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Research Article

Chemical composition of the essential oil of *Juniperus maritima*: An exploratory chemotaxonomic comparison with published *Juniperus scopulorum* essential oils

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Chemotaxonomy, phenylpropanoids, sabinene, elemicin, gas chromatography, enantioselective, chiral.

Abstract

World Flora Online and Flora of North America treat *Juniperus maritima* and *Juniperus scopulorum* as conspecifics. However, Robert P. Adams concluded that these two species are separate. This study aimed to compare the foliar essential oils of six *J. maritima* samples, collected from two habitats in the Puget Sound area of northwestern Washington state, with the essential oils of *J. scopulorum* reported in the literature. The essential oils were obtained by hydrodistillation and characterized by gas chromatography and enantioselective gas chromatography. The essential oil compositions of *J. maritima* and *J. scopulorum* were considerably different with elemicin dominating *J. maritima* essential oils (19.9-34.7%), but apparently not observed in previously published *J. scopulorum*; the essential oils of *J. scopulorum*, on the other hand, were rich in sabinene (29.8-86.5%). The enantiomeric distributions of chiral monoterpenoids in *J. maritima* are consistent with the distributions observed in other *Juniperus* species. However, a limitation of this study is the small number of samples available; the findings are exploratory and limited in scope.

1. Introduction

Essential oils are mixtures of volatile terpenoids, phenylpropanoids, and other secondary metabolites [1]. The compositions of essential oils have been exploited as chemotaxonomic markers to differentiate closely related taxa [2]. Essential oil compositions with chemometric analyses such as principal component analysis (PCA) or hierarchical cluster analysis (HCA), have been used to discriminate taxa where morphological differentiation is subtle or cryptic, such as in *Piper* [3], *Eugenia* [4], and *Aniba* [5] species. Studies of essential oil compositions coupled with chemometrics have also been conducted on coniferous species such as *Pinus* [6] and *Sabina* [7] as well as *Juniperus* [8–10].

The World Flora Online treats *Juniperus maritima* R.P. Adams (Puget Sound juniper, Cupressaceae) as conspecific with *Juniperus scopulorum* Sarg. (Rocky Mountain juniper) [11]. The natural range of *J. maritima* includes the coastal areas of southwestern British Columbia, Canada, and northwestern Washington, U.S.A., whereas the range of *J. scopulorum* is widespread in western North America

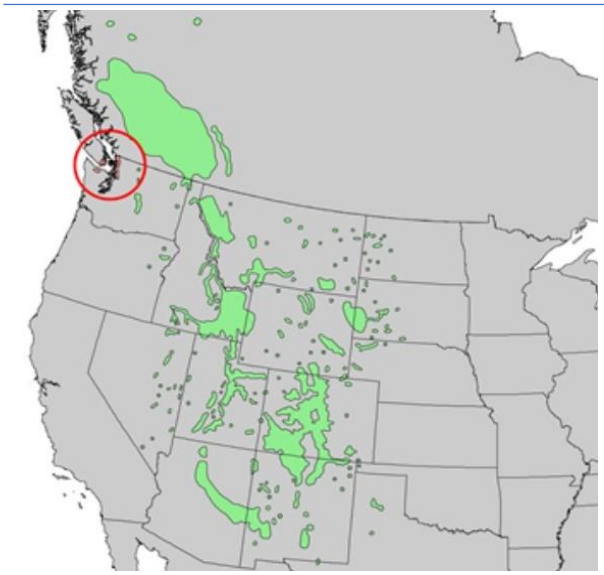

Figure 1. Native ranges of *Juniperus scopulorum* Sarg. (green) and *Juniperus maritima* R.P. Adams (red) [13]. This work is in the public domain in the United States because it is a work prepared by an officer or employee of the United States Federal Government as part of that person's official duties under the terms of Title 17, Chapter 1, Section 105 of the US Code.

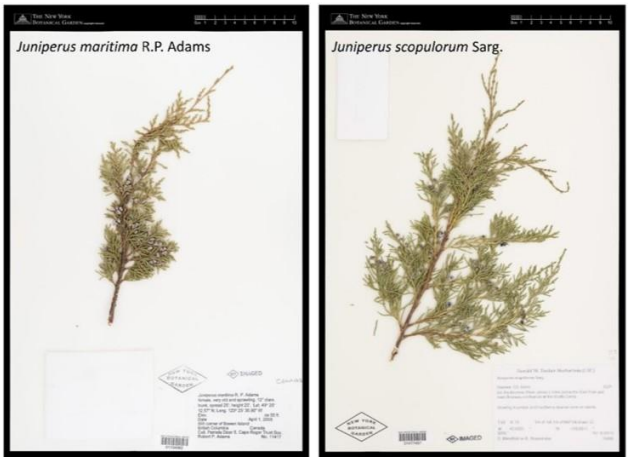

Figure 2. Herbarium specimens of *Juniperus maritima* R.P. Adams (image 01104983) and *Juniperus scopulorum* Sarg. (image 01477457) from the C.V. Starr Virtual Herbarium of the New York Botanical Garden [14].

(Fig. 1). The morphology of the two species is barely distinguishable (Fig. 2), but Adams, using nuclear ribosomal DNA and single nucleotide polymorphisms concluded that *J. maritima* should be considered a species separate from *J. scopulorum* [12]. This study aimed to examine the essential oil composition, including the enantiomeric distributions of chiral monoterpenoids, of *J. maritima* from Puget Sound, Washington, and compare it with previously published essential oil compositions of *J. scopulorum*. We hypothesized that differences in essential oil compositions could provide complementary chemotaxonomic evidence for the separation of the two *Juniperus* species.

2. Materials and methods

2.1. Plant material

Foliage of *J. maritima* was collected on 2 September 2025, from three individual mature trees located on sand dunes near Deception Pass (mid-day, 11:50-12:10) and from three mature individuals on a bluff overlooking Burrows Bay (early afternoon, 13:50-14:10), Washington (Fig. 3, Table 1). The plants were identified by W.N. Setzer in the field and verified by comparison with herbarium samples from the C.V. Starr Virtual Herbarium of the New York Botanical Garden [14]. Voucher samples (WNS-Jmar-2609 and WNS-Jmar-2631) were deposited at the University of Alabama in Huntsville Herbarium. Fresh plant materials were immediately frozen and stored (–20 °C) until processing. The fresh/frozen foliage of the six *J. maritima* samples were subjected to hydrodistillation (4 h) using a Likens-Nickerson apparatus (500-mL distillation flask, foliage, and sufficient distilled water to cover the plant material) with continuous extraction of the distillate with dichloromethane (20 mL) to obtain colorless essential oils (Table 1). The obtained essential oils were stored at –20 °C until analysis.

2.2. Gas chromatographic analysis

The *J. maritima* foliar essential oils were analyzed by GC-MS and chiral GC-MS, as previously described [15, 16]. The instrumental details for GC-MS and chiral GC-MS are summarized in Table 2. Each essential oil was analyzed by GC-MS and chiral GC-MS using a single injection for each measurement. Retention index values were calculated using the van den Dool and Kratz method [17]. The components of the essential oils were determined by comparing the retention indices (within 5 RI units) and mass spectral fragmentation (similarity index > 80%) with those in the databases of Adams [18], Satyal [19], Mondello [20], and NIST20 [21]. The percentages of the essential oil components were calculated based on peak integration without standardization.

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Figure 3. Collection sites of *Juniperus maritima* from northwestern Washington (Google Earth Pro, v. 7.3.7, accessed on 6 March, 2026).

Table 1. Collection and hydrodistillation details of *Juniperus maritima* from northwestern Washington.

Sample	Collection location	Mass foliage (g)	Mass essential oil (g)	Yield[d] (%)
J.m. #1[a]	48°23'44" N, 122°39'51" W, 4 m asl	123.11	4.456	3.62
J.m. #2[a]	48°23'42" N, 122°39'51" W, 5 m asl	106.72	3.110	2.91
J.m. #3[b]	48°23'41" N, 122°39'50" W, 4 m asl	126.61	4.059	3.21
J.m. #4[b]	48°29'30" N, 122°41'36" W, 65 m asl	108.37	4.030	3.72
J.m. #5[b]	48°29'31" N, 122°41'37" W, 63 m asl	95.18	1.928	2.03
J.m. #6[c]	48°29'30" N, 122°41'36" W, 65 m asl	153.34	3.785	2.47

[a] Male tree. [b] Gender not determined. [c] Female tree. Yield[d] (%) = 100 × mass essential oil/mass fresh/frozen foliage.

2.3. Statistical analyses

Agglomerative hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). For the HCA, the major components (α-pinene, sabinene, myrcene, limonene, γ-terpinene, terpinen-4-ol, pregeijerene B, safrole, methyl eugenol, α-elemol, elemicin, α-cadinol, and 8α-acetoxyelemol) were used for the analysis, dissimilarity was used to determine clustering based on the Euclidean distance, and Ward's method was used to define the agglomeration. For the PCA, a Pearson correlation analysis was used to corroborate the results of the HCA using the same major components. Analysis of variance (ANOVA), followed by Tukey's post hoc test, and Student's *t*-test were carried out using Minitab® v. 22.4.0 (Minitab Inc., State College, PA, USA). Normal distributions were assumed and no corrections were applied. Differences were considered statistically significant at $p < 0.05$.

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Table 2. Instrument details for the gas chromatographic analyses of *Juniperus maritima*.

Gas Chromatography - Mass Spectrometry (GC-MS)	
Instrument	Shimadzu GC-MS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD, USA)
GC Column	Zebron ZB-5ms fused silica capillary column (60 m × 0.25 mm × 0.25 μm film thickness) (Phenomenex, Torrance, CA, USA)
MS Detector Conditions	Electron impact (EI) mode, electron energy = 70 eV, scan range = 40–400 atomic mass units, scan rate = 3.0 scans/second
Carrier Gas, Conditions	Helium, column head pressure = 208.3 kPa, flow rate = 2.00 mL/min
Injector, Detector Temperatures	Injector temperature = 260 °C, interface temperature = 260 °C, ion source temperature = 260 °C
GC Oven Temperature Program	Initial temperature = 50 °C, ramp 2 °C/min to 260 °C, hold 260 °C for 5 min
Sample Concentration, Volume Injected	5% (in dichloromethane), 0.1 μL volume
Split Mode	24.5 : 1.0
Chiral Gas Chromatography - Mass Spectrometry	
Instrument	Shimadzu GCMS-QP2010S (Shimadzu Scientific Instruments, Columbia, MD, USA)
GC Column	Restek B-Dex 325 chiral GC column (30 m × 0.25 mm × 0.25 μm film thickness) (Restek Corp., Bellefonte, PA, USA)
MS Detector Conditions	Electron impact (EI) mode, electron energy = 70 eV, scan range = 40–400 atomic mass units, scan rate = 3.0 scans/second
Carrier Gas, Conditions	Helium, column head pressure = 53.6 kPa, flow rate = 1.00 mL/min
Injector, Detector Temperatures	Injector temperature = 240 °C, interface temperature = 240 °C, ion source temperature = 240 °C
GC Oven Temperature Program	Initial temperature = 50 °C, hold for 5 min, ramp 1 °C/min to 100 °C, ramp 2 °C/min to 220 °C
Sample Concentration, Volume Injected	5% (in dichloromethane), 0.3 μL volume
Split Mode	24.0 : 1.0

3. Results and discussion

3.1. Chemical composition of Juniperus maritima essential oil

The clear colorless essential oils were obtained by hydrodistillation of the foliage, with yields of 2.03-3.72% (w/w). The *J. maritima* essential oils were analyzed by GC-MS. A total of 149 compounds were identified in the six essential oil samples, which accounted for 98.9-99.8% of the compositions (Table 3).

The *J. maritima* foliage was collected from trees growing in different habitats. Samples #1, #2, and #3 were collected from trees growing on sand dunes near the water's edge, whereas samples #4, #5, and #6 were collected from trees growing on a sandstone bluff overlooking the bay (Fig. 3). The major components in the essential oils were sabinene (4.6-11.0% for the dunes trees, 15.4-21.8% for the bluff trees), limonene (19.9-33.2% for the dunes trees, but only 0.6-1.3% for the bluff trees), terpinen-4-ol (2.6-4.8% for the dunes trees, 6.0-12.2% for the bluff trees), safrole (0.4-4.6%), methyl eugenol (2.4-15.6%), elemicin (19.9-34.7%), and 8α-acetoxyelemol (4.7-10.1%).

3.2. Comparison with published Juniperus scopulorum essential oils

Although both World Flora Online [11] and Flora of North America [22] treat the two as conspecific, Adams concluded that *J. maritima* and *J. scopulorum* are different species based on essential oil composition, leaf morphology, and DNA sequence data [12]. A comparison of the major essential oil components of *J. maritima* with *J. scopulorum* shows noteworthy differences in support of the treatment by Adams. However, we cannot rule out potential hybridization [23], abiotic environmental characteristics of the collection sites [24, 25], or biotic factors, such as herbivory [26, 27] or fungal infection [28].

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Table 3. Foliar essential oil compositions (percentages) of *Juniperus maritima* from northwestern Washington, U.S.A.

RI_{calc}	RI_{db}	Compounds	J.m. #1	J.m. #2	J.m. #3	J.m. #4	J.m. #5	J.m. #6
783	780	Methyl 2-methylbutanoate	0.1	tr	0.1	0.2	0.1	tr
801	801	Hexanal	tr	0.1	tr	tr	tr	tr
848	850	(2*E*)-Hexenal	0.1	0.1	tr	0.1	0.1	0.1
849	853	(3*Z*)-Hexenol	0.1	tr	tr	0.1	0.1	tr
922	923	Tricyclene	tr	tr	tr	tr	tr	tr
925	925	α-Thujene	0.4	0.3	0.3	0.8	0.7	0.9
932	**932**	**α-Pinene**	**0.5**	**0.3**	**0.4**	**0.8**	**0.5**	**0.8**
941	950	4-Methyl-1-hexanol	tr	-	0.1	tr	-	tr
942	942	Isopropyl butanoate	-	-	-	-	tr	-
947	948	α-Fenchene	tr	tr	tr	tr	tr	tr
948	950	Camphene	tr	tr	tr	tr	tr	tr
954	956	(2*E*)-Heptenal	-	tr	tr	tr	tr	tr
968	968	Isopentyl propanoate	-	-	-	tr	-	-
971	**971**	**Sabinene**	**11.0**	**4.6**	**9.8**	**21.8**	**15.4**	**16.7**
977	978	β-Pinene	-	tr	tr	-	-	-
977	978	1-Octen-3-ol	0.2	0.1	0.2	0.4	0.4	0.3
983	983	Octan-3-one	-	-	-	-	-	tr
988	**989**	**Myrcene**	**1.1**	**0.8**	**1.0**	**1.4**	**0.7**	**1.0**
992	992	6-Methyl-5-hepten-2-ol (= Sulcatol)	-	-	-	-	tr	-
995	996	Butyl butanoate	tr	tr	0.1	-	tr	tr
996	992	Methyl (2*E*)-heptenoate	0.1	0.1	0.5	1.0	tr	tr
998	997	Ethyl hexanoate	-	-	-	tr	tr	tr
1004	1005	(3*Z*)-Hexenyl acetate	-	-	-	0.1	-	-
1006	1006	α-Phellandrene	tr	tr	tr	tr	tr	0.1
1008	1008	δ-3-Carene	-	-	tr	-	-	tr
1017	1017	α-Terpinene	0.9	0.5	0.7	1.7	1.2	2.5
1024	1025	*p*-Cymene	tr	tr	tr	0.1	0.1	0.1
1030	**1030**	**Limonene**	**25.3**	**33.2**	**19.9**	**1.3**	**0.6**	**1.1**
1032	1031	β-Phellandrene	0.1	0.1	0.1	0.1	0.1	0.2
1032	1033	Benzyl alcohol	-	tr	-	tr	tr	tr
1034	1034	Isopropyl hexanoate	-	-	-	tr	tr	tr
1039	1040	2-Methylbutyl butanoate	-	-	-	-	tr	tr
1045	1047	Butyl isopentanoate	-	-	-	tr	tr	tr
1054	1056	Isopentyl butanoate	0.3	tr	tr	tr	0.4	tr
1057	**1057**	**γ-Terpinene**	**1.4**	**0.8**	**1.2**	**2.6**	**1.9**	**3.8**
1063	1064	3-Methyl-2-butenyl butanoate	0.1	-	tr	tr	0.1	tr
1069	1069	*cis*-Sabinene hydrate	0.4	0.3	0.5	1.0	0.8	0.7
1081	1083	Heptyl acetate	-	-	-	0.2	-	-
1085	1086	Terpinolene	0.8	0.6	0.6	0.9	0.6	1.1
1088	1090	Fenchone	tr	tr	tr	-	-	-
1093	1094	Methyl benzoate	0.1	tr	tr	tr	tr	tr
1099	1099	Linalool	0.5	0.2	0.7	0.7	0.5	0.2
1100	1101	*trans*-Sabinene hydrate	0.7	0.3	0.6	0.9	0.7	0.7
1104	1104	Nonanal	tr	0.1	0.1	-	tr	tr
1106	1109	Isopentyl isopentanoate (= Solustrerol)	tr	tr	tr	tr	0.6	0.1
1109	1108	*cis*-Rose oxide	-	-	-	tr	tr	-
1115	1115	3-Methyl-3-butenyl isopentanoate	tr	-	tr	tr	0.2	tr
1117	1117	β-Thujone	tr	-	tr	tr	tr	tr
1118	1120	*endo*-Fenchol	0.1	tr	tr	-	-	-

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Table 3. *(Continued).*

RI_{calc}	RI_{db}	Compounds	J.m. #1	J.m. #2	J.m. #3	J.m. #4	J.m. #5	J.m. #6
1118	1120	*trans*-4-Methoxythujane	-	-	-	-	0.1	-
1123	1124	*cis*-*p*-Menth-2-en-1-ol	0.3	0.2	0.2	0.6	0.4	0.7
1125	1127	*trans*-Rose oxide	-	-	-	tr	tr	-
1125	1121	*trans*-Pinene hydrate	tr	tr	tr	-	-	-
1136	1134	*cis*-Limonene oxide	0.1	0.1	tr	-	-	-
1137	1140	3-Methyl-2-butenyl 2-methylbutanoate	-	-	-	-	tr	-
1141	1142	*trans*-*p*-Menth-2-en-1-ol	0.2	0.1	0.2	0.4	0.3	0.5
1147	1148	3-Methyl-2-butenyl pentanoate	tr	-	-	tr	0.1	tr
1151	1151	Citronellal	0.1	tr	tr	tr	tr	tr
1154	1156	Camphene hydrate	tr	-	-	-	-	-
1155	1157	Sabina ketone	-	-	-	-	-	tr
1171	1170	Borneol	0.1	tr	0.1	0.1	0.1	tr
1177	1179	2-Isopropenyl-5-methyl-4-hexenal	-	tr	tr	-	-	-
1180	**1180**	**Terpinen-4-ol**	**4.8**	**2.6**	**3.7**	**9.3**	**6.0**	**12.2**
1184	1187	(3*Z*)-Hexenyl butanoate	tr	tr	tr	tr	tr	0.1
1185	1186	*p*-Cymen-8-ol	tr	tr	tr	tr	tr	tr
1189	1190	Butyl hexanoate	-	-	-	-	-	tr
1190	1190	Methyl salicylate	tr	tr	tr	-	tr	0.1
1194	1195	α-Terpineol	0.2	0.2	0.2	0.4	0.2	0.5
1196	1196	*cis*-Piperitol	0.1	tr	tr	0.1	0.1	0.2
1197	1197	Methyl chavicol (= Estragol)	0.2	0.5	0.4	0.1	0.4	0.3
1208	1208	*trans*-Piperitol	0.1	tr	0.1	0.2	0.1	0.2
1226	1227	Citronellol	1.6	0.7	0.7	0.9	0.6	0.3
1228	1226	*iso*-Nerol	tr	-	0.7	-	tr	tr
1229	1231	(3*Z*)-Hexenyl 2-methylbutanoate	-	-	-	-	-	tr
1235	1235	(3*Z*)-Hexenyl isopentanoate	-	-	-	tr	tr	tr
1240	1242	Hexyl isopentanoate	-	-	-	-	-	tr
1242	1242	Carvone	0.1	tr	tr	-	-	-
1249	1252	Isopentyl hexanoate	tr	-	-	-	tr	-
1249	1249	Geraniol	-	-	-	tr	-	-
1255	1255	(4*Z*)-Decen-1-ol	tr	tr	0.1	tr	0.1	0.2
1256	1256	Methyl citronellate	0.2	0.3	tr	0.1	0.2	0.1
1265	1264	(*Z*)-Anethole	-	-	-	-	tr	-
1278	**1278**	**Pregeijerene B**	**2.8**	**2.3**	**2.1**	**1.5**	**1.8**	**1.6**
1283	1282	Bornyl acetate	tr	tr	tr	tr	tr	tr
1285	1285	(*E*)-Anethole	tr	0.1	tr	tr	tr	tr
1288	**1291**	**Safrole**	**2.6**	**0.4**	**4.3**	**3.9**	**2.9**	**4.6**
1292	1292	3-Methyl-2-butenyl hexanoate	tr	-	tr	-	-	-
1293	1293	(2*E*,4*Z*)-Decadienal	0.1	tr	0.1	tr	tr	tr
1309	1307	4-Methylhexenyl 2-methylbutanoate	-	-	-	-	-	tr
1312	1310	(2*E*,4*E*)-Decadienol	0.3	0.1	0.3	0.2	0.2	0.4
1317	1318	(2*E*,4*E*)-Decadienal	0.1	tr	0.1	0.1	tr	0.1
1319	1319	Methyl geranate	tr	tr	-	tr	tr	tr
1326	1327	*p*-Mentha-1,4-dien-7-ol	-	-	-	-	-	tr
1346	1348	α-Cubebene	-	-	-	tr	tr	tr
1348	1349	Citronellyl acetate	-	-	-	0.1	tr	-
1349	1356	Eugenol	0.1	0.3	0.6	0.9	0.1	0.5
1364	1362	Chavibetol	tr	tr	0.1	tr	0.1	tr
1374	1375	α-Copaene	-	-	-	tr	tr	tr

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Table 3. *(Continued).*

RI_{calc}	RI_{db}	Compounds	J.m. #1	J.m. #2	J.m. #3	J.m. #4	J.m. #5	J.m. #6
1386	1387	β-Cubebene	-	-	-	tr	tr	tr
1388	1390	*trans*-β-Elemene	-	-	-	tr	tr	tr
1398	**1403**	**Methyl eugenol**	**2.4**	**15.6**	**10.8**	**4.2**	**5.4**	**6.2**
1418	1424	(*E*)-β-Caryophyllene	0.1	0.1	tr	0.1	0.1	0.1
1427	1427	γ-Elemene	-	-	-	-	-	tr
1433	1433	*cis*-Thujopsene	-	-	-	-	0.1	tr
1447	1446	*cis*-Muurola-3,5-diene	-	-	-	0.3	0.2	0.1
1454	1454	α-Humulene	-	-	-	0.1	tr	tr
1470	1472	*trans*-Cadina-1(6),4-diene	tr	tr	tr	0.3	0.2	0.2
1473	1475	γ-Muurolene	tr	tr	tr	tr	tr	tr
1479	1480	Germacrene D	tr	tr	tr	tr	tr	tr
1490	1490	γ-Amorphene	tr	tr	tr	0.5	0.4	0.3
1493	1497	*epi*-Cubebol	tr	tr	tr	0.4	0.3	0.2
1496	1497	α-Muurolene	0.1	0.1	-	-	-	0.1
1511	1512	γ-Cadinene	0.1	tr	0.1	tr	tr	tr
1513	1515	Cubebol	tr	tr	tr	1.2	0.7	0.3
1516	1518	δ-Cadinene	0.5	0.3	0.4	0.7	0.7	0.5
1520	1519	*trans*-Calamenene	-	-	-	tr	tr	tr
1521	1521	Zonarene	-	-	-	0.2	0.2	0.1
1530	1533	*trans*-Cadina-1,4-diene	-	-	-	0.1	tr	tr
1535	1536	α-Copaen-11-ol	0.1	0.1	tr	0.1	0.1	0.1
1535	1538	α-Cadinene	tr	tr	tr	tr	tr	-
1539	1540	Liguloxide	tr	-	-	-	-	-
1548	**1549**	**α-Elemol**	**1.0**	**tr**	**1.8**	**1.2**	**1.4**	**1.2**
1550	**1551**	**Elemicin**	**27.1**	**19.9**	**27.5**	**25.6**	**34.7**	**28.8**
1557	1557	Germacrene B	tr	-	tr	tr	tr	tr
1560	1560	(*E*)-Nerolidol	tr	-	-	-	-	-
1575	1574	Germacra-1(10),5-dien-4β-ol	0.4	0.2	0.4	0.4	0.4	0.1
1580	1587	Caryophyllene oxide	tr	0.1	0.1	0.1	0.1	0.1
1591	1592	Methoxyeugenol	-	-	-	0.3	-	-
1601	1600	α-Oplopenone	0.1	0.1	0.1	0.1	0.1	0.1
1606	1606	Cedrol	tr	tr	-	-	-	-
1607	1609	Humulene epoxide II	-	tr	-	-	-	-
1613	1614	1,10-di-*epi*-Cubenol	tr	tr	tr	-	-	-
1620	1624	*epi*-γ-Eudesmol	tr	tr	tr	-	-	-
1626	1628	1-*epi*-Cubenol	tr	0.1	tr	1.2	0.9	0.7
1630	1632	γ-Eudesmol	0.3	0.2	0.2	0.2	0.2	0.3
1640	1643	τ-Cadinol	0.2	0.3	0.2	0.2	0.3	0.2
1642	1644	τ-Muurolol	0.3	0.4	0.3	0.4	0.6	0.3
1643	1645	(*E*)-*iso*-Elemicin	0.1	tr	tr	tr	tr	0.1
1644	1643	α-Muurolol (= δ-Cadinol)	0.1	0.1	0.1	0.1	0.2	0.1
1647	1647	*cis*-Guaia-3,9-dien-11-ol	tr	-	-	-	-	-
1652	1652	α-Eudesmol	0.4	0.2	0.2	0.2	0.1	0.3
1653	**1655**	**α-Cadinol**	**1.1**	**1.3**	**1.0**	**0.9**	**1.4**	**0.6**
1680	1686	Botrydiol	0.1	0.1	0.1	-	0.1	tr
1691	1689	Shyobunol	tr	tr	tr	tr	0.1	tr
1737	1740	8α,11-Elemodiol	0.1	0.1	0.1	0.1	0.1	tr
1778	**1779**	**8α-Acetoxyelemol**	**7.0**	**10.1**	**5.8**	**4.7**	**9.8**	**5.8**
2287	2295	4-*epi*-Abietal	0.1	0.1	0.1	0.2	0.1	0.1

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Table 3. *(Continued).*

	Compound classes					
	Monoterpene hydrocarbons	41.4	41.2	33.9	31.6	21.9	28.4
	Oxygenated monoterpenoids	9.3	4.8	7.7	14.7	10.0	16.4
	Sesquiterpene hydrocarbons	0.7	0.4	0.5	2.3	1.7	1.4
	Oxygenated sesquiterpenoids	11.1	13.2	10.4	11.4	16.7	10.2
	Diterpenoids	0.1	0.1	0.1	0.2	0.1	0.1
	Benzenoid aromatics	32.5	36.7	43.6	35.0	43.6	40.4
	Non-terpenoid esters	0.6	0.1	0.7	1.5	1.6	0.2
	Others	3.6	2.7	3.0	2.3	2.7	2.5
	Total identified	***99.4***	***99.2***	***99.8***	***98.9***	***98.4***	***99.6***

RI_{calc} = Retention index values determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Reference retention index values from the databases. tr = trace (< 0.05%). Compounds used for the HCA and PCA analyses are highlighted in bold.

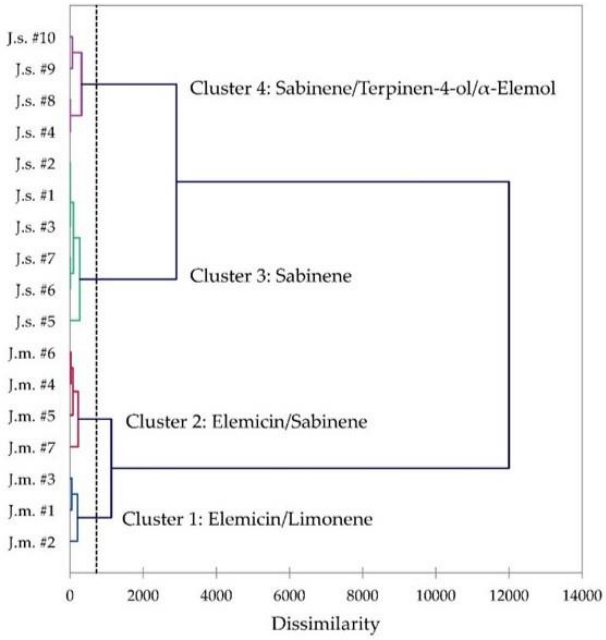

Figure 4. Dendrogram obtained from agglomerative hierarchical cluster analysis (HCA) based on the major components in the foliar essential oils of *Juniperus maritima* and *Juniperus scopulorum*. J.m. #1-#6 (this study), J.m. #7 [43], J.s. #1-#3 [44], J.s. #4 [43], J.s. #5 [45], J.s. #6 [46], J.s. #7 [47], J.s. #8 [48], J.s. #9 [49], J.s. #10 [50].

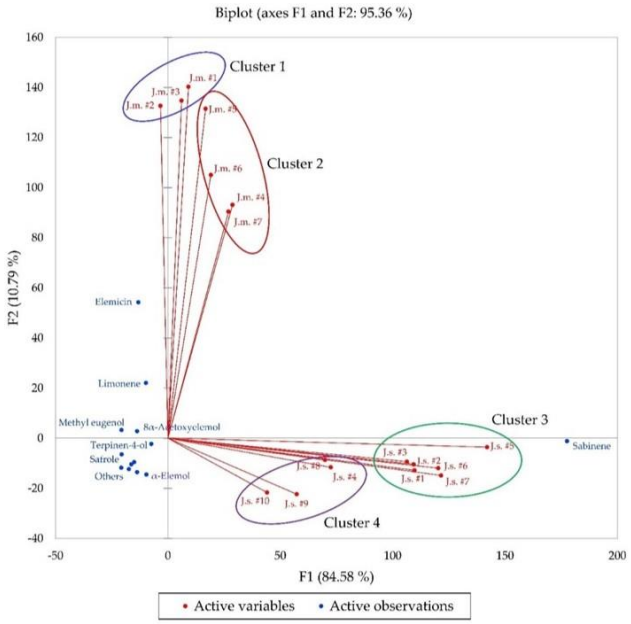

Figure 5. Biplot obtained from principal component analysis (PCA) based on the major components in the foliar essential oils of *Juniperus maritima* and *Juniperus scopulorum*. J.m. #1-#6 (this study), J.m. #7 [43], J.s. #1-#3 [44], J.s. #4 [43], J.s. #5 [45], J.s. #6 [46], J.s. #7 [47], J.s. #8 [48], J.s. #9 [49], J.s. #10 [50].

Hierarchical cluster analysis (HCA) revealed two major groupings that separated *J. maritima* from *J. scopulorum* (Fig. 4). The *J. maritima* group is rich in elemicin (apparently not observed in *J. scopulorum*), whereas the *J. scopulorum* group is rich in sabinene. The *J. maritima* group can be subdivided into two clusters, cluster 1, (elemicin/limonene) and cluster 2 (elemicin/sabinene). The *J. scopulorum* group can also be subdivided into a sabinene-rich cluster (cluster 3) and a sabinene/terpinen-4-ol/α-elemol cluster (cluster 4). The principal component analysis (PCA, Fig. 5) corroborated the HCA, showing that *J. maritima* foliar essential oils correlated strongly with elemicin and That *J. scopulorum* essential oils strongly correlated with sabinene. The ANOVA analysis further illustrates the differences among the four clusters (Fig. 6). That is, Cluster 1 (the dunes *J. maritima*) is that characterized by significantly high concentrations of limonene and elemicin, Cluster 2 (the bluff *J. maritima*) is also characterized by a significantly high concentration of elemicin, while the two *J. scopulorum* clusters have significantly high concentrations of sabinene. Note that these statistical methods provide

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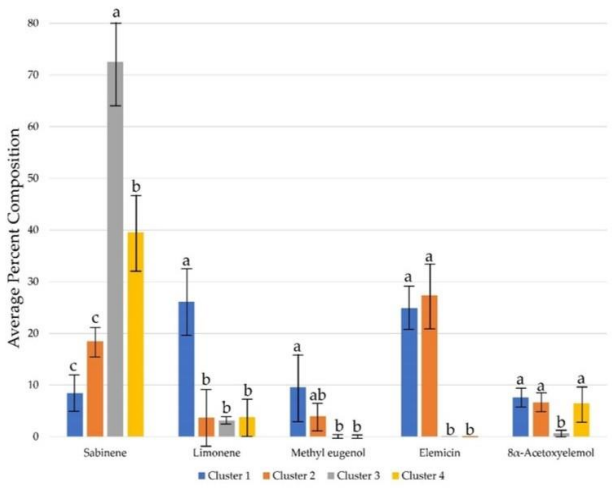

Figure 6. Average percentage compositions of the major components in *Juniperus maritima* (Clusters 1 and 2) and *Juniperus scopulorum* (Clusters 3 and 4). For each component, bars with the same numbers are not significantly different ($p > 0.05$, ANOVA followed by Tukey's test).

support for, but do not establish taxonomic separation.

3.3. Comparison between the two Juniperus maritima collection sites

Several factors have been attributed to variations in the chemical composition of essential oils within a species [29–33]. These include genetic factors [34–36], abiotic environmental characteristics of the collection sites [24, 25], seasonality/phenology [37], and biotic factors such as herbivory [26, 27] or fungal infection [28], as well as differences in processing methods. The factors responsible for the observed compositional differences in the samples from the two *J. maritima* collection sites are unclear, but they may be due to abiotic and/or biotic environmental characteristics. Exposure to salt stress may be a factor in the differences between the compositions of dunes and the bluff trees. The trees collected from the sand dunes were very near the water's edge, whereas those on the bluff were farther away and higher in elevation. Salt stress is known to affect the composition of essential oils [38]. However, biotic factors such as competition with other flora [39, 40] or arbuscular mycorrhizal fungal diversity [41, 42] may also play a role, but this is speculative at this point.

3.4. Enantiomeric distribution of chiral monoterpenoids in Juniperus maritima essential oils

The *J. maritima* essential oils were subjected to enantio-selective GC-MS analysis (Table 4). With the exception of α-thujene (100% (–)-α-thujene), the dominant enantiomer in each of the monoterpenoids detected was the dextrorotatory, (+)-enantiomer. In particular, (+)-α-pinene (98.5 ± 0.7%), (+)-sabinene (99.7 ± 0.2%), (+)-limonene (98.2 ± 1.9%), (+)-*cis*-sabinene hydrate (95.0 ± 2.6%), and (+)-*trans*-sabinene hydrate (96.8 ± 1.4%) were dominant. Interestingly, there were minor, but significant (*t*-test) differences in the distributions from the two habitats for limonene (99.8 ± 0.0% (+)-limonene for the dunes trees, 96.5 ± 1.1% for the bluff trees, $p = 0.035$) and for α-terpineol (68.0 ± 1.0% (+)-α-terpineol for the dunes trees, 52.5 ± 1.1% for the bluff trees, $p < 0.001$). These results are consistent with the distributions observed in other *Juniperus* species [16], including *J. scopulorum* [50].

4. Conclusions

There is some controversy regarding the classification of *Juniperus maritima* as a species separate from *Juniperus scopulorum*. The current study provides new compositional and chiral data for *J. maritima* and suggests distinct chemical differences compared to published *J. scopulorum* essential oil profiles, thus providing complementary supporting evidence, but not definitive taxonomic proof, for separating the species based on essential oil analysis. Multivariate analyses revealed two major clusters that separated *J. maritima* from *J. scopulorum*. The *J. maritima* cluster was rich in elemicin, whereas the *J. scopulorum* cluster was rich in sabinene. The *J. maritima* cluster showed two sub-clusters, one described as an elemicin/sabinene chemotype and the other as an elemicin/limonene chemotype. The *J. scopulorum* cluster was subdivided into a cluster dominated by sabinene, and another cluster dominated by sabinene/terpinen-4-ol. It is apparent that phenylpropanoids define the volatile phytochemistry of *J. maritima* and differentiate its essential oil from that of *J. scopulorum*. The taxonomic treatment of *J. maritima* requires additional scrutiny.

This study was preliminary and was limited by the small sample size. The HCA and PCA are used as exploratory pattern-recognition tools and are supportive of taxonomic separation rather than confirmation. Future research should include more samples from various habitats and focus on the effects of the ecological components present in the different

Table 4. Enantiomeric distributions (percent of each enantiomer) of chiral monoterpenoids in the foliar essential oils of *J. maritima* from northwestern Washington, U.S.A.

Enantiomers	RI_{calc}	RI_{db}	J.m. #1	J.m. #2	J.m. #3	J.m. #4	J.m. #5	J.m. #6
(+)-α-Thujene	n.o.	950	0.0	0.0	0.0	0.0	0.0	0.0
(−)-α-Thujene	953	951	100.0	100.0	100.0	100.0	100.0	100.0
(−)-α-Pinene	977	976	1.6	2.8	1.8	1.0	1.1	0.0
(+)-α-Pinene	981	982	98.4	97.2	98.2	99.0	98.9	99.3
(+)-Sabinene	1016	1021	99.7	99.3	99.7	99.9	99.9	99.9
(−)-Sabinene	1030	1030	0.3	0.7	0.3	0.1	0.1	0.1
(−)-Limonene	1077	1073	0.2	0.1	0.1	2.8	4.7	2.9
(+)-Limonene	1081	1081	99.8	99.9	99.9	97.2	95.3	97.1
(+)-*cis*-Sabinene hydrate	1198	1199	94.4	90.8	93.5	97.3	96.8	97.2
(−)-*cis*-Sabinene hydrate	1202	1202	5.6	9.2	6.5	2.7	3.2	2.8
(−)-Linalool	1229	1228	13.3	16.7	13.2	12.4	23.0	18.2
(+)-Linalool	1232	1231	86.7	83.3	86.8	87.6	77.0	81.7
(+)-*trans*-Sabinene hydrate	1231	1231	97.1	94.4	96.0	98.1	97.8	97.8
(−)-*trans*-Sabinene hydrate	1236	1235	2.9	5.6	4.0	1.9	2.2	2.2
(+)-Terpinen-4-ol	1294	1297	73.0	73.3	73.5	68.1	74.1	67.1
(−)-Terpinen-4-ol	1300	1300	27.0	27.7	26.5	31.9	25.9	32.9
(−)-α-Terpineol	1348	1347	32.5	30.8	32.7	46.9	46.8	48.7
(+)-α-Terpineol	1356	1356	67.5	69.2	67.3	53.1	53.2	51.3

RI_{calc} = Retention index values determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. RI_{db} = Retention index from our in-house database prepared using commercially available standards. n.o. = compound not observed.

habitats of *J. maritima*. In addition to providing chemotaxonomic evidence to support the existence of a separate species, this report provides chiral GC data identifying the enantiomeric distributions of chiral monoterpenoid components.

Disclaimer (artificial intelligence)

Authors hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, W.N.S.; methodology, P.S., W.N.S.; software, P.S.; validation, P.S., W.N.S.; formal analysis, A.P., P.S., W.N.S.; investigation, K.S., A.P., P.S., W.N.S.; resources, P.S., W.N.S.; data curation, W.N.S.; writing—original draft preparation, W.N.S.; writing—review and editing, K.S., A.P., P.S.; visualization, W.N.S.; supervision, P.S., W.N.S.; project administration, W.N.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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