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Chemical composition of the foliar essential oil of *Juniperus occidentalis* var. *occidentalis* from southeastern Oregon

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Abstract
The essential oil was obtained from the foliage of *Juniperus occidentalis* collected from Leslie Gulch, southeastern Oregon, by hydrodistillation. Gas chromatographic analysis revealed a total of 120 identified components with bornyl acetate (36.8%), *p*-cymene (9.9%), terpinen-4-ol (7.6%), and sabinene (6.2%) as major components. Enantioselective gas chromatography showed, for the first time, that (+)-α-pinene, (+)-camphene, (+)-sabinene, (+)-β-pinene, (+)-δ-3-carene, (+)-limonene, (+)-*cis*-sabinene hydrate, (+)- camphor, (+)-terpinen-4-ol, (+)-borneol, and (+)-α-terpineol were the predominant enantiomers in *J. occidentalis* essential oil.

Keywords Western juniper, gas chromatography, hydrodistillation, chiral, enantiomer

1. Introduction

Juniperus occidentalis Hook., Cupressaceae (western juniper) is a tree (4-10 m tall); the leaves are overlapping scale-like (1-6 mm long) [1] (Figure 1). The foliage is loose and open, the sub-branchlets are elongated, and the trees have a strong central axis [2]. There were two recognized varieties of, *J. occidentalis* var. *occidentalis* Hook. and *J. occidentalis* var. *australis* (Vasek) A.H. Holmgren & N.H. Holmgren [3, 4]. However, *J. occidentalis* var. *australis*, which ranges in the Sierra Nevada Mountains of California, has been re-named *Juniperus grandis* R.P. Adams [5]. *J. occidentalis* var. *occidentalis* ranges from south-central Washington, throughout Oregon, east of the Cascades, in into northern California, southwestern Idaho, and north western Nevada (Figure 2). It has been reported that western juniper is an invasive tree invading sagebrush-grasslands in the Great Basin area [6-9].

Fig 1: *Juniperus occidentalis* var. *occidentalis* from Leslie Gluch, Oregon

Western juniper is not a preferred browse for wild or domestic ungulates, but considerable browsing by mule deer (*Odocoileus hemionus* Rafinesque) does occur during winter when other forage is limited [3]. The female cones ("berries") of *J. occidentalis* are favored by several avian species such as American robin (*Turdus migratorius* Linnaeus), cedar waxwing

(*Bombycilla cedrorum* Vieillot), pinyon jay (*Gymnorhinus cyanocephalus* Wied-Neuwied), and Townsend's solitaire (*Myadestes townsendi* Audubon). Additionally, mammals such as coyote (*Canis latrans* Say), kit fox (*Vulpes macrotis* Merriam), and mule deer also eat western juniper "berries" [1]. As is the case with other *Juniperus* species, *J. occidentalis* has been important in the traditional medicine of Native Americans within its geographical range [10]. The Piute people took an infusion of the foliage to treat colds and the fumes from burning foliage was inhaled for colds; a decoction of the foliage was taken for coughs, influenza, or fevers, and was used as an antiseptic wash for sores; a poultice of the foliage was applied to treat rheumatism. The Shoshoni people took a foliar infusion for intestinal worms; a poultice of the foliage was applied to burns or to swellings; a decoction of the foliage was used as a remedy for colds and coughs as well as an antiseptic wash.

Fig 2: Range of *Juniperus occidentalis* var. *occidentalis* (based on Miller *et al*., 2005 [9] and Adams and Corbet, 2015 [17])

Several 2-hydroxyacetophenone glycosides (the Juniperosides I-IX), have been isolated from *J. occidentalis* [11]. In addition, cupressuflavone, sabinic acid, α-thujaplicin, β-thujaplicin, dihydromayurone, sabinic acid, and 7-oxo-8, 15-isopimaradien-18-oic acid have also been isolated and characterized from *J. occidentalis* [11]. The heartwood essential oil of *J. occidentalis* var. *occidentalis* was comprised of cedrol (38.9%, 49.7%), thujopsene (18.9%, 11.8%), widdrol (13.3%), α-cedrene (8.8%, 12.4%), and β-cedrene (2.6%, 3.4%) [12, 13]. The foliar essential oils of *J. occidentalis* from Oregon and California have been previously investigated [14-17]. The purpose of this current study is to describe the chemical composition of the foliage from a single *J. occidentalis* tree growing in Leslie Gulch, Oregon, which should complement the previous investigations. In addition, enantioselective gas chromatography has been carried out to determine the enantiomeric distributions of chiral monoterpenoids, which adds an element of novelty and expands our understanding of *Juniperus* essential oils.

2. Materials and Methods

2.1 Plant Material

Foliage of *J. occidentalis* var. *occidentalis* was collected from a single individual tree (only one tree found) growing in Leslie Gulch, Oregon (43°17′55″ N, 117°16′12″ W, 1045 m asl), on 27 May 2023. The fresh foliage (152.2 g) was hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane to give 5.23 g yellow essential oil.

2.2 Gas Chromatographic Analysis

The essential oil was subjected to gas chromatographic analysis as previously described [18]. GC-MS: Shimadzu GC-MS-QP2010 Ultra (Shimadzu Scientific Instruments), ZB-5ms GC column (60 m × 0.25 mm × 0.25 µm film thickness, Phenomenex); injector and detector temperatures = 260 °C, He carrier gas (column head pressure = 208.2 kPa, flow rate = 2.00 mL/min; GC oven temperature program (50-260 °C at 2 °C/min); injected 1.0 µL of a 5% (w/v) essential oil solution in CH_2Cl_2, splitting mode = 24.5:1. Retention index (RI) values were calculated using a homologous series of *n*-alkanes [19]. The essential oil components were identified by comparing RI values and MS fragmentation patterns with those from the Adams [20], FFNSC 3 [21], NIST20 [22], and Satyal [23] databases. GC-FID: Shimadzu GC 2010 with FID detector (Shimadzu Scientific Instruments), ZB-5 GC column (60 m× 0.25 mm× 0.25 µm film thickness, Phenomenex); same operating conditions as above for GC-MS. The percent compositions were determined from raw peak areas without standardization. Enantioselective GC-MS: Shimadzu GC-MS-QP2010S (Shimadzu Scientific Instruments), Restek B-Dex 325 column (30 m× 0.25 mm diameter × 0.25 µm film thickness, Restek Corp.); injector and detector temperatures = 240 °C; He carrier gas (column head pressure = 53.6 kPa, flow rate = 1.00 mL/min; GC oven program (initial temperature = 50 °C, held for 5 min, increased to 100 °C at 1.0 °C/min, then increased to 220 °C at a rate of 2 °C/min); injected 0.3 µL of a 5% (w/v) essential oil solution in CH_2Cl_2, splitting mode = 24.0:1. The enantiomers were determined by comparison of calculated retention indices with those of authentic samples (Sigma-Aldrich). Enantiomeric ratios were determined from raw peak areas.

2.2 Multivariate Analyses

Multivariate analyses were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). Agglomerative hierarchical cluster analysis (HCA) was carried out using the percentages of the 11 most abundant components (bornyl acetate, sabinene, *p*-cymene, terpinen-4-ol, β-phellandrene, α-pinene, γ-terpinene, δ-cadinene, γ-cadinene, manoyl oxide, and citronellol) from this study as well as previously reported compositions from the literature [14-17]. The nine *J. occidentalis* samples were treated as operational taxonomic units (OTUs). Pearson correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition. Principal component analysis (PCA, type Pearson

Correlation) was used to verify the similarity of essential oil samples based on the HCA analysis.

3. Results and Discussion

3.1 Essential Oil Composition: The yellow foliar essential oil of *J. occidentalis* var. *occidentalis* was obtained in 3.43% (w/w) yield. Gas chromatographic analysis (GC-MS and GC-FID) allowed for the identification of 120 components accounting for 98.8% of the essential oil composition (Table 1).

Table 1: Chemical composition (percent of total) of the foliar essential oil of *Juniperus occidentalis* var. *occidentalis* from Leslie Gulch, Oregon

RT (min)	RI_{calc}	RI_{db}	Compound	%
12.157	920	919	Hashishene	0.1
12.293	922	923	Tricyclene	2.1
12.429	925	925	α-Thujene	0.7
12.858	932	933	α-Pinene	1.4
13.671	947	948	α-Fenchene	0.1
13.781	949	950	Camphene	1.2
15.123	973	972	Sabinene	6.2
15.377	977	978	β-Pinene	0.1
16.002	988	989	Myrcene	0.9
17.080	1007	1007	α-Phellandrene	0.4
17.228	1009	1009	δ-3-Carene	0.7
17.761	1017	1018	α-Terpinene	1.4
18.350	1026	1025	*p*-Cymene	9.9
18.423	1027	1026	2-Acetyl-3-methylfuran	tr
18.586	1029	1030	Limonene	1.6
18.690	1031	1031	β-Phellandrene	1.0
18.974	1035	1035	Lavender lactone	0.1
19.618	1045	1045	(*E*)-β-Ocimene	tr
19.949	1050	1051	3-Ethylcyclohexanone	0.1
20.471	1058	1058	γ-Terpinene	2.4
21.255	1069	1069	*cis*-Sabinene hydrate	0.2
21.260	1070	1069	*cis*-Linalool oxide (furanoid)	0.2
22.265	1085	1086	Terpinolene	0.7
22.330	1086	1086	*trans*-Linalool oxide (furanoid)	tr
22.596	1091	1093	*p*-Cymenene	0.2
23.217	1100	1102	(3*E*,7*E*)-2,9-Dimethyl-3,7-decadiene	0.4
23.341	1102	1101	*trans*-Sabinene hydrate	0.2
23.472	1104	1104	Hotrienol	0.1
24.140	1113	1113	*p*-Mentha-1,3,8-triene	tr
24.510	1119	1118	β-Thujone	0.1
24.585	1120	1119	*endo*-Fenchol	tr
24.777	1123	1122	*trans*-*p*-Mentha-2,8-dien-1-ol	tr
24.977	1125	1124	*cis*-*p*-Menth-2-en-1-ol	0.6
25.125	1128	1126	α-Campholenal	tr
25.642	1135	1135	2-Vinylanisole	tr
26.104	1142	1141	*trans*-Pinocarveol	0.1
26.225	1143	1142	*trans*-*p*-Menth-2-en-1-ol	0.4
26.550	1148	1149	Camphor	0.4
27.108	1156	1156	Camphene hydrate	0.4
27.272	1158	1157	Sabina ketone	0.4
27.615	1163	1164	Pinocarvone	tr
27.680	1164	1165	*iso*-Borneol	0.1
28.167	1171	1165	Coahuilensol	0.3
28.337	1174	1173	Borneol	1.5
29.021	1182	1180	Terpinen-4-ol	7.6
29.095	1185	1184	Thuj-3-en-10-al	tr
29.205	1186	1188	*p*-Methylacetophenone	0.2
29.384	1189	1189	*p*-Cymen-8-ol	1.1
29.715	1193	1191	Myrtenol	0.1
29.917	1196	1195	α-Terpineol	0.4
30.019	1198	1198	*cis*-Piperitol	0.2
30.722	1208	1208	Verbenone	tr
30.871	1210	1209	*trans*-Piperitol	0.2
31.392	1218	1219	*endo*-Fenchyl acetate	tr
31.552	1220	1221	3-Isopropylbenzaldehyde	0.3
32.165	1229	1228	3-Isopropylphenol	0.1
32.304	1231	1230	*cis*-*p*-Mentha-1(7),8-dien-2-ol	tr
33.149	1243	1242	Cuminal	0.2
33.265	1245	1246	Carvone	0.1
33.294	1245	1246	*cis*-Linalool oxide acetate	0.1
33.581	1250	1250	Linalyl acetate	0.2

33.928	1255	1254	Piperitone	0.1
34.087	1257	1257	Carvenone	0.1
34.180	1258	1255	*cis*-Piperitone oxide	0.1
35.587	1279	1277	Phellandral	0.1
36.243	1286	1285	Bornyl acetate	36.8
36.310	1289	1287	*iso*-Bornyl acetate	0.9
36.520	1292	1291	*p*-Cymen-7-ol	0.5
36.769	1296	1296	Terpinen-4-yl acetate	0.1
36.946	1299	1293	Thymol	0.5
37.401	1305	1300	Carvacrol	0.4
38.421	1321	1320	Methyl geranate	0.4
38.958	1329	1327	*p*-Mentha-1,4-dien-7-ol	0.3
40.115	1346	1346	α-Cubebene	0.1
41.960	1375	1377	Isobornyl propionate	0.1
42.005	1376	1375	α-Copaene	0.6
42.536	1384	1382	β-Bourbonene	0.1
42.793	1388	1387	β-Cubebene	tr
42.890	1389	1390	*trans*-β-Elemene	tr
43.065	1392	1391	(*E*)-α-Damascone	tr
43.330	1396	1386	Methyl (2*E*,4*Z*)-decadienoate	0.1
44.822	1419	1417	(*E*)-β-Caryophyllene	0.1
45.465	1430	1430	β-Copaene	tr
45.676	1433	1432	6-Oxobornyl acetate	tr
46.671	1449	1448	*cis*-Muurola-3,5-diene	0.1
47.078	1456	1454	α-Humulene	0.1
47.493	1462	1463	*cis*-Muurola-4(14),5-diene	tr
47.890	1469	---	Methyl (2*E*,4*E*)-3,7-dimethylocta-2,4,6-trienoate [a]	0.2
48.105	1472	1472	*cis*-Cadina-1(6),4-diene	0.2
48.291	1475	1478	γ-Muurolene	0.3
48.678	1481	1480	Germacrene D	0.2
49.349	1492	1496	*trans*-Muurola-4(14),5-diene	0.2
49.599	1496	1497	*epi*-Cubebol	0.1
49.760	1498	1497	α-Muurolene	0.5
50.285	1507	1507	*iso*-Bornyl isovalerate	0.1
50.668	1513	1512	γ-Cadinene	1.1
50.793	1515	1515	Cubebol	tr
50.905	1517	1523	*endo*-1-Bourbonanol	0.2
50.981	1519	1518	δ-Cadinene	1.7
51.170	1522	1519	*trans*-Calamenene	0.5
51.230	1523	1521	Zonarene	0.1
51.800	1532	1533	Cadina-1,4-diene	0.1
52.050	1537	1538	α-Cadinene	0.2
52.322	1541	1541	α-Calacorene	0.1
53.556	1562	1560	β-Calacorene	0.1
54.429	1577	1574	Germacra-1(10),5-dien-4β-ol	0.2
55.028	1587	1584	Gleenol	0.1
56.007	1603	1607	β-Oplopenone	0.2
56.679	1615	1614	1,10-di-*epi*-Cubenol	0.1
57.330	1626	1624	Muurola-4,10(14)-dien-1β-ol	0.1
57.405	1628	1628	1-*epi*-Cubenol	0.7
58.221	1642	1643	τ-Cadinol	0.6
58.330	1644	1644	τ-Muurolol	0.5
58.467	1646	1645	α-Muurolol (= δ-Cadinol)	0.2
58.981	1655	1655	α-Cadinol	0.8
59.922	1672	1677	Cadalene	0.1
63.256	1732	1735	Oplopanone	0.1
76.448	1990	1989	Manoyl oxide	0.2
80.743	2082	2086	Abietadiene	tr
90.378	2301	2314	Abieta-7,13-dien-3-one	0.1
			Monoterpene hydrocarbons	31.1
			Oxygenated monoterpenoids	55.3
			Sesquiterpene hydrocarbons	6.3
			Oxygenated sesquiterpenoids	4.0
			Diterpenoids	0.4
			Benzenoid aromatics	0.9
			Others	0.9
			Total identified	98.8

RT = Retention Time in minutes. RI_{calc} = Retention Index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Reference Retention Index from the databases [20-23]. tr = trace (< 0.05%). [a] Although there was a good MS match (90% similarity), a reference RI was not available

The *J. occidentalis* var *occidentalis* foliar essential oil was dominated by bornyl acetate (36.8%), followed by *p*-cymene (9.9%), terpinen-4-ol (7.6%), and sabinene (6.2%). von Rudloff and co-workers [14] and Adams and co-workers [15-17] have previously examined the foliar essential oil of *J. occidentalis*. In order to provide a comparison between the current study and the previous investigations, hierarchical cluster analysis (HCA) and principal component analysis (PCA), were carried out (Figures 3 and 4).

Although the different samples of *J. occidentalis* foliar essential oils are qualitatively similar, there are quantitative differences that are reflected in the multivariate analyses. The HCA shows three distinct clusters, a bornyl acetate / sabinene cluster (samples from Redmond OR, Burns OR, Baker OR, and Bend OR), a bornyl acetate / *p*-cymene cluster (samples from Prineville OR, Leslie Gulch OR, Sage Hen Pass CA, and a shrub-form from Bend OR), and a sabinene cluster (single sample from Trinity Alps CA).

The PCA analysis confirms the three different clusters. The Trinity Alps CA sample, rich in sabinene (20.4%) but only a trace of bornyl acetate, is dissimilar to the other samples; the collection site is remote from the normal range of *J. occidentalis*. The other samples included in the HCA analysis show 70% similarity and all lie within the normal geographical range of *J. occidentalis*.

Several of the major components have demonstrated biological activities relevant to the Native American traditional uses of *J. occidentalis*. For example, bornyl acetate has shown anti-inflammatory [24-26] and antimicrobial [27] activities, sabinene has shown anti-inflammatory and anti-dermatophyte activities [28], *p*-cymene has analgesic [29, 30] and anti-inflammatory [29] properties, and terpinen-4-ol has also shown anti-inflammatory [31-33] and antibacterial [34] activities as well as anthelmintic activity [35, 36]. These biological activities are consistent with the traditional uses of *J. occidentalis* by the Paiute and Shoshoni Native Americans.

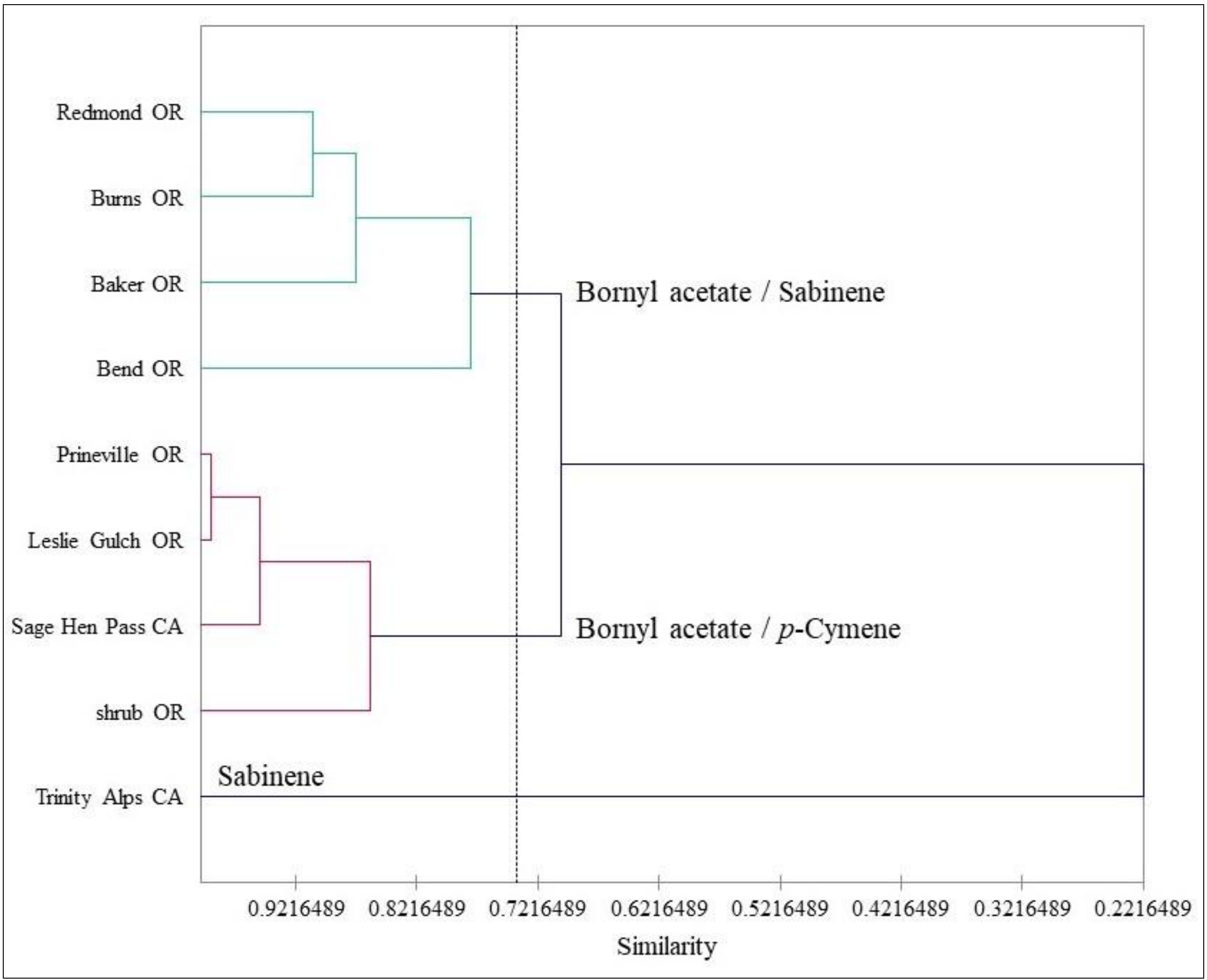

Fig 3: Dendrogram based on the agglomerative hierarchical cluster analysis (HCA) of foliar essential oil compositions of *Juniperus occidentalis*. The sample labeled Bend OR is from Adams & Kauffmann, 2010 [15], samples shrub OR (a shrub-like form from Bend OR) and Trinity Alps CA are from Adams, 2012 [16], samples Baker OR, Burns OR, and Sage Hen Pass CA are from Adams & Corbet, 2015 [17], samples Redmond OR and Prineville OR are from von Rudloff *et al*., 1980 [14], and the sample Leslie Gulch OR is from this current study

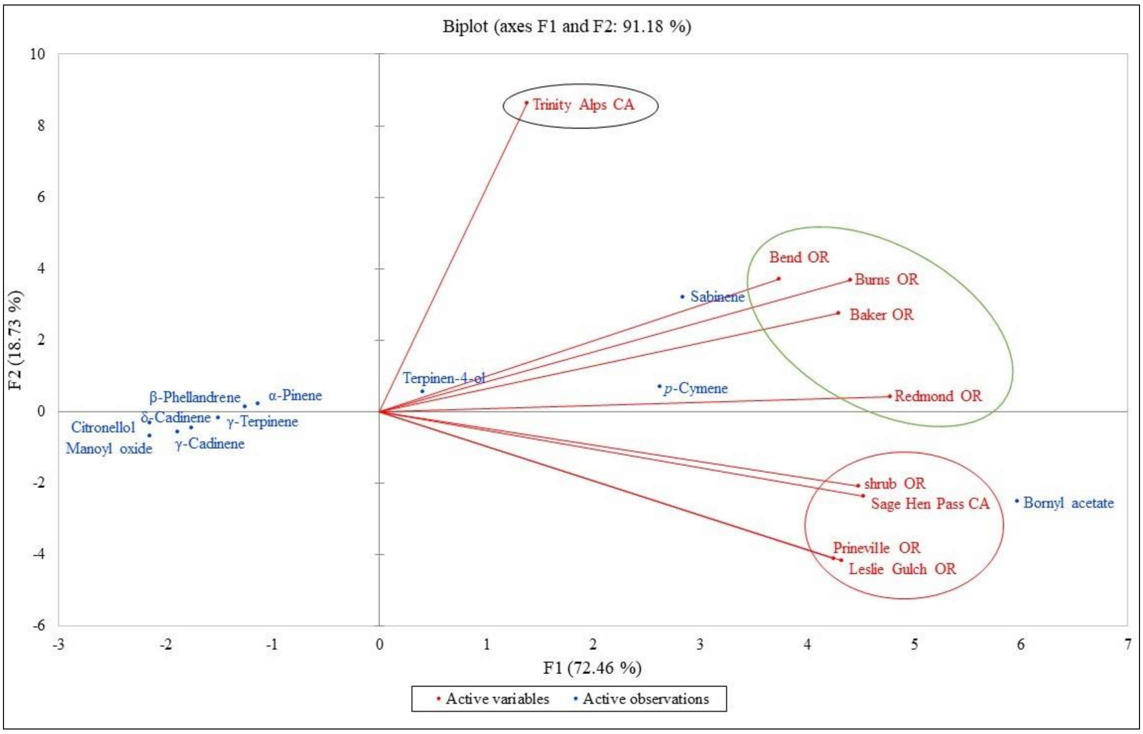

Fig 4: Biplot based on the principal component analysis (PCA) of foliar essential oil compositions of *Juniperus occidentalis*. The sample labeled Bend OR is from Adams & Kauffmann, 2010 [15], samples shrub OR (a shrub-like form from Bend OR) and Trinity Alps CA are from Adams, 2012 [16], samples Baker OR, Burns OR, and Sage Hen Pass CA are from Adams & Corbet, 2015 [17], samples Redmond OR and Prineville OR are from von Rudloff *et al*., 1980 [14], and the sample Leslie Gulch OR is from this current study

3.2 Enantiomeric Distribution

An enantioselective GC-MS analysis on the foliar essential oil of *J. occidentalis* was carried out in order to determine the enantiomeric distributions of chiral monoterpenoid components (Table 2).

The dextrotoratory enantiomers predominated in α-pinene (85.3%), camphene (88.8%), sabinene (100%), β-pinene (100%), δ-3-carene (100%), limonene (93.7%), *cis*-sabinene hydrate (100%), camphor (100%), terpinen-4-ol (67.7%), borneol (100%), and α-terpineol (100%). However, only (-)-β-phellandrene was detected in *J. occidentalis* essential oil. Although only one peak was observed for α-thujene, the proximity of the reference RI values for (+)- and (-)-α-thujene may preclude separation of the α-thujene enantiomers. Similarly, the peak for bornyl acetate was very large, so separation of individual enantiomers of bornyl acetate is not likely possible.

The previous examinations of *J. occidentalis* foliar essential oils did not include chiral GC-MS analyses. Nevertheless, there have been some studies on the enantiomers in other *Juniperus* species [37-40]. (+)-Limonene is the major enantiomer in *Juniperus* essential oils, in contrast to what is observed in members of the Pinaceae [41-45] where (-)-limonene is the dominant enantiomer. Except for a sample of *Juniperus brevifolia* (Seub.) Antoine [37], (+)-α-pinene is the predominant enantiomer in *Juniperus* essential oils.

Table 2: Enantiomeric distribution (% enantiomer) of chiral monoterpenoids in the foliar essential oil of *Juniperus occidentalis* var. *occidentalis*

Compound	RI_{db}	RI_{calc}	ED (%)
(+)-α-Thujene	950	nd	0.0
(-)-α-Thujene	951	951	100.0
(-)-α-Pinene	976	976	14.7
(+)-α-Pinene	982	980	85.3
(-)-Camphene	998	999	11.2
(+)-Camphene	1005	1004	88.8
(+)-Sabinene	1021	1018	100.0
(-)-Sabinene	1030	nd	0.0
(+)-β-Pinene	1027	1027	100.0
(-)-β-Pinene	1031	nd	0.0
(+)-δ-3-Carene	1052	1051	100.0
(-)-δ-3-Carene	na	nd	0.0
(-)-Limonene	1073	1077	6.3
(+)-Limonene	1081	1081	93.7
(-)-β-Phellandrene	1083	1084	100.0
(+)-β-Phellandrene	1089	nd	0.0
(+)-*cis*-Sabinene hydrate	1199	1192	100.0
(-)-*cis*-Sabinene hydrate	1202	nd	0.0
(-)-Camphor	1253	nd	0.0
(+)-Camphor	1259	1260	100.0
(+)-Terpinen-4-ol	1297	1293	67.7

(-)-Terpinen-4-ol	1300	1297	32.3
(-)-Borneol	1335	nd	0.0
(+)-Borneol	1340	1340	100.0
(-)-Bornyl acetate	1344	1347	100.0
(+)-Bornyl acetate	na	nd	0.0
(-)-α-Terpineol	1347	nd	0.0
(+)-α-Terpineol	1356	1353	100.0

RI_{db} = Retention index from our in-house database developed using commercially available samples on a Restek B-Dex 325 column. RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 column. na = reference compound not available. nd = compound not observed

4. Conclusion
An obvious limitation to this study is the fact that only one sample of *J. occidentalis* was obtained. Unfortunately, no other *J. occidentalis* trees were found in Leslie Gulch, Oregon. Nevertheless, the chemical composition of this essential oil and the enantiomeric distribution of monoterpenoids adds to our general understanding of the phytochemistry of the species. The chemical compositions of *J. occidentalis* var. *occidentalis* are relatively consistent throughout its normal geographical range and the bioactivities of the major components support the traditional ethnopharmacology of the tree.

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7. Conflicts of Interest
The authors declare no conflicts of interest.

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