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Chemical composition of *Artemisia frigida* Willd. from Southwestern Idaho

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Abstract
Artemisia frigida is an aromatic medicinal plant native to North America, Eastern Europe, and Asia. As part of our interest in aromatic and medicinal plants of western North America, we have investigated the essential oil of *A. frigida* growing in southwestern Idaho, the first report of *A. frigida* essential oil from the western United States. The essential oils from the aerial parts of three individual *A. frigida* plants were obtained by hydrodistillation and analyzed by gas chromatography and enantioselective gas chromatography. The essential oils showed wide variation in composition; the major components were 1,8-cineole (18.9-22.8%), camphor (0.1-37.5%), davanone (0.0-35.6%), *trans*-chrysanthenol (0.0-18.5%), and *trans*-chrysanthenyl acetate (0.0-17.5%). A hierarchical cluster analysis based on major components of the essential oils from this work (New World) and from Old World samples, revealed at least six chemical groups depending on the concentrations of camphor, 1,8-cineole, borneol, camphene, and davanone, indicating wide chemical variation throughout the geographical range of *A. frigida*. The (-)-enantiomers were dominant for the observed chiral monoterpenoids (α-pinene, camphene, sabinene, β-pinene, limonene, *cis*-sabinene hydrate, camphor, terpinen-4-ol, borneol, and α-terpineol), but there are few trends in enantiomeric distributions compared to other *Artemisia* species.

Keywords Fringed sagebrush, prairie sagewort, hierarchical cluster analysis, gas chromatography, chiral, enantiomer

1. Introduction
There are some 525 species of *Artemisia* (Asteraceae) according to the World Flora Online [1] with 82 species occurring in North America [2] and 15 species found in the intermountain western United States [3, 4]. *Artemisia frigida* Willd. (fringed sagebrush, prairie sagewort) is found in the steppes of Asia (Kyrgyzstan, Mongolia, Russia, Tajikistan, and China), as well as southwestern Asia and eastern Europe [5]. It is also found in North America, including the high desert region of western United States [6, 7]. The plant is a perennial sub-shrub, strongly aromatic, growing up to 40 cm tall. The plant has a silky silvery-gray appearance due to abundant silvery hairs. The leaves are small (5-7 mm × 5-7 mm), with 2-3 lobes. The inflorescence is made up of numerous spherical flower heads with several ray florets and many disc florets (Figure 1) [5, 6].
In North America, *A. frigida* is important as a traditional medicine by Native American tribes. An infusion or decoction of the plant is used by the Arapaho, Blackfoot, Montana, Navajo, and Okanagan people as a cough medicine and to treat colds and other lung problems [8]. As part of our interest in aromatic and medicinal plants of the intermountain western United States, the purpose of this study is to investigate the essential oil composition of *A. frigida* growing in southwestern Idaho.

2. Materials and Methods
2.1 Plant Material and Essential Oil
Three individual *Artemisia frigida* potted plants were purchased from High Country Gardens (Clinton, Utah, USA) and planted in the spring of 2023 (Kuna, Idaho, 43°30′44″ N, 116°24′9″ W, 907 m asl). Aerial parts from each plant were harvested on 8 July 2024 (flowering period). The fresh plant material was frozen (-20 °C) and stored frozen until processed. The fresh/frozen plant material was hydrodistilled for four hours using a Likens-Nickerson apparatus with continuous extraction of the distillate with dichloromethane. Evaporation of the dichloromethane gave pale-yellow essential oils (Table 1).

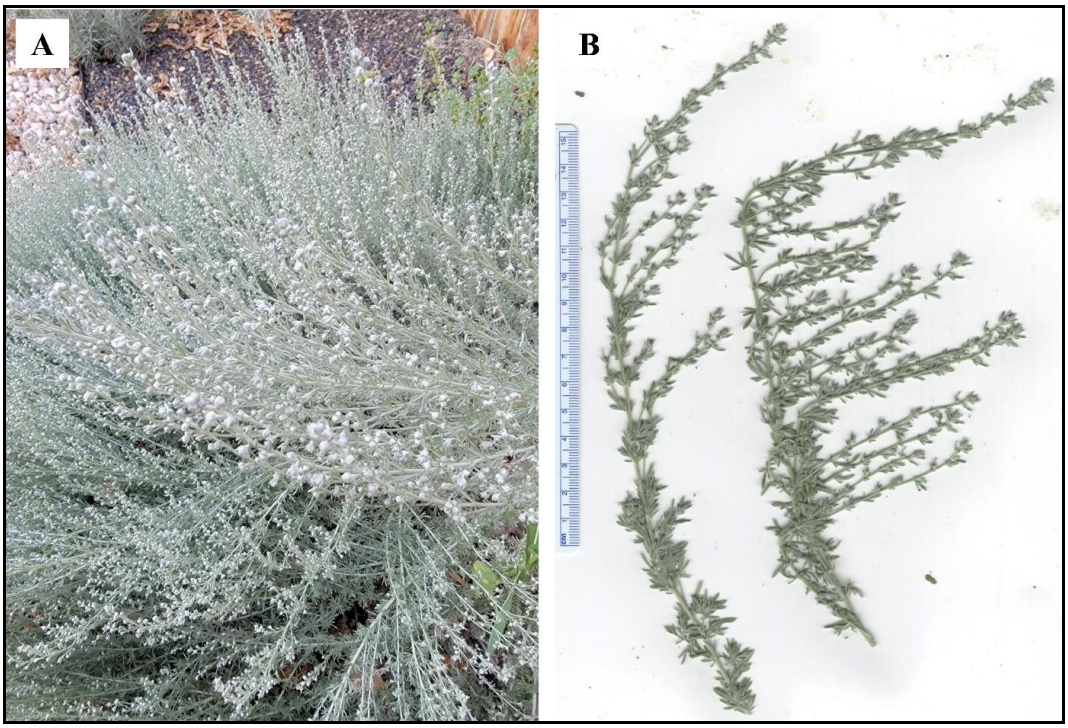

Fig 1: *Artemisia frigida* Willd. from southwestern Idaho. A: Photographs of plant A taken at the time of collection. B: Scan of pressed sample of plant B.

Table 1: Hydrodistillation of *Artemisia frigida* aerial parts.

Sample	Mass aerial parts (g)	Mass essential oil (g)	Yield (%)
A	85.51	3.4371	4.020
B	146.17	6.9164	4.732
C	103.50	5.6783	5.486

2.2 Gas Chromatographic Analysis

Gas chromatography-mass spectrometry (GC-MS), gas chromatography with flame ionization detection (GC-FID), and enantioselective GC-MS were carried out on the *A. frigida* essential oil samples as previously reported [9].

2.3 Hierarchical Cluster Analysis

Hierarchical cluster analysis (HCA) was carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The HCA analysis was carried out using the percentages of the 19 most abundant components, those most likely to define the different chemotypes, (camphene, yomogi alcohol, 1,8-cineole, *trans*-chrysanthenol, chrysanthenone, *cis*-*p*-menth-2-en-1-ol, camphor, borneol, lavandulol, terpinen-4-ol, α-terpineol, myrtenol, *trans*-chrysanthenyl acetate, bornyl acetate, germacrene D, bicyclogermacrene, davanone, spathulenol, and gymnomitrone) from this study and essential oil compositions from other geographical locations [10-15].

3. Results and Discussion

The pale-yellow *A. frigida* essential oils were obtained in yields of 4.02-5.49%. Gas chromatographic analysis of the essential oils allowed for identification of 107 components, which constituted 96.1%, 98.2%, and 86.4% of the total compositions. The three essential oils showed wide variation in their compositions (Table 2), which cannot be explained by edaphic, climatic, or seasonal variation.

Table 2: Chemical compositions (percent) of the essential oils from the aerial parts of *Artemisia frigida* grown in southwestern Idaho.

RI_{calc}	RI_{db}	Compounds	Plant samples		
			A	B	C
800	801	Hexanal	0.1	0.1	0.1
849	849	(2*E*)-Hexenal	0.2	0.2	0.4
909	911	5-Ethyl-2-methyl-2-vinyltetrahydrofuran	-	-	0.3
914	915	2-Methyl-2,5-divinyltetrahydrofuran	-	-	0.7
922	923	Tricyclene	0.4	0.1	-
925	925	α-Thujene	0.1	0.1	0.1
933	933	α-Pinene	3.4	1.7	0.5
944	---	Unidentified [a]	-	-	1.3
946	945	4,4-Dimethyl-2-butenolide	-	0.2	3.8
949	950	Camphene	7.7	1.8	-
962	960	Benzaldehyde	-	tr	-
972	972	Sabinene	0.2	0.3	-
977	978	β-Pinene	1.8	0.5	0.3
979	979	Hexanoic acid	0.1	0.2	0.3
989	989	Myrcene	tr	1.0	-
990	990	Dehydro-1,8-cineole	0.1	0.1	0.3
1004	1004	Octanal	-	tr	0.1
1009	1009	δ-3-Carene	tr	-	-
1017	1017	α-Terpinene	0.1	-	-
1024	1025	*p*-Cymene	0.4	0.7	1.8
1029	1030	Limonene	0.5	0.1	0.1
1033	1032	1,8-Cineole	22.0	22.8	18.9
1035	1034	(*Z*)-β-Ocimene	tr	-	-
1036	1035	γ-Vinyl-γ-valerolactone	-	0.1	0.9
1043	1043	Phenylacetaldehyde	0.1	tr	tr
1046	1045	(*E*)-β-Ocimene	tr	0.1	-
1049	1049	*cis*-Arbusculone	-	0.1	1.0
1058	1058	γ-Terpinene	1.1	1.1	0.5
1068	1068	*trans*-Arbusculone	-	tr	0.2
1070	1069	*cis*-Sabinene hydrate	0.5	0.4	1.2
1074	1074	(2*E*)-Octen-1-ol	-	-	tr
1083	1083	Camphenilone	0.1	tr	-
1085	1086	Terpinolene	0.1	-	tr
1090	1091	*p*-Cymenene	tr	tr	-
1096	1099	6-Camphenone	0.2	-	-
1099	1103	Isoamyl isovalerate	-	-	0.1
1100	1103	Filifolone	-	0.1	-
1102	1101	*trans*-Sabinene hydrate	0.3	0.3	0.2
1105	1104	Nonanal	-	-	0.1
1107	1107	1-Octen-3-yl acetate	-	-	0.1
1116	1114	*trans*-Chrysanthenol	-	18.5	-
1121	1122	Chrysanthenone	0.1	0.4	-
1123	1122	*trans*-*p*-Mentha-2,8-dien-1-ol	0.1	-	-
1125	1124	*cis*-*p*-Menth-2-en-1-ol	0.1	0.1	0.2
1127	1127	α-Campholenal	0.2	0.1	0.1
1136	1144	*cis*-Chrysanthenol	-	2.4	-
1138	1139	Nopinone	0.1	-	-
1145	1142	*trans*-*p*-Menth-2-en-1-ol	0.1	-	-
1147	1145	Camphor	37.5	9.6	0.1
1157	1159	Benzoic acid	-	-	0.3
1162	1164	Pinocarvone	0.8	0.6	0.1
1170	1170	δ-Terpineol	0.3	0.4	0.2
1176	1173	Borneol	3.8	2.8	-
1181	1180	Terpinen-4-ol	3.5	3.4	2.4
1187	1186	*p*-Cymen-8-ol	-	0.1	0.1
1196	1195	α-Terpineol	0.5	0.8	0.6
1206	1206	Decanal	-	0.1	0.1
1213	1208	Verbenone	tr	-	-
1214	1217	2-Oxocineole	0.1	-	0.2
1224	1224	*nor*-Davanone	-	-	1.0
1230	1231	*trans*-Chrysanthenyl acetate	-	17.5	0.2
1240	1240	Ascaridole	0.2	0.2	0.1
1245	1246	Carvone	tr	-	-
1256	1257	*cis*-Chrysanthenyl acetate	-	0.1	-
1267	1264	2,5-Bornanedione	0.5	0.1	-

1285	1285	Bornyl acetate	5.4	2.4	-
1290	1289	Thymol	-	0.1	-
1305	1306	*iso*-Ascaridole	0.1	0.2	0.1
1320	1354	*cis*-Chrysanthenyl propionate	-	0.1	-
1333	1332	*trans*-Carvyl acetate	tr	-	-
1338	1337	*exo*-2-Hydroxycineole acetate	0.2	0.1	1.2
1351	1356	Eugenol	0.2	0.1	-
1353	1376	3-(5-Methyl-5-vinyltetrahydrofuran-2-yl)butan-2-ol	-	-	0.3
1358	1361	Neryl acetate	tr	-	-
1358	---	*endo*-2-Hydroxycineole acetate [b]	-	0.1	0.1
1368	1367	Cyclosativene	-	-	0.2
1376	1375	α-Copaene	0.3	0.1	0.5
1378	1378	Geranyl acetate	0.1	-	-
1384	1382	β-Bourbonene	tr	-	-
1389	1390	*trans*-β-Elemene	tr	0.1	-
1392	1392	(*Z*)-Jasmone	0.3	-	-
1400	1400	4-(4-Methylphenyl)pentanal	-	0.1	-
1448	1448	5-Oxobornyl acetate	0.5	0.1	-
1458	1460	Cabreuva oxide B	tr	-	-
1473	1476	Selina-4,11-diene	0.1	0.1	0.2
1475	1477	Cabreuva oxide D	0.1	-	-
1475	1478	γ-Muurolene	-	-	0.3
1481	1480	Germacrene D	0.6	1.0	0.1
1486	1484	3,5,11-Eudesmatriene	0.1	-	-
1488	1489	β-Selinene	0.1	-	-
1495	1497	Bicyclogermacrene	0.1	0.1	-
1498	1497	α-Muurolene	-	-	0.1
1515	1516	Artedouglasia oxide C	-	-	0.3
1518	1518	δ-Cadinene	tr	0.1	0.3
1527	1529	Artedouglasia oxide A	-	-	0.4
1556	1556	Davanone C	-	-	0.2
1562	1562	Davanone D	-	-	0.5
1573	1573	Artedouglasia oxide B	-	-	0.3
1578	1577	Davanone	-	3.8	35.6
1618	---	Unidentified [c]	2.2	0.4	-
1633	1631	Longifolenaldehyde	-	-	0.4
1636	---	Unidentified [d]	-	-	1.0
1638	1637	Gymnomitrone	-	-	6.1
1641	1642	Methyl (*Z*)-jasmonate	0.1	-	0.2
1650	---	Unidentified [e]	-	-	1.7
1657	1655	α-Cadinol	0.2	0.3	-
1684	---	Unidentified [f]	-	-	5.5
1710	1708	β-Davanone-2-ol	-	-	1.2
1753	1760	*cis*-Lanceol	-	0.2	-
1777	1769	Methyl isocostate	0.1	0.1	-
1845	1853	*trans*-Valerenyl acetate	0.2	0.1	-
1868	1865	2α-Acetoxy-11-methoxyamorpha-4,7-diene	0.4	-	0.4
		Monoterpene hydrocarbons	15.7	7.4	3.2
		Oxygenated monoterpenoids	77.2	83.8	27.3
		Sesquiterpene hydrocarbons	1.2	1.4	1.6
		Oxygenated sesquiterpenoids	1.0	4.4	45.4
		Benzenoid aromatics	0.2	0.2	0.3
		Others	0.8	0.9	8.6
		Total identified	96.1	98.2	86.4

RI_{calc} = Calculated retention index using the formula of van den Dool and Kratz [16]. RI_{db} = Reference retention index values from the databases [17-20]. tr = trace (< 0.05%). [a] MS(EI): 140(4%), 125(16%), 111(100%), 96(32%), 82(80%), 67(66%), 55(81%), 43(97%), 41(45%). [b] A reference RI value was not available; however, the reference MS showed a 95% match. [c] MS(EI): 152(17%), 150(12%), 134(43%), 119(26%), 108(57%), 95(31%), 93(33%), 92(38%), 91(30%), 81(14%), 79(14%), 67(14%), 55(10%), 43(100%), 41(15%). [d] MS(EI): 221(8%), 139(15%), 126(15%),125(13%), 121(10%), 111(88%), 98(61%), 97(98%), 93(63%), 81(37%), 69(47%), 57(33%), 55(76%), 43(100%). [e] MS(EI): 221(12%), 139(14%), 125(15%), 111(87%), 98(44%), 97(100%), 93(77%), 81(27%), 69(47%), 55(79%), 43(79%), 41(39%). [f] MS(EI): 250(1%), 235(3%), 140(12%), 125(18%), 111(100%), 93(58%), 83(18%), 69(37%), 55(46%), 43(23%), 41(28%).

There have been several previous investigations of *A. frigida* essential oils from different geographical locations. In order to probe the chemical relationships between the essential oils, a hierarchical cluster analysis (HCA) was carried out (Figure 2). The cluster analysis reveals at least six groups: Group 1, 2, and 3 are all rich in both camphor and 1,8-cineole; Group 6 is rich in camphor, borneol, and camphene; Group 4 is not dominated by any particular component, but these essential oils had a low number of identified components; and Group 5 had only one sample, dominated by davanone.

Interestingly, Group 1 is composed primarily of samples from the Altai Republic of Russia, the Tuva Republic of Russia,

Mongolia, and Inner Mongolia, as well as sample B from Idaho. Samples from Russia as well as a sample from Alberta, Canada, and a sample from Kazakhstan make up Group 2. Group 3 is made up of samples from Russia, China, and Idaho sample A. Samples from China and from Russia are found in Group 4, and samples from Russia are found in Group 6. It is apparent that the chemical composition of *A. frigida* is variable throughout it range and habitats. Genetic factors may play a role in the essential oil variations within a given geographical location [21]. There are more than 500 species of *Artemisia* currently recognized by World Flora Online [1]. Numerous species of *Artemisia* have either 1,8-cineole, camphor, chrysanthenol, chrysanthenyl acetate, or davanone as major components [22-25].

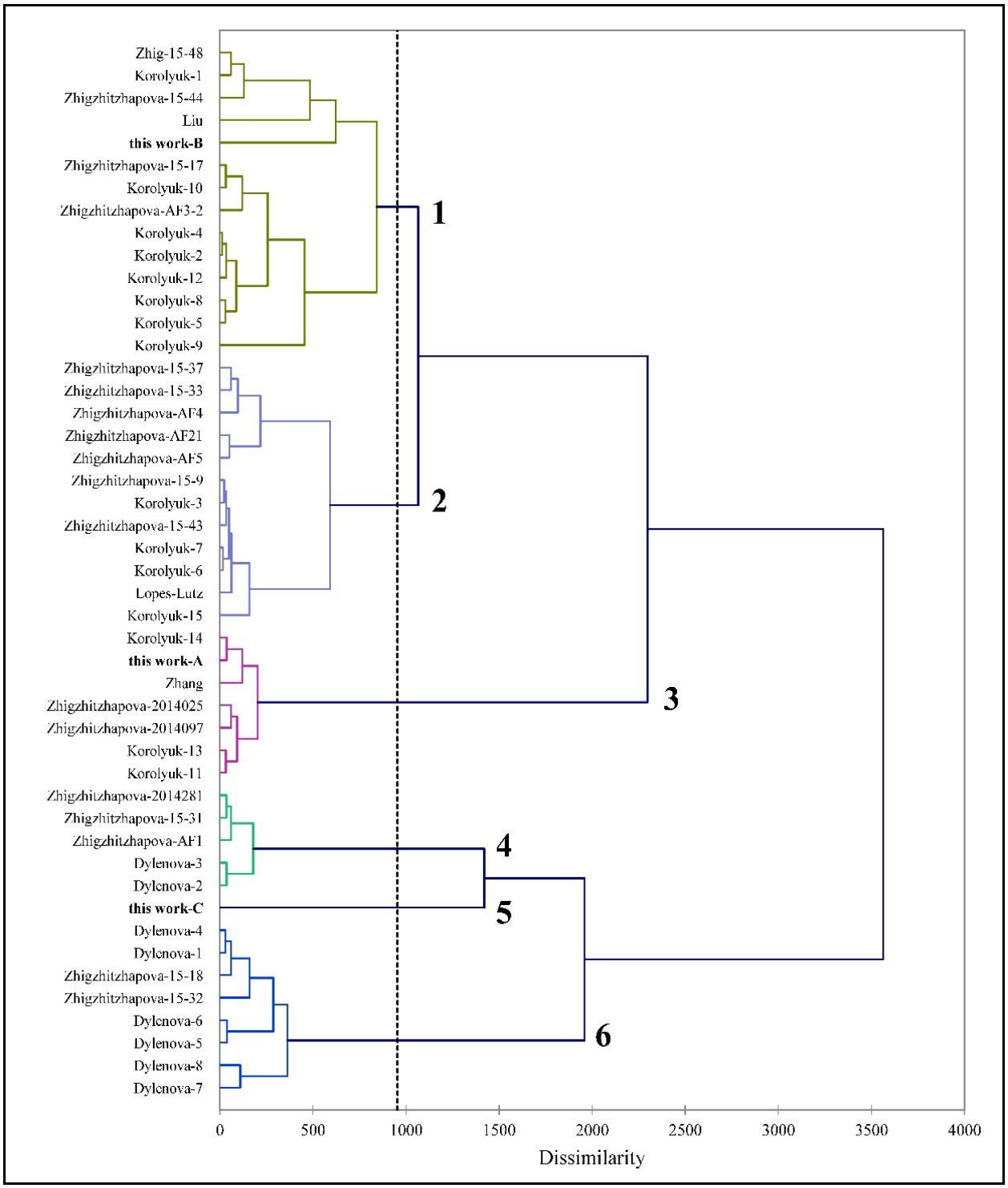

Fig 2: Dendrogram based on the hierarchical cluster analysis of *Artemisia frigida* major components. Dylenova = Dylenova *et al*., 2023 [10], Korolyuk = Korolyuk & Tkachev, 2010 [11], Lopes-Lutz = Lopes-Lutz *et al*., 2008 [12], Liu = Liu *et al*., 2014 [13], Zhang = Zhang *et al*., 2019 [14], Zhigzhitzhapova = Zhigzhitzhapova *et al*., 2017 [15].

The major components in *A. frigida* from Idaho, 1,8-cineole, *trans*-chrysanthenol, camphor, *trans*-chrysanthenyl acetate, and davanone are noteworthy. 1,8-Cineole has shown bronchodilation, mucus reduction, anti-inflammatory, and airway clearance activities [26-28], while camphor has shown antitussive effects in Guinea pig models [29, 30]. Consistent with these effects, camphor is predicted to be a respiratory analeptic and antiviral (influenza) [31]. Similarly,

chrysanthenol, chrysanthenyl acetate, and davanone are predicted to show mucomembranous protective, anti-inflammatory, and respiratory analeptic activities, and davanone is also predicted to show antiviral activity against rhinovirus [31]. Thus, the major components found in North American *A. frigida* are consistent with the Native American traditional uses of the plant.

Enantioselective GC-MS was carried out to further characterize the *A. frigida* essential oils, allowing determination of the enantiomeric distribution of chiral monoterpenoid components (Table 3). For each monoterpenoid identified (α-pinene, camphene, sabinene, β-pinene, limonene, *cis*-sabinene hydrate, camphor, terpinen-4-ol, borneol, and α-terpineol), the (-)-enantiomer was the dominant. Interestingly, a comparison with other high-desert *Artemisia* species, the dominance of (-)-enantiomers is not consistent (Table 4). The enantiomeric distribution in *A. frigida* closely mirrors those found in *A. ludoviciana* and *A. tridentata* subsp. *vaseyana*, while *A. dracunculus*, *A tridentata* subsp. *tridentata*, and *A. tridentata* subsp. *wyomingensis* show differences. In *A. dracunculus*, (+)-α-pinene and (+)-limonene predominated; in *A. tridentata* subsp. *tridentata*, (+)-α-pinene and (+)-β-pinene were the dominant enantiomers; and in *A. tridentata* subsp. *wyomingensis*, (+)-camphor and (+)-terpinen-4-ol were dominant. (-)-Sabinene, (-)-*cis*-sabinene hydrate, and (-)-α-terpineol concentrations were consistent across the *Artemisia* species.

Table 3: Enantiomeric distribution (percent) of chiral monoterpenoid components in the aerial parts essential oil of *Artemisia frigida* from Idaho.

Enantiomers	RI_{calc}	RI_{db}	Plant samples		
			A	B	C
(-)-α-Pinene	977	976	87.1	77.2	99.5
(+)-α-Pinene	982	982	12.9	22.8	0.5
(-)-Camphene	998	998	99.2	86.1	-
(+)-Camphene	1004	1005	0.8	13.9	-
(+)-Sabinene	1021	1021	25.7	29.8	-
(-)-Sabinene	1030	1030	74.3	70.2	-
(+)-β-Pinene	1027	1027	12.1	13.3	5.1
(-)-β-Pinene	1032	1031	87.9	86.7	94.9
(-)-Limonene	1074	1073	81.9	77.4	-
(+)-Limonene	1081	1081	18.1	22.6	-
(+)-*cis*-Sabinene hydrate	1191	1199	9.7	15.2	-
(-)-*cis*-Sabinene hydrate	1195	1202	90.3	84.8	-
(-)-Camphor	1260	1253	96.7	69.7	-
(+)-Camphor	1265	1259	3.3	30.3	-
(+)-Terpinen-4-ol	1301	1297	44.6	38.3	35.5
(-)-Terpinen-4-ol	1304	1300	55.4	61.7	64.5
(-)-Borneol	1333	1335	99.6	85.3	-
(+)-Borneol	1341	1340	0.4	14.7	-
(-)-α-Terpineol	1344	1347	-	-	85.9
(+)-α-Terpineol	1351	1356	-	-	14.1

RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 column. RI_{db} = Retention index from our own in-house database.

Table 4: Comparison of (-)-enantiomers for high-desert *Artemisia* species.

Compound	*A. frigida*	*A. dracunculus* [32]	*A. ludoviciana* [33]	*A. tridentata* subsp. *tridentata* [34]	*A. tridentata* subsp. *wyomingensis* [34]	*A. tridentata* subsp. *vaseyana* [34]
(-)-α-Pinene	88.0±11.2	3.8	73.0±12.7	0.0	65.7±23.7	75.6±18.3
(-)-Camphene	92.6±9.3	-	97.2±5.5	39.6±39.9	87.2±32.6	99.8±0.2
(-)-Sabinene	72.3±2.0	-	74.6±21.3	-	-	60.9±22.6
(-)-β-Pinene	89.9±4.4	66.0	86.8±7.4	0.0	-	75.8±26.6
(-)-Limonene	79.6±3.2	22.0	75.4±27.3	-	-	91.6±1.6
(-)-*cis*-Sabinene hydrate	87.6±3.9	-	85.1±9.2	-	-	73.8±6.8
(-)-Camphor	83.2±19.1	-	93.9±19.3	22.5±31.9	16.7±21.2	100.0
(-)-Terpinen-4-ol	60.5±4.6	-	68.0±19.7	72.3±2.1	0.0	68.9±9.6
(-)-Borneol	92.5±10.1	-	100.0	37.6±47.1	100.0	100.0
(-)-α-Terpineol	85.9	56.6	77.0±16.2	-	-	90.9±11.8

4. Conclusions

This is the first report of the essential oil composition of *A. frigida* growing in the western United States. The chemical composition shows wide variation within the three samples in this work and there is wide variation within samples from Alberta, Canada, eastern Europe, and Asia. (-)-enantiomers were dominant for the chiral monoterpenoids in *A. frigida*, but are not consistent throughout the *Artemisia* genus.

5. Acknowledgments

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6. Funding

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7. Conflicts of Interest

The authors declare no conflicts of interest.

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