



Research Article

The essential oil characterization of *Achillea millefolium* var. *occidentalis* DC. from the Great Basin of North America

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Abstract

Achillea millefolium is native to temperate regions of the northern hemisphere and is important in traditional herbal medicines throughout its range. The purpose of this work is to evaluate the essential oil composition of the North American variety, *A. millefolium* var. *occidentalis*, and to test the hypothesis that var. *occidentalis* has a chemical composition different from Old World *A. millefolium*. Six samples of *A. millefolium* were collected from different locations in the Great Basin region of eastern Oregon and southern Idaho. The essential oils were obtained by hydrodistillation and analyzed by gas chromatographic methods. The essential oils showed wide variation in composition with the major components (–)-camphor (0.5-59.6%), (–)- α -thujone (0.0-40.5%), terpinen-4-ol (0.9-23.4%), artemisia ketone (0.0-18.7%), 1,8-cineole (5.3-16.4%), and (+)-(*E*)-nerolidol (0.4-13.5%). The essential oil compositions of western North American *A. millefolium* cannot be distinguished from the samples from Old World samples of *A. millefolium*; there is no phytochemical support for infraspecific differentiation of the North American variety.

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1. Introduction

Achillea millefolium L. (Asteraceae) is a polymorphous species with a complex taxonomy [1, 2]. *World Flora Online* currently lists 161 synonyms for the species [3]. The plant is native to the temperate regions of the northern hemisphere, including Europe, western Asia, and North America [4], but has been introduced and cultivated worldwide. The plant is characterized by deeply divided pubescent leaves with flowers gathered in capitula, which are grouped in corymbs (Fig. 1). The western North American plant, known as *Achillea millefolium* var. *occidentalis* DC. (western yarrow), ranges from western Canada, south through the Dakotas, Colorado, and New Mexico, and into northern Mexico; and west to the Pacific Coast [5]. The *Flora of North America*, however, does not recognize infraspecific taxa of *A. millefolium* [6].

Achillea millefolium has been used in traditional medicine throughout its global distribution [7]. The Shoshoni Native Americans applied a poultice of the boiled whole plant externally to treat sores, while a decoction of the plant was used as a liniment for sores and rashes. The Shoshoni prepared a decoction of the plant and took it to treat colds [8]. Similarly, the Paiute took an infusion of *A. millefolium* as a cough medicine [9]. In Europe, the plant is traditionally taken orally for gastrointestinal disorders or used topically to treat skin disorders [1]. In Iran, the plant is used for menstruation problems, to stimulate menstruation, to stop bleeding, for wound healing, to relieve asthma and constipation, and as diuretic, anthelmintic, and carminative [10]. In Colombia and Peru, *A. millefolium* is used to relieve gastrointestinal problems such as

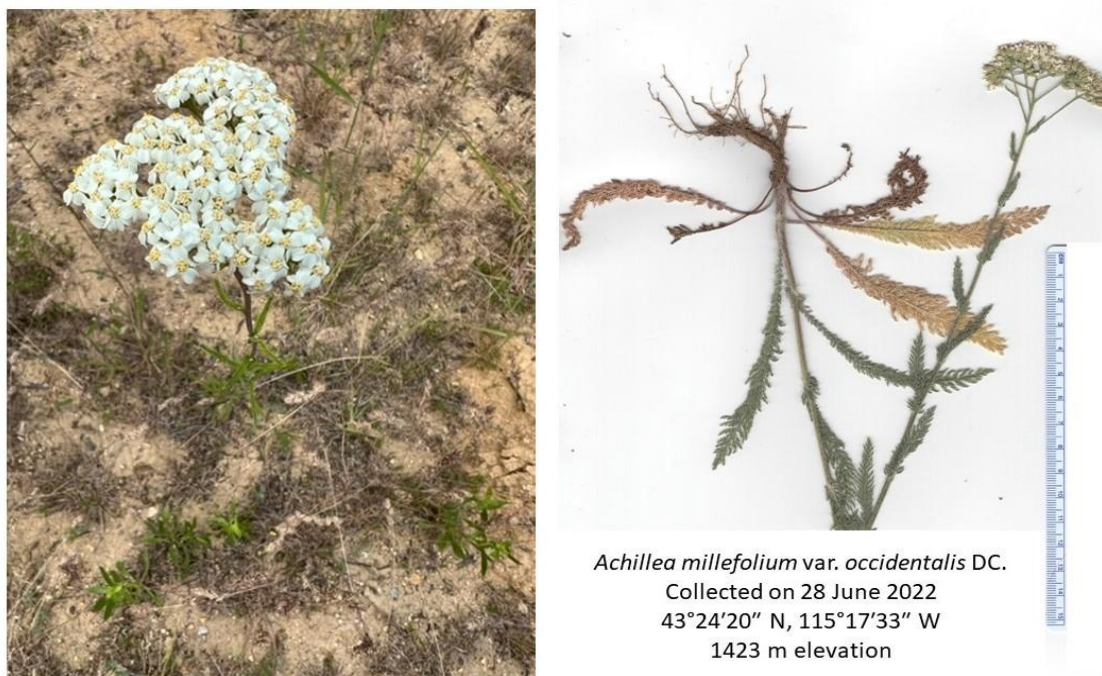


Figure 1. *Achillea millefolium* var. *occidentalis*. **A:** Photograph of plant (by K. Swor). **B:** Scan of pressed plant (by W.N. Setzer).

indigestion, flatulence, colitis, and gastritis [11]. The whole plant is used in Colombia to treat skin disorders, including acne, boils, botfly infestations, skin ulcers, and sores [11].

Achillea millefolium has been shown to be a source of sesquiterpene lactones, including 8-acetylgelelolide, 8-angeloylgelelolide, 8-angeloyloxyleucodin, acetylbalchanolide, achillicin, achillin, achillinin A, achillifolin, badkhyzin, millefin, millifolide C, isoachifolidiene, leukodin, dihydroparthenolide, and balchanolide; several polyacetylenes such as anacycline, dehydroanacycline, 2,9-pentadecadiene-12,14-diy-noic acid isobutylamide, 2,4-tetradecadiene-8,10-diy-noic acid 3-methylbutylamide, 2,4-undecadiene-8,10-diy-noic acid 2,3-dehydropiperidide, and 2,4-undecadiene-8,10-diy-noic acid isobutylamide; and flavonoids apigenin, artemetin, casticin, centauredidin, chrysoeriol, isorhamnetin, luteolin, and quercetin, as well as an azulene-containing essential oil [1, 7, 12, 13].

Although the taxonomic treatment in *Flora of North America* suggests little support for infraspecific taxa [6], it was deemed important to examine the essential oil compositions of several samples of *A. millefolium* collected in the wild from several locations in the Great Basin area of western North America to test the hypothesis that chemical composition differences

may differentiate the *A. millefolium* var. *occidentalis* from Old World samples of *A. millefolium*.

2. Materials and methods

2.1. Plant Material

Aerial parts of *A. millefolium* were collected from six individual plants growing in southern Idaho and eastern Oregon (Table 1). The plants were identified by W.N. Setzer in the field and verified by comparison with samples from the New York Botanical Garden [14]. Voucher specimens (WNS-Amo-5675 and WNS-Amo-7746) have been deposited at the University of Alabama in Huntsville herbarium. The fresh aerial parts were frozen and stored frozen (−20 °C) until processed.

2.2. Essential oil

For each sample of *A. millefolium*, the fresh/frozen aerial parts were finely chopped and hydrodistilled for three hours using a Likens-Nickerson apparatus [15–17] with continuous extraction of the distillate with dichloromethane to give the essential oils (Table 1).

2.3. Gas chromatographic analysis

The *A. millefolium* essential oils were analyzed by GC-MS, GC-FID, and enantioselective GC-MS as previously reported [18].

Table 1. Collection and hydrodistillation details of *Achillea millefolium* var. *occidentalis*.

Sample number	Collection date	Collection site	Mass of plant material (g)	Mass of essential oil (mg)	Essential oil color
#1	28 June 2022	Anderson Ranch, Idaho 43°24'20"N, 115°17'33"W, 1423 m asl	73.21	236.2	deep blue
#2	7 July 2022	Bogus Basin, Idaho 43°43'58"N, 116°7'42"W, 1673 m asl	74.48	198.5	deep blue
#3	27 May 2023	Leslie Gulch, Oregon 43°18'13"N, 117°17'17"W, 971 m asl	98.25	1034.4	deep blue
#4	8 August 2023	Redfish Lake, Idaho 44°8'40"N, 114°54'42"W, 2001 m asl	83.14	145.9	yellow
#5	11 August 2023	Silver City, Idaho 43°2'37"N, 116°46'33"W, 1788 m asl	59.75	563.0	blue
#6	26 August 2023	Boise Foothills, Idaho 43°41'20"N, 116°2'48"W, 1355 m asl	32.76	475.7	deep blue

2.4. Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) was carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The concentrations of the 28 most abundant components (α -pinene, camphene, sabinene, β -pinene, yomogi alcohol, *p*-cymene, 1,8-cineole, artemisia ketone, γ -terpinene, *cis*-sabinene hydrate, terpinolene, α -thujone, β -thujone, camphor, borneol, terpinen-4-ol, α -terpineol, chrysanthenyl acetate, bornyl acetate, α -copaene, (*E*)- β -caryophyllene, germacrene D, (*E*)-nerolidol, spathulenol, caryophyllene oxide, γ -eudesmol, α -eudesmol, and chamazulene) from this study as well as previously reported compositions from the literature [19–52] and commercial samples from the Aromatic Plant Research Center (APRC) collection were used for the analysis. Dissimilarity was used to determine clusters considering Euclidean distance and Ward's method was used to define agglomeration.

3. Results and discussion

Hydrodistillation of *A. millefolium* samples #1–#6 gave essential oils in yields of 0.323%, 0.267%, 1.053%, 0.175%, 0.942%, and 1.452%, respectively. Samples #1 and #2 showed the lowest yields; sample #6 showed the highest yield. It is not known what factors may be responsible for the yield variations; the samples were collected in different years, however, with the 2022 samples showing lower yields than the 2023 samples. The gas chromatographic analysis allowed the determination of a total of 152 compounds, which accounted for 95.0–99.1% of the compositions (Table 2).

The major components were camphor (0.5–59.6%, mean 18.5%), α -thujone (0.0–40.5%, mean 9.3%), terpinen-4-ol (0.9–23.4%, mean 7.7%), artemisia ketone (0.0–18.7%, mean 4.8%), 1,8-cineole (5.3–16.4%, mean 9.7%), (*E*)-nerolidol (0.4–13.5%, mean 4.3%), yomogi alcohol (0.0–8.8%, mean 2.6%), camphene (0.1–7.3%, mean 1.8%), chamazulene (0.0–7.0%, mean 2.3%), β -pinene (0.3–6.3%, mean 1.2%), β -thujone (0.0–5.6%, mean 1.2%), and bornyl acetate (0.1–5.4%, mean 1.8%). The *A. millefolium* essential oil compositions show little similarity. However, samples #1 and #3 were rich in α -thujone, samples #2 and #6 were rich in camphor, and samples #4 and #5 were rich in terpinen-4-ol. Essential oil samples #1, #2, #3, #5, and #6 were blue in color, owing to the presence of chamazulene. Essential oil sample #4, which lacked chamazulene, was yellow in color.

In order to compare the essential oil compositions of the Great Basin samples in this work with the chemical compositions of *A. millefolium* from Old World locations, a hierarchical cluster analysis was carried out based on the 28 most abundant components. The cluster analysis shows much variation in essential oil compositions (Fig. 2), but four major groupings are evident: (1) a germacrene D/sabinene group, (2) a β -pinene/caryophyllene oxide group, (3) a 1,8-cineole/camphor group, and (4) a chamazulene group. Great Basin samples #1, #2, #4, #5, and #6 fall into the 1,8-cineole/camphor group, while sample #3 falls into the β -pinene/caryophyllene oxide group. Interestingly, the 1,8-cineole/camphor group is dominated by samples from Iran. The β -

Table 2. Chemical compositions (percent of total) of *Achillea millefolium* var. *occidentalis* essential oils.

RI _{calc}	RI _{db}	Compounds	#1	#2	#3	#4	#5	#6
847	846	(Z)-Salvene	-	-	0.1	-	-	-
874	873	2-Methylbutyl acetate	-	-	0.3	-	-	-
902	902	Santolina triene	-	0.1	0.3	-	0.1	-
922	922	Artemisia triene	-	-	0.2	tr	tr	-
923	923	Tricyclene	tr	tr	-	tr	0.1	0.3
925	927	α-Thujene	tr	tr	0.2	tr	0.2	0.1
932	932	α-Pinene	0.3	0.4	0.9	0.2	1.4	2.2
945	942	5,5-Dimethylbut-3-enolide	tr	tr	-	-	0.1	-
949	950	Camphene	0.4	1.0	0.1	0.4	1.7	7.3
960	959	Benzaldehyde	tr	tr	0.1	0.1	0.1	tr
969	967	Isoamyl propionate	-	tr	0.1	-	tr	tr
972	972	Sabinene	3.2	1.1	3.1	0.8	2.3	0.7
977	978	β-Pinene	0.5	0.5	2.2	0.3	6.3	1.6
988	989	Myrcene	0.1	0.1	0.2	tr	0.1	0.1
989	990	Dehydro-1,8-cineole	0.1	tr	0.1	0.1	0.2	0.1
995	996	Yomogi alcohol	-	1.0	8.8	2.1	3.6	tr
1015	1015	Isoamyl isobutyrate	0.1	0.1	0.3	0.1	0.1	0.1
1017	1017	α-Terpinene	0.2	0.2	1.9	1.1	1.5	0.1
1024	1025	p-Cymene	0.1	0.5	2.6	1.1	4.3	0.7
1028	1026	2-Acetyl-3-methylfuran	-	-	-	0.1	tr	tr
1029	1030	Limonene	0.2	0.4	0.3	0.1	1.1	0.5
1030	1031	β-Phellandrene	tr	tr	0.1	tr	0.1	tr
1032	1032	1,8-Cineole	10.8	7.5	7.6	16.4	10.7	5.3
1035	1036	Benzyl alcohol	-	-	0.1	0.2	-	-
1037	1034	Lavender lactone	-	-	-	-	0.1	tr
1042	1043	Phenylacetaldehyde	0.1	0.1	tr	0.1	0.2	0.2
1058	1056	Artemisia Ketone	-	-	4.2	5.7	18.7	-
1059	1058	γ-Terpinene	0.5	1.9	4.2	1.4	-	0.4
1069	1069	cis-Sabinene hydrate	0.7	1.9	1.1	4.4	1.0	1.4
1079	1079	Artemisia alcohol	-	2.0	3.8	0.2	1.0	-
1085	1086	Terpinolene	0.1	0.1	0.7	0.8	0.6	0.1
1095	1096	Methyl benzoate	-	-	0.1	0.2	-	-
1100	1101	Linalool	0.8	1.0	-	-	0.1	tr
1101	1101	trans-Sabinene hydrate	-	0.3	0.7	2.2	0.6	0.5
1103	1103	2-Methylbutyl isovalerate	-	0.8	1.1	-	0.5	0.5
1103	1104	Hotrienol	-	-	-	-	0.3	0.1
1105	1104	Nonanal	-	-	-	0.1	0.1	tr
1106	1105	α-Thujone	40.5	0.1	15.0	-	-	-
1108	1103	2-Methylbutyl isovalerate	-	-	-	0.1	tr	tr
1112	1111	Phenethyl alcohol	0.1	0.2	0.2	-	-	-
1118	1118	β-Thujone	5.6	tr	1.8	-	-	-
1120	1118	Dehydrosabina ketone	-	-	-	0.1	tr	-
1121	1122	trans-p-Mentha-2,8-dien-1-ol	tr	tr	tr	0.1	0.1	0.1
1124	1124	cis-p-Menth-2-en-1-ol	0.1	0.1	0.5	1.3	0.5	0.1
1126	1126	α-Campholenal	tr	tr	-	0.1	0.1	0.2
1130	1131	Limona ketone	tr	-	-	tr	tr	-
1136	1138	cis-p-Mentha-2,8-dien-1-ol	tr	-	-	-	0.1	0.1
1139	1139	Nopinone	-	-	-	-	0.1	-
1140	1140	trans-Sabinol	0.3	-	0.1	-	-	-
1141	1141	trans-Pinocarveol	-	-	0.1	0.4	0.1	0.4
1142	1142	trans-p-Menth-2-en-1-ol	0.1	0.1	0.3	1.0	0.4	tr

Table 2. (Continued)

RI _{calc}	RI _{db}	Compounds	#1	#2	#3	#4	#5	#6
1148	1149	Camphor	7.2	19.4	0.5	15.8	8.8	59.6
1155	n.a.	3,3,6-Trimethyl-4,5-heptadien-2-one	-	-	-	0.2	-	-
1156	1155	<i>trans</i> -Chrysanthemol	-	0.9	-	-	0.1	-
1157	1157	Sabina ketone	0.1	-	-	0.2	0.1	0.1
1159	1165	<i>iso</i> -Borneol	0.1	-	-	-	-	-
1160	1162	(<i>Z</i>)- <i>iso</i> -Citral	-	-	-	0.1	-	-
1162	1164	Pinocarvone	0.1	tr	-	0.4	0.3	0.6
1164	1164	β -Artemisyl acetate	0.1	1.6	3.8	0.9	1.5	-
1169	1168	α -Phellandrene epoxide	0.1	-	-	-	-	-
1170	1170	δ -Terpineol	0.2	-	0.1	0.5	0.1	0.1
1172	1173	Borneol	0.6	3.5	-	0.3	0.7	1.3
1181	1180	Terpinen-4-ol	1.7	2.4	9.0	23.4	8.9	0.9
1183	1183	Thuj-3-en-10-al	0.1	-	-	-	0.1	tr
1187	1186	<i>p</i> -Cymen-8-ol	tr	tr	tr	0.1	0.2	0.1
1188	1191	1-Dodecene	0.1	-	-	-	-	-
1195	1195	α -Terpineol	2.5	2.9	1.1	1.8	0.8	0.3
1196	1196	Myrtenal	-	-	-	-	0.9	0.7
1198	1187	Cryptone	0.2	0.1	-	-	0.2	0.1
1198	1196	<i>cis</i> -Piperitol	-	-	0.1	0.1	-	-
1207	1208	Verbenone	-	-	-	-	-	0.1
1208	1207	(<i>3E</i>)-Octenyl acetate	0.1	-	-	-	-	-
1208	1208	<i>trans</i> -Piperitol	0.1	tr	0.2	-	-	-
1218	1217	7-Ethylidene-bicyclo[3.3.0]octan-3-one	0.3	-	-	0.1	-	-
1237	1237	Pulegone	-	-	-	-	0.1	-
1240	1240	Ascaridole	-	-	-	0.1	-	-
1241	1242	Cuminal	0.1	-	-	0.1	0.1	-
1243	1242	Carvone	-	-	-	-	0.1	0.1
1269	1264	2,5-Bornanedione	-	-	-	-	-	0.1
1272	1266	Chrystanthemyl acetate	-	3.4	0.1	0.1	0.1	0.6
1283	1284	Lavandulyl acetate	-	-	0.4	0.1	0.1	-
1284	1285	Bornyl acetate	0.6	5.4	0.1	0.7	1.7	2.2
1287	1286	<i>trans</i> -Sabinylyl acetate	0.3	-	0.3	-	-	-
1290	1291	<i>p</i> -Cymen-7-ol	0.1	0.1	0.1	-	-	-
1296	1296	Terpin-1-en-4-yl acetate	-	-	0.1	0.1	0.2	-
1298	1300	Carvacrol	-	tr	0.1	0.1	0.1	tr
1304	1306	<i>iso</i> -Ascaridole	-	-	-	0.1	tr	-
1327	1327	<i>p</i> -Mentha-1,4-dien-7-ol	0.2	tr	-	-	-	-
1334	1335	δ -Elemene	0.1	-	-	-	-	-
1352	1356	Eugenol	0.4	0.6	0.2	0.3	0.2	0.1
1356	1349	α -Terpinyl acetate	0.1	-	-	-	-	-
1358	1361	Neryl acetate	-	0.1	-	-	-	-
1367	1367	Cyclosativene	-	0.1	-	-	-	-
1374	1375	α -Copaene	-	0.2	0.2	0.1	-	0.1
1377	1378	Geranyl acetate	-	0.3	-	-	-	-
1383	1385	(<i>E</i>)-Jasmone	0.1	0.1	-	0.1	-	-
1384	1384	Benzyl pentanoate	0.1	0.2	0.1	tr	-	-
1386	1385	α -Isocomene	0.1	-	-	-	-	0.1
1388	1390	<i>trans</i> - β -Elemene	tr	0.1	-	-	-	tr
1391	1392	(<i>Z</i>)-Jasmone	0.2	1.0	0.2	0.2	0.1	tr
1396	1396	(<i>2E</i>)-1,3,7-Trimethyl-2,6-octadienyl acetate	-	0.4	-	-	0.1	-
1410	1413	β -Isocomene	0.1	-	-	-	-	-

Table 2. (Continued)

RI _{calc}	RI _{db}	Compounds	#1	#2	#3	#4	#5	#6
1419	1417	(E)-β-Caryophyllene	0.6	1.7	0.5	0.2	0.5	0.5
1453	1452	(E)-β-Farnesene	0.1	0.1	0.2	0.1	0.1	0.1
1455	1454	α-Humulene	0.1	0.3	0.1	tr	0.1	0.1
1481	1480	Germacrene D	2.0	2.6	3.2	1.8	1.6	1.5
1483	1483	Davana ether 1	0.1	0.1	-	-	-	-
1484	1483	Phenethyl 2-methylbutyrate	-	0.1	0.1	-	0.1	0.1
1488	1489	β-Selinene	-	-	-	-	-	0.1
1491	1489	(Z,E)-α-Farnesene	-	0.1	-	-	-	-
1494	1497	Bicyclogermacrene	0.1	0.1	0.2	0.1	0.1	0.1
1494	1497	α-Selinene	-	-	-	-	-	0.1
1498	1497	α-Muurolene	-	0.3	0.2	tr	-	0.1
1502	1502	Davana ether 2	0.3	0.2	-	-	-	-
1504	1504	Davana ether 3	0.1	-	-	-	-	-
1504	1503	(E,E)-α-Farnesene	tr	0.1	0.2	-	tr	-
1512	1512	γ-Cadinene	tr	tr	tr	-	-	0.1
1514	1515	Cubebol	-	-	tr	-	-	0.1
1517	1518	δ-Cadinene	0.1	0.2	0.1	0.1	0.1	0.2
1521	1521	Davana ether 4	0.2	0.1	-	-	-	-
1523	1523	β-Sesquiphellandrene	0.1	-	0.1	-	-	-
1547	1549	α-Elemol	0.1	-	0.4	0.5	-	-
1560	1561	(E)-Nerolidol	5.7	13.5	4.4	0.4	1.4	0.5
1569	1570	Neryl 2-methylbutanoate	0.3	-	0.1	tr	0.1	-
1572	1569	Longipinocarvone	0.2	0.2	-	0.3	0.5	0.3
1577	1580	Neryl isovalerate	0.3	-	0.3	0.1	0.1	0.2
1581	1587	Caryophyllene oxide	0.7	0.8	0.5	0.5	1.3	1.0
1591	1593	Salvial-4(14)-en-1-one	-	-	-	0.1	0.1	-
1597	1597	Fokienol	-	0.8	0.8	0.1	0.7	0.2
1608	1613	Copaborneol	-	0.3	0.2	-	-	-
1610	1613	Humulene epoxide II	-	-	-	-	tr	tr
1612	1609	Rosifoliol	-	-	0.2	0.1	-	-
1621	1632	Humulenol II	-	-	-	0.5	1.7	0.7
1629	1627	Eremoligenol	-	-	0.4	0.1	-	-
1631	1632	γ-Eudesmol	-	-	3.0	-	-	-
1632	1630	Caryophylla-4(12),8(13)-dien-5α-ol	0.2	0.3	0.3	0.2	0.8	0.5
1635	1635	Caryophylla-4(12),8(13)-dien-5β-ol	0.5	0.4	0.4	0.6	0.8	0.4
1641	1643	τ-Cadinol	-	-	-	-	-	0.4
1642	1642	Methyl (Z)-jasmonate	0.1	0.9	0.2	-	0.2	0.1
1654	1655	α-Eudesmol	-	-	0.7	3.0	-	0.1
1655	1655	α-Cadinol	0.4	0.3	-	-	-	0.1
1671	1671	14-Hydroxy-9-epi-(E)-caryophyllene	-	-	-	0.5	-	0.1
1680	1676	8-Hydroxyisobornyl isobutanoate	-	-	-	0.2	1.2	0.6
1684	1683	Germacra-4(15),5,10(14)-trien-1α-ol	-	-	0.3	1.3	-	-
1716	1716	(2E,6E)-Farnesol	-	0.1	0.2	0.1	-	-
1730	1730	Chamazulene	4.4	7.0	0.8	-	0.7	0.8
1775	1784	Dehydrochamazulene	-	0.1	-	-	-	-
1832	1832	(2Z,6E)-Farnesyl acetate	-	0.1	-	-	-	-
1932	1938	Pellitorine	-	-	-	-	-	0.4
2300	2300	Tricosane	0.1	0.1	-	0.1	0.1	tr
2500	2500	Pentacosane	0.1	0.1	-	0.1	0.1	0.1
2700	2700	Heptacosane	0.1	0.1	-	0.1	0.1	tr

Table 2. (Continued)

RI _{calc}	RI _{db}	Compounds	#1	#2	#3	#4	#5	#6
Compound Classes								
		<i>Monoterpene hydrocarbons</i>	5.7	6.5	17.0	6.2	19.7	13.8
		<i>Oxygenated monoterpenoids</i>	74.3	53.8	60.3	79.7	64.0	75.8
		<i>Sesquiterpene hydrocarbons</i>	7.8	12.8	5.7	2.2	3.1	3.8
		<i>Oxygenated sesquiterpenoids</i>	8.5	17.3	11.7	8.3	7.2	4.3
		<i>Benzenoid aromatics</i>	0.6	1.1	0.8	0.8	0.5	0.4
		<i>Others</i>	1.1	3.5	2.3	1.0	1.7	1.1
		Total identified	97.9	95.0	97.8	98.2	96.2	99.1

RI_{calc} = Retention index determined using a homologous series of n-alkanes on a ZB-5ms column [53]. RI_{db} = Reference retention index from the databases [54–57]. tr = trace (< 0.05%). - = not detected. Major components are highlighted in bold.

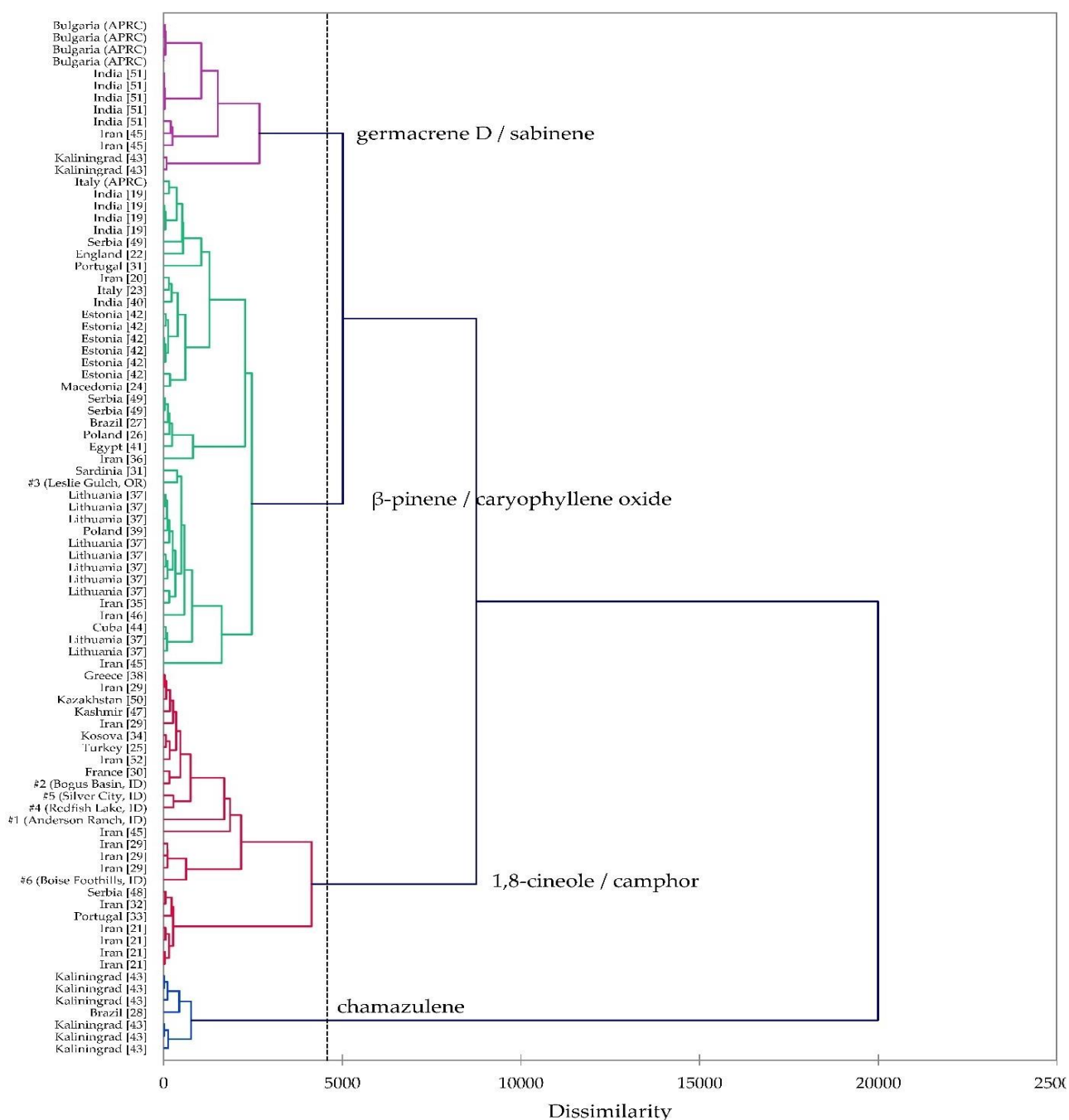


Figure 2. Dendrogram based on hierarchical cluster analysis of *Achillea millefolium* essential oil compositions.

pinene/caryophyllene oxide group, on the other hand, is dominated by samples from Europe. It is apparent that there is much chemical diversity in the essential oils of *A. millefolium* and that essential oil chemical compositions cannot distinguish New World (i.e., var. *occidentalis*) samples of *A. millefolium* from Old World samples.

Kokkalou and co-workers had noted wide variation in *A. millefolium* essential oil compositions when comparing samples of European origin with samples cultivated in North America [38]. Likewise, Farajpour and co-workers had observed wide variation in *A. millefolium* essential oil compositions from Iran [58].

These investigators identified five chemotypes: 1,8-cineole/(*E*)-nerolidol, high cubenol, high germacrene-D/isospathulenol, high camphor/cubenol, and high 1,8-cineole/ β -thujone/cubenol. Furthermore, Raudone and co-workers investigated different morphotypes of *A. millefoium* and found notable differences in triterpenoid and phenolic components [2].

The *A. millefolium* essential oils were analyzed by enantioselective GC-MS (Table 3). The dominant enantiomers were (-)- α -pinene ($83.2 \pm 5.4\%$), (-)-camphene ($98.5 \pm 2.2\%$), (-)- β -pinene ($89.5 \pm 6.1\%$), and (-)- α -terpineol ($81.8 \pm 4.3\%$). Sabinene, limonene, *cis*-sabinene hydrate, and *trans*-sabinene hydrate had variable enantiomeric distributions. The sesquiterpenoids (-)-(*E*)- β -caryophyllene, (-)-germacrene D, and (+)-(*E*)-nerolidol were the only enantiomers observed. (-)-Camphor was the only enantiomer observed. When detected, only (-)- β -phellandrene, (-)- α -thujone, (+)- β -thujone, and (-)-borneol were observed. Orth and co-workers carried out enantioselective GC-MS on *A. millefolium* from Kaliningrad [43]. These investigators found (-)- β -pinene to dominate, but sabinene and α -pinene distributions were variable, although (-)-sabinene and (-)- α -pinene were often dominant.

There have been several investigations on enantioselective GC-MS analyses of Asteraceae essential oils. However, there are no obvious trends in the enantiomeric distribution of monoterpene hydrocarbons or of oxygenated monoterpenoids in the family [59]. When detected, (-)-(*E*)- β -caryophyllene is the only enantiomer observed [60–62]. This enantiomer is found in numerous higher plants, while (+)-(*E*)- β -caryophyllene is apparently found in liver-

worts [13]. Likewise, (-)-germacrene D and (+)-(*E*)-nerolidol are the dominant, if not exclusive, enantiomers found in the Asteraceae [59, 61, 62].

Several of the major components in *A. millefolium* have shown relevant biological activities. For example, 1,8-cineole, α -thujone, β -thujone, and camphor have shown analgesic-like activity [63, 64]. Camphor and 1,8-cineole have shown antitussive effects in a Guinea-pig model [65]. A combination of (-)-camphor and 1,8-cineole has shown synergistic antimicrobial activity against *Candida albicans* [66]. Terpinen-4-ol has shown antimicrobial activity against several pathogenic micro-organisms [67]. Terpinen-4-ol has also shown anti-inflammatory effects [68], attributed to suppression of pro-inflammatory cytokine production [69, 70]. (*E*)-Nerolidol has shown antinociceptive activity involving the GABAergic system and not to the opioidergic or ATP-sensitive K⁺ channels [71]. Nerolidol has also shown anti-inflammatory activity attributed to suppression of TNF- α and IL-1 β pro-inflammatory cytokines [71]. Thus, the bioactivities of the major components in *A. millefolium* essential oil are consistent with the traditional uses of the plant by Native Americans.

4. Conclusions

The essential oils of *Achillea millefolium* var. *occidentalis*, collected from several sites in the Great Basin of North America, show wide variation in their chemical compositions, including broadly variable concentrations of camphor, α -thujone, terpinen-4-ol, artemisia ketone, 1,8-cineole, and (*E*)-nerolidol. A survey of the literature also reveals wide variation in *A. millefolium* essential oil compositions in samples from Europe and Asia. Furthermore, because of the wide variation in essential oil compositions of *A. millefolium* from the Great Basin as well as the wide variation in compositions from samples of European or Asian origin, essential oil compositions cannot distinguish between New-World and Old-World *A. millefolium* essential oils. Thus, there is no phytochemical support for infraspecific separation of this species. Future research using DNA barcoding may provide evidence for the separation of infraspecific taxa. Enantioselective gas chromatography has shown (-)- α -pinene, (-)-camphene, (-)- β -pinene, (-)- α -terpineol, (-)-camphor, (-)- β -phellandrene, (-)- α -thujone, (+)- β -thujone, and (-)-borneol

Table 3. Enantiomeric distribution (percent of each enantiomer) of chiral terpenoid components in *Achillea millefolium* var. *occidentalis*.

Compounds	RI _{db}	RI _{calc}	#1	#2	#3	#4	#5	#6
(+)- α -Thujene	950	950	nd	nd	24.2	nd	0.0	nd
(-)- α -Thujene	951	951	nd	nd	75.8	nd	100.0	nd
(-)- α -Pinene	976	977	72.8	85.7	82.0	86.0	85.4	87.3
(+)- α -Pinene	982	983	27.2	14.3	18.0	14.0	14.6	12.7
(-)-Camphene	998	1000	100.0	100.0	94.3	99.2	98.2	99.3
(+)-Camphene	1005	1003	0.0	0.0	5.7	0.8	1.8	0.7
(+)-Sabinene	1021	1020	56.1	11.8	47.2	15.7	42.6	15.8
(-)-Sabinene	1030	1029	43.9	88.2	52.8	84.3	57.4	84.2
(+)- β -Pinene	1027	1027	18.8	10.5	10.6	7.7	1.1	14.6
(-)- β -Pinene	1031	1031	81.2	89.5	89.4	92.3	98.9	85.4
(-)-Limonene	1073	1074	68.9	83.1	78.9	80.4	31.8	74.7
(+)-Limonene	1081	1081	31.1	16.9	21.1	19.6	68.2	25.3
(-)- β -Phellandrene	1083	1082	nd	nd	100.0	nd	100.0	nd
(+)- β -Phellandrene	1089	nd	nd	nd	0.0	nd	0.0	nd
(+)-cis-Sabinene hydrate	1199	1199	75.4	88.1	7.7	16.8	23.8	79.1
(-)-cis-Sabinene hydrate	1202	1201	24.6	11.9	92.3	83.2	76.2	20.9
(+)- α -Thujone	1213	nd	0.0	0.0	0.0	nd	nd	nd
(-)- α -Thujone	1222	1221	100.0	100.0	100.0	nd	nd	nd
(-)-Linalool	1228	1231	100.0	16.5	nd	nd	nd	nd
(+)-Linalool	1231	1234	0.0	83.5	nd	nd	nd	nd
(+)- <i>trans</i> -Sabinene hydrate	1231	1229	nd	nd	8.1	25.9	18.5	61.5
(-)- <i>trans</i> -Sabinene hydrate	1235	1233	nd	nd	91.9	74.1	81.5	38.5
(+)- β -Thujone	1230	1232	100.0	nd	100.0	nd	nd	nd
(-)- β -Thujone	na	nd	0.0	nd	0.0	nd	nd	nd
(-)-Camphor	1253	1254	100.0	100.0	100.0	100.0	100.0	100.0
(+)-Camphor	1259	nd	0.0	0.0	0.0	0.0	0.0	0.0
(+)-Terpinen-4-ol	1297	1295	60.5	59.3	26.1	21.5	31.0	61.5
(-)-Terpinen-4-ol	1300	1297	39.5	40.7	73.9	78.5	69.0	38.5
(-)-Borneol	1335	1336	100.0	100.0	nd	nd	100.0	100.0
(+)-Borneol	1340	nd	0.0	0.0	nd	nd	0.0	0.0
(-)- α -Terpineol	1347	1346	87.6	86.1	81.7	76.9	78.1	80.7
(+)- α -Terpineol	1356	1355	12.4	13.9	18.3	23.1	21.9	19.3
(-)-(<i>E</i>)- β -Caryophyllene	1461	1462	100.0	100.0	100.0	100.0	100.0	100.0
(+)-(<i>E</i>)- β -Caryophyllene	na	nd	0.0	0.0	0.0	0.0	0.0	0.0
(+)-Germacrene D	1519	nd	0.0	0.0	0.0	0.0	0.0	0.0
(-)-Germacrene D	1522	1523	100.0	100.0	100.0	100.0	100.0	100.0
(-)-(<i>E</i>)-Nerolidol	1677	nd	0.0	0.0	0.0	0.0	0.0	0.0
(+)-(<i>E</i>)-Nerolidol	1680	1679	100.0	100.0	100.0	100.0	100.0	100.0

RI_{db} = Retention index from our in-house database. RI_{calc} = Calculated retention index based on a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. na = reference compound not available. nd = compound not detected.

to be the dominant monoterpenoid enantiomers in *A. millefolium* from western North America. However, there are no obvious trends in the enantiomeric distribution of monoterpenoids in the Asteraceae family overall. Additional research using enantioselective gas chromatography may be able to identify trends within individual genera, however.

Authors' contributions

Conceptualization, W.N.S.; Methodology, A.P., P.S.,

and W.N.S.; Software, P.S.; Validation, W.N.S., Formal Analysis, A.P., and W.N.S.; Investigation, A.P., P.S., K.S., and W.N.S.; Resources, P.S. and W.N.S.; Data Curation, W.N.S.; Writing – Original Draft Preparation, W.N.S.; Writing – Review & Editing, P.S. and W.N.S.; Project Administration, W.N.S.

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Availability of data and materials

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Conflicts of interest

The authors declare no conflict of interest.

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