-Dex 325 column (30 m length, 0.25 mm diameter, 0.25 μm film thickness), injector and detector temperatures 240 °C, He carrier gas with column head pressure of 53.8 kPa and flow rate of 1.00 mL/min, GC oven temperature program 50 °C, held for 5 min, increased 1.0 °C/min to 100 °C, then increased 2.0 °C/min to 220 °C; and 0.3 μL injection of 5% solution of essential oil samples in dichloromethane (split mode, 24:1) was injected. The enantiomeric distributions were determined by comparison of retention times with authentic samples obtained from Sigma-Aldrich (Milwaukee, WI, USA). The relative enantiomer percentages were calculated from peak areas.

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Statement of Human and Animal Rights

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Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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