Natural Product Communications
Volume 18(2): 1–14
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DOI: 10.1177/1934578X231154965
journals.sagepub.com/home/npx
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Chemical Characterization of Three *Artemisia tridentata* Essential Oils and Multivariate Analyses: A Preliminary Investigation

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Abstract

Background/Objective: Big sagebrush (*Artemisia tridentata* Nutt., Asteraceae) is a conspicuous shrub growing in arid and semi-arid regions of intermountain western North America, from eastern Washington and Oregon, through Nevada, Idaho, and Utah, into northern Arizona and New Mexico, and east as far as the western Dakotas, and Colorado. At least three subspecies of *A. tridentata* have been recognized, including *A. tridentata* subsp. *tridentata* Nutt. (basin big sagebrush), *A. tridentata* subsp. *wyomingensis* Beetle & A.L.Young (Wyoming big sagebrush), and *A. tridentata* subsp. *vaseyana* (Rydb.) Beetle (mountain big sagebrush). Although big sagebrush is an important food source and cover for several animal species, the volatile phytochemistry has shown wide variation in composition. The purpose of this work was to compare and contrast the essential oil compositions of the three subspecies of *A. tridentata* growing in southwestern Idaho. **Methods/Results:** Several samples of *A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *vaseyana* were collected, the essential oils obtained by hydrodistillation using a Likens-Nickerson apparatus in yields of 2.33%-4.45% and 0.90%-1.73%, respectively, and the essential oils analyzed by gas chromatographic techniques. Although *A. tridentata* shows wide variation in essential oil compositions, multivariate analysis (hierarchical cluster analysis and principal component analysis) indicated *A. tridentata* subsp. *tridentata* to be characterized by high concentrations of yomogi alcohol (5.8%-30.8%) and camphor (5.2%-20.1%), while camphor was the defining component in *A. tridentata* subsp. *wyomingensis* (10.0%-30.1%) and subsp. *vaseyana* (16.1%-40.2%). One sample of *A. tridentata* subsp. *vaseyana* was dominated by α-thujone (76.1%), however. **Conclusion:** There is much variation in essential oil compositions of *A. tridentata*, both within subspecies and between geographical locations; much additional investigation is necessary to more fully characterize the volatile phytochemistry of this plant.

Keywords

big sagebrush, basin sagebrush, Wyoming sagebrush, mountain sagebrush, enantiomeric distribution, multivariate analysis

Received: December 21st, 2022; Accepted: January 17th, 2023.

Introduction

Big sagebrush, *Artemisia tridentata* Nutt., Asteraceae, is a conspicuous shrub growing in much of the western United States (Figure 1). The plant is an essential food source and cover for the pygmy rabbit, *Brachylagus idahoensis*[1]; the Gunnison sage-grouse, *Centrocercus minimus*; and the greater sage-grouse, *Centrocercus urophasianus*,[2,3] which rely on this plant for up to 99% of their winter forage. In addition, several wild ungulates, including elk, *Cervus canadensis*; mule deer, *Odocoileus hemionus*[4]; and pronghorn, *Antilocapra americana*,[5] forage on big sagebrush.

The essential oil compositions of *A. tridentata* have been previously reported,[6] and the plant has been characterized by the major components camphor (20%-45%), camphene (3%-21%), 1,8-cineole (12%-30%), thujone (6%), and borneol (5%).[7-12] Additionally, a number of irregular monoterpenoids have been isolated and characterized.[13-17] Sesquiterpene lactones have been characterized in the non-volatile extracts of *A. tridentata*.[18]

There is debate regarding the varieties and subspecies of *A. tridentata*. Nevertheless, there are three widely recognized subspecies: *A. tridentata* subsp. *tridentata* Nutt., *A. tridentata* subsp. *wyomingensis* Beetle & A.L.Young, and *A. tridentata* subsp. *vaseyana* (Rydb.) Beetle.[19,20] It has been observed that the three different sagebrush subspecies have different palatability attributes with respect to herbivory. The *vaseyana* and *wyomingensis* subspecies are generally more palatable to herbivores, while the *tridentata* subspecies is the least preferable.[21] The chemical

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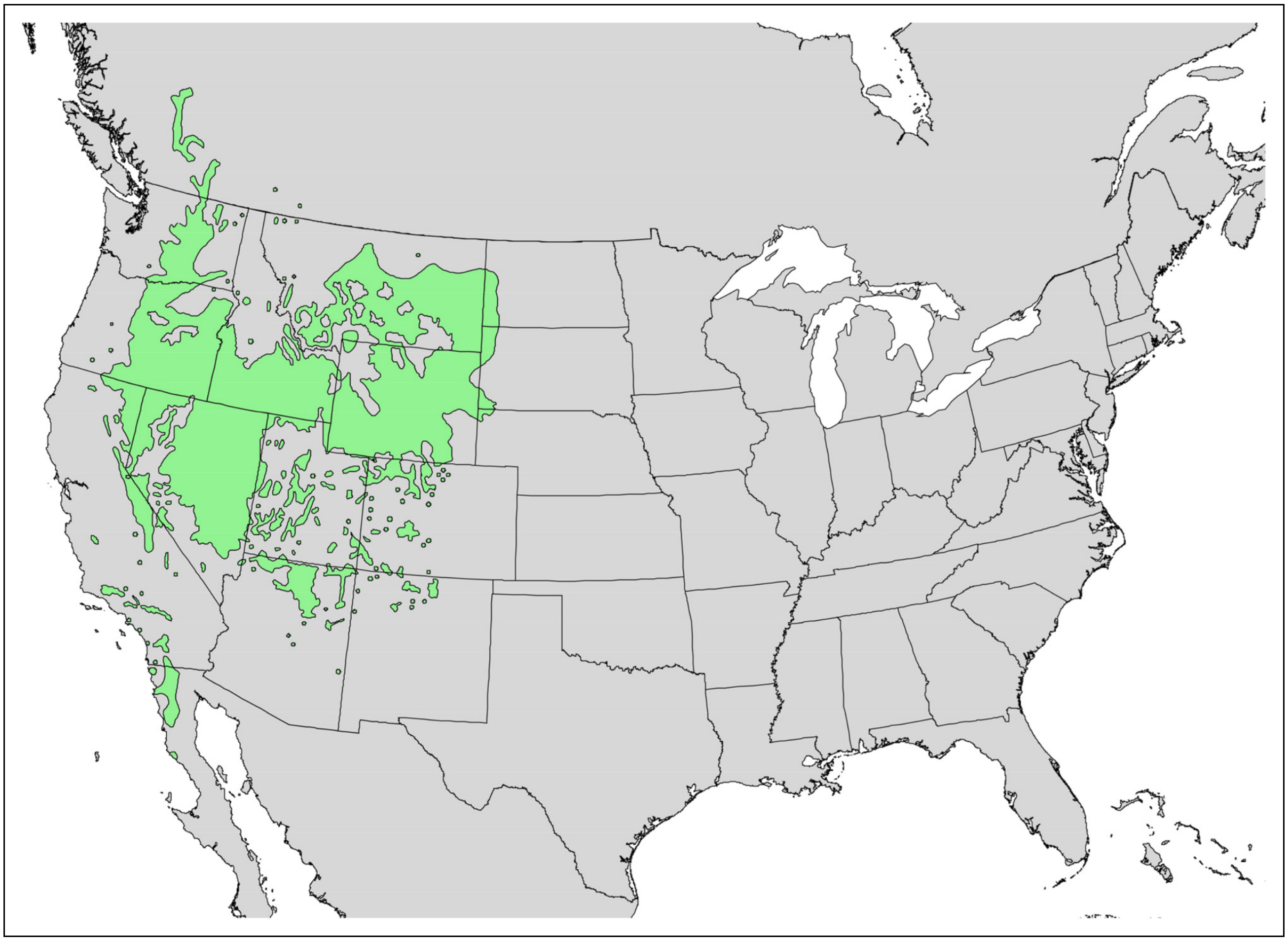

Figure 1. Range of *Artemisia tridentata* in North America.

constituents of the three subspecies most likely play a role in the observed herbivory.[21-23]

To complement our previous examination of *A. tridentata* subsp. *tridentata*,[6] the purpose of this study was to characterize the essential oils of *A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *vaseyana* growing in southwestern Idaho using gas chromatographic techniques and to compare and contrast the three subspecies using multivariate analyses.

Results and Discussion

Essential Oil Composition

A. tridentata *subsp.* tridentata. The essential oil composition of *A. tridentata* subsp. *tridentata* from southwestern Idaho has been previously reported.[6] The major components were yomogi alcohol (5.8%-30.8%), santolina epoxide (1.7%-10.5%), camphor (5.2%-20.1%), and (*Z*)-tagetone (0.9%-8.9%).

A. tridentata subsp. *wyomingensis*

The aerial parts of *A. tridentata* subsp. *wyomingensis* were hydrodistilled to give pale yellow essential oils in 2.34% to 4.45% yield. Gas chromatographic analysis of the six essential oil samples led to identification of a total of 53 compounds, which accounted for 73.4%-84.7% of the total compositions. The essential oil compositions of *A. tridentata* subsp. *wyomingensis* are compiled in Table 1.

The major components found in the essential oil of *A. tridentata* subsp. *wyomingensis* were camphor (10.0%-30.1%), artemiseole (2.9%-16.6%), methyl santolinate (2.6%-13.6%), 1,8-cineole (1.8%-11.1%), santolina triene (1.8%-12.6%), β-artemisyl acetate (0.8%-10.0%), and an unidentified component (retention index [RI] = 1234, 2.5%-7.3%).

A. tridentata *subsp.* vaseyana. Hydrodistillation of *A. tridentata* subsp. *vaseyana* gave yellow essential oils (0.89%-1.73% yield). An aqueous leaf extract of each *A. tridentata* subsp. *vaseyana* sample gave a bright blue-white fluorescence under illumination with UV light indicating the presence of coumarins (eg, isoscopoletin, scopoletin, and esculetin) in the leaves.[9] Coumarins are known to be abundant in *A. tridentata* subsp. *vaseyana*, but are present in only trace amounts in subsp. *tridentata* or subsp. *wyomingensis*.[19] The chemical components of *A. tridentata* subsp. *vaseyana* essential oils are listed in Table 2.

Table 1. Chemical Composition (%) of the Essential Oils From the Aerial Parts of *Artemisia tridentata* subsp. *wyomingensis*.

RI_{calc}	RI_{db}	Compound[a]	Atw#1	Atw#2	Atw#3	Atw#4	Atw#5	Atw#6
888	888	Ethyl pent-4-enoate	1.6	0.4	0.8	0.3	0.3	0.5
901	902	Santolina triene	6.2	1.8	5.1	4.7	5.1	12.6
902	909	2-Acetylfuran	1.9	1.4	1.1	-	1.2	-
922	922	Artemisia triene	0.2	0.1	-	0.2	0.1	0.1
932	933	α-Pinene	-	-	-	0.3	0.1	0.2
932	-	Unidentified (932)[b]	-	4.0	-	-	-	-
946	944	4,4-Dimethyl-2-buten-4-olide	-	8.4	0.2	-	-	-
950	954	Camphene	0.3	-	1.3	4.7	1.4	0.4
974	971	Artemiseole	2.9	4.8	7.9	9.9	16.6	3.1
980	978	β-Pinene	-	-	-	0.4	-	-
990	990	Dehydro-1,8-cineole	-	-	-	-	-	0.2
995	996	Yomogi alcohol	7.8	3.5	5.3	2.8	0.9	6.6
1008	1008	Isomaltol	-	-	-	0.2	-	-
1009	1003	Isobutyl 2-methylbutanoate	1.2	0.9	1.0	0.3	0.5	0.2
1025	1025	*p*-Cymene	0.9	0.4	0.2	0.3	0.3	1.0
1028	-	Unidentified (1028)[c]	1.0	0.6	1.4	1.4	1.5	1.4
1031	1031	Santolina alcohol	0.3	0.2	0.2	0.2	0.3	0.3
1033	1032	1,8-Cineole	6.3	7.2	1.8	4.5	4.9	11.1
1039	1035	γ-Valerolactone	-	1.7	-	-	-	-
1047	1047	Santolina epoxide	5.9	3.6	4.2	1.5	1.5	7.1
1051	1049	*cis*-Arbusculone	0.2	1.1	0.6	0.6	1.1	0.6
1052	1049	*o*-Cresol	0.2	0.3	0.2	0.1	0.2	-
1054	1053	*epi*-Isolyratol	0.6	1.0	0.8	0.7	0.8	1.0
1058	1058	γ-Terpinene	-	0.3	-	-	-	0.3
1068	1070	Isolyratol	1.5	2.3	2.2	1.9	2.1	2.6
1072	1072	α-Santolina alcohol	3.3	2.0	2.4	0.8	1.0	2.2
1074	1075	*epi*-α-Santolina alcohol	1.6	1.2	1.3	0.6	0.9	1.0
1080	1079	Artemisia alcohol	8.6	1.4	2.3	0.8	0.4	2.0
1110	-	Unidentified (1110)[d]	2.4	1.4	1.8	0.7	0.9	1.6
1116	1115	3-Methyl-3-vinylcyclohexanone	0.5	0.7	0.7	0.5	0.8	0.4
1133	1129	Methyl santolinate	2.6	5.0	6.6	6.9	13.6	2.6
1141	-	Unidentified (1141)[e]	1.2	1.7	1.9	1.3	2.6	1.5
1150	1149	Camphor	10.0	13.4	13.4	30.1	14.0	10.5
1166	1164	β-Artemisyl acetate	3.4	5.2	6.2	5.6	0.8	10.0
1174	1173	Borneol	-	-	1.2	0.4	0.2	0.1
1181	1180	Terpinen-4-ol	0.6	0.6	0.2	0.4	0.4	0.8
1187	1188	*p*-Cymen-8-ol	0.3	0.4	0.3	0.2	-	0.4
1199	1197	Myrtenal	-	-	-	0.4	-	-
1211	1208	Verbenone	-	-	-	0.3	-	-
1234	-	Unidentified (1234)[f]	7.3	5.2	6.2	2.5	4.3	5.4
1241	-	Unidentified (1241)[g]	0.3	-	1.1	0.8	1.5	-
1251	1253	*trans*-Chrysanthenyl acetate	0.5	0.4	1.4	0.8	1.3	0.2
1257	1255	Geraniol	0.7	0.4	0.5	0.2	0.2	0.4
1261	1258	Santolinolide B’	1.4	1.7	0.2	0.8	1.3	0.8
1265	1259	Santolinolide B	-	0.7	0.6	0.6	1.3	0.2
1269	1266	*cis*-Chrysanthenyl acetate	1.2	0.6	1.6	0.8	1.0	0.2
1293	1292	Santolinolide C	0.6	0.5	0.4	-	0.2	0.3
1367	-	Unidentified (1367)[h]	1.3	1.5	1.9	0.4	0.6	0.3
1396	1396	(2*E*)-13,7-Trimethyl-2,6-octadienyl acetate	0.2	-	-	-	-	-
1419	1424	(*E*)-β-Caryophyllene	0.4	0.4	0.1	0.2	0.2	0.2
1439	-	Verbenyl 2-methylpropionate	0.6	0.5	0.4	0.3	0.5	0.4
1481	1480	Germacrene D	0.3	0.3	0.2	0.2	0.2	0.2
1555	-	Unidentified (1555)[i]	2.3	0.7	1.3	1.3	1.9	0.6
1562	1562	(*E*)-Nerolidol	-	0.3	-	-	-	-
1584	1587	Caryophyllene oxide	0.4	0.6	0.2	0.2	-	0.6
1639	1636	Caryophylla-4(12),8(13)-dien-5β-ol	-	0.4	0.2	0.2	0.4	0.5
1645	1647	Methyl (*Z*)-jasmonate	0.5	0.3	0.5	0.3	0.4	0.5
1657	1655	α-Bisabolol oxide B	-	-	-	-	-	0.3

(Continued)

Table 1. Continued.

RI_{calc}	RI_{db}	Compound[a]	Atw#1	Atw#2	Atw#3	Atw#4	Atw#5	Atw#6
1676	1676	Methyl *epi*-(*E*)-jasmonate	0.2	-	-	-	-	-
1686	1683	Germacra-4(15),5,10(14)-trien-1α-ol	-	-	-	-	-	0.2
1805	-	Unidentified (1805)[j]	1.1	1.0	1.5	1.2	1.0	1.2
2045	2045	Kaurene	0.1	0.1	0.1	0.1	0.1	0.1
		Monoterpene hydrocarbons	9.1	4.5	8.9	12.1	9.1	16.9
		Oxygenated monoterpenoids	59.2	54.2	58.6	69.5	61.6	61.5
		Sesquiterpene hydrocarbons	0.7	0.7	0.3	0.4	0.4	0.4
		Oxygenated sesquiterpenoids	0.4	1.3	0.4	0.4	0.4	1.5
		Benzenoid aromatics	0.2	0.3	0.2	0.1	0.2	0.0
		Diterpenoids	0.1	0.1	0.1	0.1	0.1	0.1
		Others	6.4	14.9	5.0	2.3	4.2	2.2
		Total identified	76.2	76.0	73.4	84.7	76.0	82.6
		Number identified	38	41	39	42	38	42

Abbreviations: RI_{calc}, retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column using the linear equation of van den Dool and Kratz[24]; RI_{db}, reference retention index obtained from the databases.[25-28]

[a]Mass spectra of unidentified components are available as a supplementary figure (Figure S1).

[b]MS(EI): 136(2%), 113(47%), 93(32%), 71(99%), 67(100%), 55(40%), 43(63%), 41(35%).

[c]MS(EI): 135(3%), 107(5%), 94(5%), 79(9%), 69(100%), 53(8%), 41(84%).

[d]MS(EI): 134(3%), 121(45%), 107(14%), 105(15%), 93(58%), 91(44%), 79(100%), 77(42%), 43(21%), 41(29%).

[e]MS(EI): 137(9%), 123(26%), 119(100%), 105(28%), 95(35%), 93(35%), 91(93%), 79(64%), 77(46%), 67(60%), 55(42%), 43(40%), 41(68%).

[f]MS(EI): 139(6%), 121(4%), 96(94%), 81(100%), 75(62%), 57(61%), 43(63%).

[g]MS(EI): 137(9%), 123(26%), 119(100%), 105(28%), 95(35%), 93(35%), 91(93%), 79(64%), 77(46%), 67(60%), 55(42%), 43(40%), 41(68%).

[h]MS(EI): 139(3%), 117(37%), 96(30%), 81(30%), 75(30%), 67(9%), 55(9%), 43(100%).

[i]MS(EI): 150(16%), 149(25%), 134(10%), 107(12%), 91(11%), 79(10%), 43(100%).

[j]MS(EI): 192(3%), 179(3%), 151(8%), 141(27%), 123(14%), 108(67%), 80(19%), 79(19%), 69(100%), 55(10%), 43(29%), 41(59%).

The essential oils of *A. tridentata* subsp. *vaseyana* showed wide variation in composition, but major components included α-thujone (0.1%-76.1%), camphor (0.1%-40.2%), and 1,8-cineole (1.0%-24.6%).

Multivariate Analysis

To provide insight into possible chemical differentiation between the three subspecies of *A. tridentata* collected in southwestern Idaho, both agglomerative hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out using the percent compositions of the major components. The compositions of *A. tridentata* subsp. *tridentata*[6] are also included in the analyses. The HCA is shown in Figure 2, and the PCA is shown in Figure 3.

The HCA revealed three well-defined clusters: (a) A cluster defined by relatively high concentrations of yomogi alcohol and camphor and made up of the three *A. tridentata* subsp. *tridentata* samples,[6] (b) a cluster dominated by camphor and comprised of all six *A. tridentata* subsp. *wyomingensis* samples as well as three *A. tridentata* subsp. *vaseyana* samples, and (c) a single *A. tridentata* subsp. *vaseyana* sample dominated by α-thujone. The PCA corroborates the HCA with the *A. tridentata* subsp. *tridentata* group positively correlating with yomogi alcohol and camphor. There is large grouping that strongly correlates with camphor and corresponds to the camphor-rich cluster from the HCA. Finally, *A. tridentata* subsp. *vaseyana* sample #2 strongly correlates with α-thujone.

To further probe the volatile components that differentiate the three *A. tridentata subspecies*, an analysis of variance (ANOVA) was carried out on each of the major components in *A. tridentata* essential oils. The chemical components that exhibited statistical differences between the subspecies are illustrated in Figure 4.

As observed in the HCA and PCA, the high concentration of yomogi alcohol in *A. tridentata* subsp. *tridentata* differentiates that subspecies from the *wyomingensis* and *vaseyana* subspecies. Furthermore, the (*Z*)-tagetone concentration differentiates *A. t. tridentata* from *A. t. wyomingensis* and *A. t. vaseyana*. On the other hand, the concentration of unidentified component (RI = 1234) in *A. t. wyomingensis* is significantly higher than those in *A. t. tridentata* or *A. t. vaseyana*. At this point, it is difficult to explain the chemical differences between the subspecies. There are likely genetic differences, but the collection sites were also different, so there may be environmental effects.

The essential oils of *A. tridentata* subsp. *tridentata*, *A. tridentata* subsp. *wyomingensis*, and *A. tridentata* subsp. *vaseyana* from Wyoming have been published.[23] A comparison of the major components of *A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *vaseyana* from Idaho and from Wyoming is summarized in Table 3.

There are some notable differences between the concentrations of the major components of the samples from Idaho and the samples from Wyoming. A perusal of Table 3 reveals that both the Idaho samples and the Wyoming samples of *A. tridentata* subsp. *wyomingensis* were rich in santolina triene, artemiseole, 1,8-cineole, and camphor. However, the Wyoming samples had significantly higher concentrations of artemiseole and 1,8-cineole. The camphor concentrations were statistically comparable. In addition, the Wyoming samples of *A. tridentata* subsp. *wyomingensis* showed significantly higher percentages of α-pinene, camphene, *cis*-

Table 2. Chemical Composition (%) of the Essential Oils From the Aerial Parts of *Artemisia tridentata* subsp. *vaseyana*.

RI_{calc}	RI_{db}	Compound[a]	Atv#1	Atv#2	Atv#3	Atv#4
900	902	Santolina triene	-	-	4.5	4.0
910	912	2,5-Diethenyl-2-methyltetrahydrofuran	-	-	0.1	-
920	926	Tricyclene	0.2	tr	0.3	0.2
922	927	α-Thujene	0.1	0.1	tr	-
931	932	α-Pinene	2.0	0.5	2.1	0.6
941	943	Thuja-2,4(10)-diene	tr	tr	-	-
946	942	4,4-Dimethyl-2-butenolide	-	-	0.7	5.1
949	953	Camphene	5.3	tr	7.6	1.9
972	972	Sabinene	2.3	1.1	0.2	-
973	971	Artemiseole	-	-	4.4	9.2
978	978	β-Pinene	1.2	0.3	1.1	0.5
989	990	Dehydro-1,8-cineole	0.5	0.3	-	0.2
990	991	Myrcene	0.2	0.3	2.6	0.1
994	996	Yomogi alcohol	-	-	-	0.1
1006	1007	α-Phellandrene	-	-	-	0.1
1016	1018	α-Terpinene	0.3	0.2	-	-
1023	1025	*p*-Cymene	0.2	0.2	0.2	0.5
1026	1026	2-Acetyl-3-methylfuran	tr	tr	0.1	-
1029	1030	Limonene	0.3	0.1	0.3	0.2
1031	1031	β-Phellandrene	-	0.1	-	-
1031	1031	Santolina alcohol	-	-	-	0.1
1033	1032	1,8-Cineole	24.6	10.4	1.0	7.0
1035	1035	γ-Valerolactone	-	-	0.1	0.4
1044	1045	Benzene acetaldehyde	0.1	tr	-	-
1045	1047	Santolina epoxide	-	-	2.2	0.6
1050	1049	*cis*-Arbusculone	-	-	-	4.2
1052	-	Unidentified (1052)[b]	-	-	-	3.9
1053	1053	*epi*-Isolyratol	-	-	0.1	0.1
1058	1058	γ-Terpinene	0.6	0.4	-	0.1
1067	1070	Isolyratol	-	-	0.3	0.3
1068	1068	*trans*-Arbusculone	-	-	0.1	2.3
1070	1069	*cis*-Sabinene hydrate	0.5	0.4	-	0.3
1070	1072	α-Santolina alcohol	-	-	0.1	0.3
1072	1075	*epi*-α-Santolina alcohol	-	-	0.1	-
1080	1079	Artemisia alcohol	-	-	tr	0.1
1086	1086	Terpinolene	0.1	0.1	-	tr
1101	1101	Linalool	0.2	-	0.1	0.1
1102	1101	*trans*-Sabinene hydrate	0.3	-	-	0.1
1108	1105	α-Thujone	16.3	76.1	1.9	0.1
1113	1113	Phenethyl alcohol	-	-	-	tr
1118	1118	β-Thujone	1.5	3.5	0.1	-
1121	1122	Dehydrosabina ketone	0.1	tr	-	-
1122	1122	Chrysanthenone	tr	tr	0.1	0.3
1123	1122	*trans-p*-Mentha-2,8-dien-1-ol	0.1	0.1	-	-
1126	1124	*cis-p*-Menth-2-en-1-ol	0.1	-	-	-
1127	1126	α-Campholenal	tr	-	-	-
1127	-	Unidentified (1127)[c]	-	-	1.3	-
1133	1129	Methyl santolinate	-	-	0.2	1.1
1139	1144	*trans*-Tagetone	-	0.1	0.4	1.4
1140	1138	*cis-p*-Mentha-2,8-dien-1-ol	0.1	-	-	-
1140	1140	*exo*-Isocitral	0.1	-	-	-
1141	1138	*trans*-Sabinol	-	0.3	-	-
1143	1141	*trans*-Pinocarveol	0.7	0.1	-	-
1144	1142	*trans-p*-Menth-2-en-1-ol	0.1	tr	-	-
1144	1145	*trans*-Verbenol	-	tr	0.9	0.9
1144	1141	*trans*-Pinocarveol	-	-	-	0.1
1148	-	Unidentified (1148)[d]	-	-	1.0	-
1149	1149	Camphor	28.7	0.1	40.2	16.1

(Continued)

Table 2. Continued.

RI_{calc}	RI_{db}	Compound[a]	Atv#1	Atv#2	Atv#3	Atv#4
1153	1152	Nerol oxide	-	-	0.3	0.2
1154	1153	*neo*-Thujol	-	0.2	-	-
1156	1156	β-Pinene oxide	0.1	0.1	-	-
1158	1157	Sabina ketone	tr	-	-	-
1164	1164	Pinocarvone	0.7	0.2	-	-
1164	1164	Lyratol	-	-	0.7	0.7
1170	1168	α-Phellandrene epoxide	0.1	-	-	-
1171	1169	Thujol	-	0.3	-	-
1172	1170	δ-Terpineol	0.3	0.1	-	0.1
1174	1173	Borneol	2.2	-	1.9	0.9
1182	1180	Terpinen-4-ol	1.9	1.4	0.4	0.6
1184	1184	Thuj-3-en-10-al	0.1	tr	-	-
1189	1187	*trans-p*-Mentha-1(7),8-dien-2-ol	0.1	-	-	-
1189	1186	*p*-Cymen-8-ol	-	0.1	-	-
1193	1202	Myrtenol	0.1	-	-	-
1197	1195	α-Terpineol	2.0	0.9	0.3	0.9
1200	1198	*cis*-Dihydrocarvone	0.1	tr	0.3	-
1203	1213	4-Hydroxy-α-thujone	-	tr	-	-
1205	1202	*cis*-Sabinol	-	tr	-	-
1220	1218	*trans*-Carveol	0.1	tr	0.1	-
1226	1229	Nerol	0.3	0.1	0.1	0.2
1228	1232	Citronellol	-	-	0.2	-
1231	1230	*cis-p*-Mentha-1(7),8-dien-2-ol	0.1	-	-	-
1234	1232	*cis*-Carveol	tr	tr	tr	-
1239	1238	Neral	0.1	tr	-	-
1244	1242	Cuminal	tr	tr	-	-
1249	1253	*trans*-Chrysanthenyl acetate	-	-	1.2	2.2
1251	1249	Geraniol	tr	tr	-	-
1255	-	Unidentified (1255)[e]	-	-	0.6	1.3
1267	1266	*cis*-Chrysanthenyl acetate	-	-	0.4	0.5
1269	1268	Geranial	0.1	tr	0.1	-
1285	1284	Lavandulyl acetate	-	-	0.2	0.3
1286	1285	Bornyl acetate	0.3	-	0.3	0.1
1291	1291	*trans*-Sabinyl acetate	-	tr	-	-
1293	1298	Thujyl acetate	-	tr	-	-
1294	1291	*p*-Cymen-7-ol	-	tr	-	-
1320	1326	Myrtenyl acetate	-	tr	-	-
1349	1349	α-Terpinyl acetate	0.5	tr	0.1	-
1352	1350	Citronellyl acetate	-	tr	0.1	-
1361	1361	Neryl acetate	1.2	0.5	2.5	2.8
1367	-	Unidentified (1367)[f]	-	-	0.4	1.0
1380	1380	Geranyl acetate	0.1	0.1	0.5	0.3
1424	1424	(*E*)-β-Caryophyllene	0.8	0.3	0.6	0.2
1447	1448	Neryl propionate	tr	tr	-	-
1456	1453	α-Humulene	0.1	tr	-	-
1478	1482	γ-Curcumene	0.1	tr	-	-
1482	1483	Germacrene D	0.2	0.2	-	0.3
1483	1483	Davana ether 1	-	-	-	2.9
1484	1485	Neryl isobutyrate	0.1	tr	-	-
1492	1500	*trans*-Chrysanthenyl 2-methylbutanoate	-	-	0.2	-
1497	1497	Bicyclogermacrene	0.1	0.1	-	-
1504	1502	Davana ether 2	-	-	-	8.9
1505	1504	Davana ether 3	-	-	-	1.4
1520	1520	δ-Cadinene	tr	tr	-	-
1523	1521	Davana ether 4	-	-	-	4.7
1529	1529	Artedouglasia oxide A	-	-	0.1	-
1551	1557	Davanone B	-	-	0.1	-
1561	1562	(*E*)-Nerolidol	-	-	-	1.0

(Continued)

Table 2. Continued.

RI_{calc}	RI_{db}	Compound[a]	Atv#1	Atv#2	Atv#3	Atv#4
1572	1582	Neryl isovalerate	0.1	0.1	-	-
1579	1578	Spathulenol	-	0.1	-	-
1583	1582	Davanone D	-	-	8.5	0.3
1585	1587	Caryophyllene oxide	0.1	tr	0.7	-
1638	1632	Caryophylla-4(12),8(13)-dien-5α-ol	tr	-	0.1	-
1641	1636	Caryophylla-4(12),8(13)-dien-5β-ol	0.1	tr	0.5	-
1644	1642	Methyl (*Z*)-jasmonate	0.3	0.2	0.3	0.3
1659	1655	α-Eudesmol	0.1	0.1	0.1	-
1676	1673	Methyl *epi*-(*E*)-jasmonate	0.1	tr	0.1	0.2
1714	1718	β-Davanon-2-ol	-	-	0.2	-
		Monoterpene hydrocarbons	12.6	3.2	18.6	8.0
		Oxygenated monoterpenoids	84.5	95.2	62.0	48.2
		Sesquiterpene hydrocarbons	1.2	0.6	0.6	0.4
		Oxygenated sesquiterpenoids	0.3	0.1	10.3	19.2
		Benzenoid aromatics	0.1	traces	0.0	traces
		Others	0.7	0.2	1.7	12.7
		Total identified	99.5	99.4	93.3	88.5
		Number identified	60	39	59	57

Abbreviations: RI_{calc}, retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column using the linear equation of van den Dool and Kratz[24]; RI_{db}, reference retention index obtained from the databases[25-28]; tr, trace (<0.05%).
[a]Mass spectra of unidentified components are available as a supplementary figure (Figure S2).
[b]MS(EI): 137(30%), 107(26%), 95(100%), 79(32%), 67(88%), 57(27%), 41(44%).
[c]MS(EI): 135(19%), 81(70%), 79(31%), 69(56%), 53(16%), 41(100%).
[d]MS(EI): 119(4%), 105(2%), 84(100%), 83(31%), 69(15%), 56(14%), 43(26%), 41(24%).
[e]MS(EI): 136(8%), 121(22%), 95(100%), 93(16%), 79(10%), 67(38%), 55(15%), 43(41%), 41(14%).
[f]MS(EI): 139(3%), 117(35%), 96(29%), 81(30%), 77(28%), 67(8%), 55(8%), 43(100%).

arbusculone, *trans*-arbusculone, chrysanthenone, and borneol. Conversely, the concentrations of yomogi alcohol, santolina epoxide, methyl santolinate, and β-artemisyl acetate were significantly higher in the Idaho samples.

There are wide variations in the concentrations of the major components in *A. tridentata* subsp. *vaseyana*, in both the samples from Idaho and the samples from Wyoming. Nevertheless, the concentrations of 1,8-cineole and camphor were generally high in samples from both locations. α-Thujone was particularly abundant in *A. tridentata* subsp. *vaseyana* from Idaho, while significantly lower in samples from Wyoming. In addition, santolina epoxide and methyl santolinate concentrations were significantly higher in the *A. tridentata* subsp. *vaseyana* samples from Idaho. In a common garden experiment, Welch and McArthur[9] found α-thujone content to be significantly higher in *A. tridentata* susbp. *vaseyana* while camphor concentration was higher in *A. tridentata* subsp. *tridentata*. There were, however, large variations in monoterpenoid concentrations.

Interestingly, there were three compounds that were found in high concentrations in several samples of *A. tridentata* subsp. *vaseyana* from Wyoming[23] that were not detected in this subspecies from Idaho: Fragranol, *trans*-α-necrodyl acetate, and grandisol. Fragranol was first isolated and characterized from *Artemisia fragrans*.[29] It has been subsequently identified in several essential oils of the Asteraceae, including *Tanacetum dumosum*,[30] *Achillea falcata*,[31] *Achillea ligustica*,[32] and *Achillea lingulata*.[33] *trans*-α-Necrodyl acetate is a major component of *Lavandula luisieri*,[34,35] but it has also been found in the essential oils of *Cladanthus mixtus* (Asteraceae)[36] and *Achillea ageratifolia* (Asteraceae).[37] Grandisol is a sex pheromone of the boll weevil (*Anthonomus grandis*) and the pine weevil (*Pissodes castaneus*).[18] It was also found in high concentration in *Achillea falcata* (Asteraceae)[31] and *Artemisia vestita* (Asteraceae).[38]

Terpenoid Enantiomeric Distributions

To further characterize the volatile phytochemistry of *A. tridentata*, chiral GC-MS was carried out on the essential oils of *A. t. tridentata*,[6] *A. t. wyomingensis*, and *A. t. vaseyana* (see Table 4).

A perusal of Table 4 reveals a few trends in enantiomeric distribution of terpenoids in *A. tridentata* essential oils, so differentiation between subspecies based on enantiomers does not seem possible. (+)-α-Pinene was the exclusive enantiomer in *A. tridentata* subsp. *tridentata*, but the enantiomeric distribution varied widely in the *wyomingensis* and *vaseyana* subspecies. The enantiomeric distribution of camphene varied widely within subspecies. Sabinene was only detected in *A. tridentata* subsp. *vaseyana*, but the enantiomeric distribution was variable. Limonene was only detected in *A. tridentata* subsp. *vaseyana* and (–)-limonene was the major enantiomer. Only the (+)-enantiomers of α- and β-thujone were observed in *A. tridentata* subsp. *vaseyana*. (+)-Camphor generally dominated the essential oils of *A. tridentata*. (–)-Terpinen-4-ol was the major enantiomer in *A. tridentata* subsp. *tridentata* and *A. tridentata* subsp. *vaseyana*. When observed, only (+)-terpinen-4-ol was

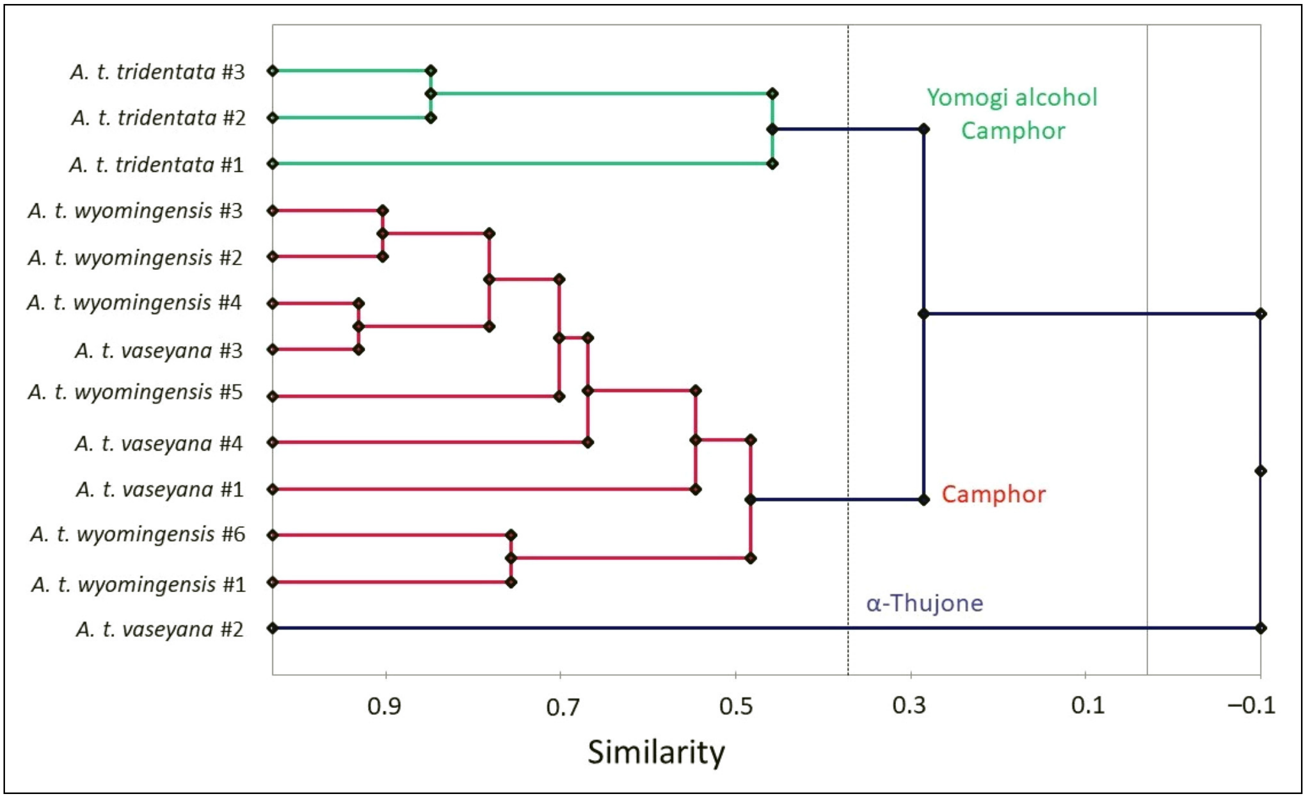

Figure 2. Dendrogram based on hierarchical cluster analysis of *Artemisia tridentata* chemical compositions.

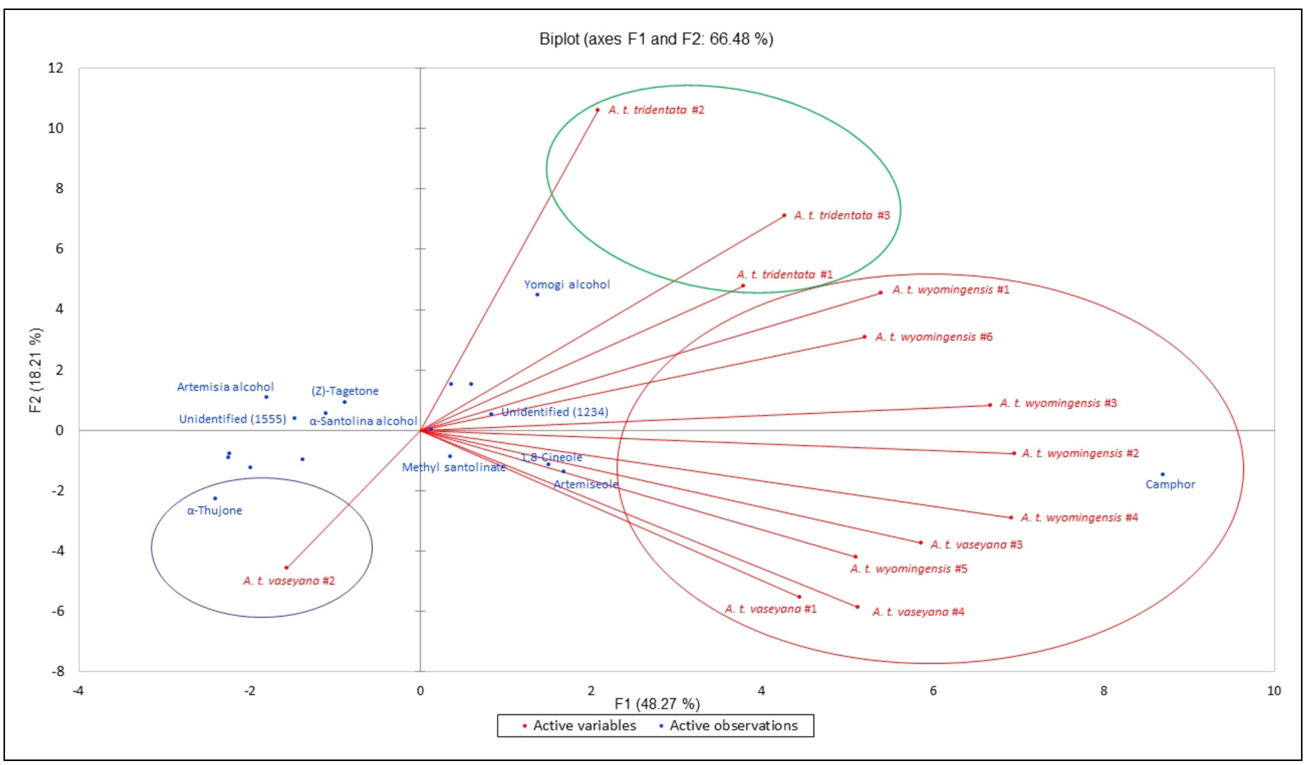

Figure 3. Biplot based on principal component analysis of *Artemisia tridentata* chemical compositions.

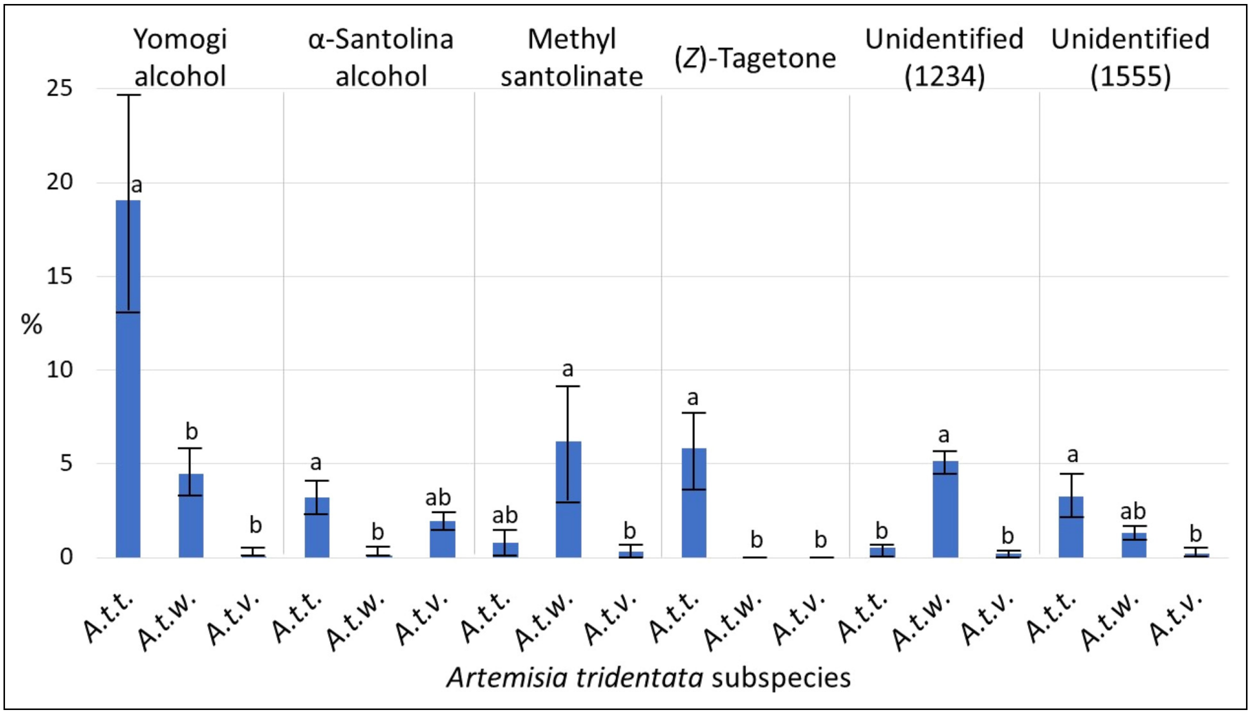

Figure 4. Percentage differences of volatile chemical components in the essential oils of three subspecies of *Artemisia tridentata* from Southwest Idaho, USA. Bars with same letter are not significantly different at $P \leq 0.05$.
Abbreviations: *A.t.t., Artemisia tridentata* subsp. *tridentata*; *A.t.w., Artemisia tridentata* subsp. *wyomingensis*; *A.t.v., Artemisia tridentata* subsp. *vaseyana*.

Table 3. Major Essential oil Components of *Artemisia tridentata* Subsp. *wyomingensis* and *Artemisia tridentata* Subsp. *vaseyana* from Southwestern Idaho and North-Central (Bighorn Mountains) Wyoming.

	Artemisia tridentata subsp. *wyomingensis*				*Artemisia tridentata* subsp. *vaseyana*			
	Idaho		Wyoming [23]		Idaho		Wyoming [23]	
Compound	Range (n = 6)	Ave	Range (n = 3)	Ave	Range (n = 4)	Ave	Range (n = 12)	Ave
Santolina triene	1.8-12.6	5.9	4.5-6.0	5.3	0.0-4.5	2.1	0.0-2.5	0.5
α-Pinene	0.0-0.3	0.1	1.1-4.0	2.6[a]	0.5-2.1	1.3	0.0-35.5	7.4
Camphene	0.0-4.7	1.4	6.6-8.0	7.3[a]	0.0-7.6	3.7	0.0-13.8	5.1
Artemiseole	2.9-16.6	7.5	17.4-20.2	18.8[a]	0.0-9.2	3.4	0.0-13.0	1.7
Yomogi alcohol	0.9-7.8	4.5[b]	nd	-	0.0-0.1	0.0	0.0-0.2	0.1
1,8-Cineole	1.8-11.1	6.0	12.4-13.0	12.7[a]	1.0-24.6	10.7	0.0-17.9	7.2
cis-Arbusculone	0.2-1.1	0.7	1.0-5.6	3.3[a]	0.0-4.2	1.0	0.0-0.2	0.0
Santolina epoxide	1.5-7.1	4.0[b]	nd	-	0.0-2.2	0.7[b]	0.0-0.3	0.0
trans-Arbusculone	nd	-	5.1-8.6	6.8[a]	0.0-2.3	0.6	0.0-2.0	0.4
α-Santolina alcohol	0.8-3.3	2.0	13.0-14.8	13.9[a]	0.0-0.3	0.1	0.0-9.7	1.1
Artemisia alcohol	0.4-8.6	2.6	nd	-	0.0-0.1	0.0	0.0-0.2	0.0
α-Thujone	nd	-	nd	-	0.1-76.1	23.6[b]	0.0-7.5	1.0
Methyl santolinate	2.6-13.6	6.2[b]	nd	-	0.0-1.1	0.3[b]	nd	-
Chrysanthenone	nd	-	1.0-6.4	3.7[a]	0.0-0.3	0.1	0.0-18.0	3.1
Camphor	10.0-30.1	15.2	20.6-21.7	21.1	0.1-40.2	21.3	0.0-45.4	15.4
Borneol	0.0-1.2	0.3	1.2-7.2	4.2[a]	0.0-2.2	1.3	0.0-2.0	0.6
β-Artemisyl acetate	0.8-10.0	5.2[b]	nd	-	nd	-	nd	-
Fragranol	nd	-	nd	-	nd	-	0.0-20.3	5.8
trans-α-Necrodyl acetate	nd	-	nd	-	nd	-	0.0-45.2	11.5
Grandisol	nd	-	nd	-	nd	-	0.0-36.2	10.0

Abbreviation: nd, not detected.
[a]The concentrations were significantly greater (ANOVA, $P < 0.05$) in the Wyoming samples than the Idaho samples.
[b]The concentrations were significantly greater (ANOVA, $P < 0.05$) in the Idaho samples than the Wyoming samples.

Table 4. Enantiomeric Distribution of Chiral Terpenoid Components (Percentage of Each Enantiomer) in *Artemisia tridentata* Essential Oils.

Compound	RT (min)[a]	*A.t.t.* #1[b]	*A.t.t.* #2[b]	*A.t.t.* #3[b]	*A.t.w.* #1	*A.t.w.* #2	*A.t.w.* #3	*A.t.w.* #4	*A.t.w.* #5	*A.t.w.* #6	*A.t.v.* #1	*A.t.v.* #2	*A.t.v.* #3	*A.t.v.* #4
(+)-α-Thujene	13.92	nd[c]	nd	nd	nd	nd	nd	nd	nd	nd	15.0	nd	nd	nd
(−)-α-Thujene	13.99										85.0			
(−)-α-Pinene	15.92	0.0	0.0	0.0	52.7	89.1	73.9	84.8	27.8	49.4	88.1	48.9	87.1	78.3
(+)-α-Pinene	16.40	100.0	100.0	100.0	47.3	10.9	26.1	15.2	72.2	50.6	11.9	51.1	12.9	21.7
(−)-Camphene	17.73	82.6	32.2	3.9	60.6	98.8	99.2	99.5	78.0	17.2	99.6	nd	100.0	99.6
(+)-Camphene	18.30	17.4	67.8	96.1	39.4	1.2	0.8	0.5	22.0	82.8	0.4		0.0	0.4
(+)-Sabinene	19.74	nd	nd	nd	nd	nd	nd	nd	nd	nd	45.3	67.5	28.5	15.0
(−)-Sabinene	20.60										54.7	32.5	71.5	85.0
(+)-β-Pinene	20.27	100.0	100.0	nd	nd	nd	nd	nd	nd	nd	8.6	13.7	10.4	64.0
(−)-β-Pinene	20.62	0.0	0.0								91.4	86.3	89.6	36.0
(−)-α-Phellandrene	22.59	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0
(+)-α-Phellandrene	22.81													100.0
(−)-Limonene	25.06	nd	nd	nd	nd	nd	nd	nd	nd	nd	89.8	nd	92.8	92.2
(+)-Limonene	25.99										10.2		7.2	7.8
(−)-β-Phellandrene	26.15	nd	nd	nd	nd	nd	nd	nd	nd	nd	100.0	nd	nd	nd
(+)-β-Phellandrene	26.88										0.0			
(+)-*cis*-Sabinene hydrate	40.70	nd	nd	nd	nd	nd	nd	nd	nd	nd	21.4	31.0	nd	nd
(−)-*cis*-Sabinene hydrate	41.25										78.6	69.0		
(+)-α-Thujone	43.32	nd	nd	nd	nd	nd	nd	nd	nd	nd	100.0	100.0	100.0	nd
(−)-α-Thujone	44.88										0.0	0.0	0.0	
(+)-β-Thujone	46.06	nd	nd	nd	nd	nd	nd	nd	nd	nd	100.0	100.0	nd	nd
(−)-β-Thujone	[d]										0.0	0.0		
(+)-*trans*-Sabinene hydrate	46.15	nd	nd	nd	nd	nd	nd	nd	nd	nd	22.6	nd	nd	nd
(−)-*trans*-Sabinene hydrate	46.84										77.4			
(−)-Camphor	49.31	59.0	8.4	0.0[e]	38.8	0.0[e]	0.0[e]	0.0[e]	44.8	4.0	0.0[e]	nd	0.0[e]	0.0[e]
(+)-Camphor	50.12	41.0	91.6	100.0	61.2	100.0	100.0	100.0	55.2	96.0	100.0		100.0	100.0
(+)-Terpinen-4-ol	54.64	27.7	29.8	25.7	100.0	100.0	nd	nd	nd	100.0	30.5	44.4	27.2	22.1
(−)-Terpinen-4-ol	54.93	72.3	70.2	74.3	0.0	0.0				0.0	69.5	55.6	72.8	77.9
(−)-Borneol	58.59	90.4	22.3	0.0	nd	nd	100.0	100.0	100.0	nd	100.0	nd	100.0	100.0
(+)-Borneol	59.11	9.6	77.7	100.0			0.0	0.0	0.0		0.0		0.0	0.0
(−)-Bornyl acetate	59.46	100.0	100.0	nd	nd	nd	100.0	100.0	nd	nd	100.0	nd	100.0	100.0
(+)-Bornyl acetate	[f]	0.0	0.0				0.0	0.0			0.0		0.0	0.0
(−)-α-Terpineol	59.73	nd	nd	nd	nd	nd	nd	nd	nd	nd	88.3	75.2	100.0	100.0
(+)-α-Terpineol	60.58										11.7	24.8	0.0	0.0
(−)-Verbenone	61.70	0.0	0.0	0.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(+)-Verbenone	62.73	100.0	100.0	100.0										
(−)-(*E*)-β-Caryophyllene	69.33	nd	nd	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
(+)-(*E*)-β-Caryophyllene	g			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(+)-Germacrene D	73.48	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0	0.0	nd	0.0
(−)-Germacrene D	73.73										100.0	100.0		100.0
(−)-δ-Cadinene	76.50	100.0	100.0	100.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(+)-δ-Cadinene	77.33	0.0	0.0	0.0										

(Continued)

Table 4. Continued.

Compound	RT (min)[a]	A.t.t. #1[b]	A.t.t. #2[b]	A.t.t. #3[b]	A.t.w. #1	A.t.w. #2	A.t.w. #3	A.t.w. #4	A.t.w. #5	A.t.w. #6	A.t.v. #1	A.t.v. #2	A.t.v. #3	A.t.v. #4
(+)-(*E*)-Nerolidol	83.40	nd	8.1	7.9	nd	0.0	nd	nd	nd	nd	nd	nd	nd	0.0
(−)-(*E*)-Nerolidol	83.59		91.9	92.1		100.0								100.0

[a] *A.t.t.*, *Artemisia tridentata* subsp. *tridentata*; *A.t.w.*, *Artemisia tridentata* subsp. *wyomingensis*; *A.t.v.*, *Artemisia tridentata* subsp. *vaseyana*; RT, retention time in minutes.
[b] The data for *Artemisia tridentata* subsp. *Tridentata* has been previously published[6] but included here for comparison.
[c] nd, compound not detected.
[d] Reference enantiomer not available; according to the *Dictionary of Natural Products*,[18] (+)-β-thujone is a "constituent of many essential oils," but (−)-β-thujone is not.
[e] The peak for camphor is very broad; it is uncertain whether there is (−)-camphor in the sample.
[f] Reference enantiomer not available; only (−)-borneol reported in the *Dictionary of Natural Products*.[18]
[g] Reference enantiomer not available; apparently only (−)-(*E*)-β caryophyllene seen in higher plants, but (+)-(*E*)-β caryophyllene has been found in some liverworts.[18]

found in *A. tridentata* subsp. *wyomingensis*. (−)-Borneol was the major enantiomer in *A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *vaseyana*. Only (−)-bornyl acetate was found in *A. tridentata* essential oils. α-Terpineol was only found in *A. tridentata* subsp. *vaseyana* and the (−)-enantiomer predominated. Only (−)-(*E*)-β-caryophyllene was detected in *A. tridentata* essential oils.

Conclusions

The essential oil compositions of three subspecies of big sagebrush, *A. tridentata* subsp. *tridentata*, *A. tridentata* subsp. *wyomingensis*, and *A. tridentata* subsp. *vaseyana* have shown chemical profiles that may be useful in differentiating these subspecies. However, there is much variation in essential oil compositions both within subspecies and between geographical locations. In this work, the essential oils were examined in different stages of development, which likely affects the compositions. It is apparent that much additional investigation is necessary to more fully characterize the volatile phytochemistry of *A. tridentata*, and we plan future studies to include collections in the same calendar year, at several different months, and different geographical locations, to examine seasonal and geographical variations as well as subspecies variations.

Materials and Methods

Plant Material

Aerial parts from six individuals of *A. tridentata* subsp. *wyomingensis* were collected on August 1, 2022 (flowering stage) from the Snake River Birds of Prey National Conservation Area, Idaho (see Table 5). Hydrodistillation of *A. tridentata* subsp. *wyomingensis* gave pale-yellow essential oils (Table 5). Note that an aqueous leaf extract of *A. tridentata* subsp. *wyomingensis* was not fluorescent under UV light, indicating the absence of coumarins.

Two individuals of *A. tridentata* subsp. *vaseyana* were collected near Pine, Idaho on June 28, 2022 (pre-flowering) and two individuals were collected near Bogus Basin Ski Resort, Idaho on July 7, 2022 (pre-flowering) (see Table 5). Hydrodistillation of the four samples gave yellow essential oils. Note that aqueous leaf extracts of each *A. tridentata* subsp. *vaseyana* showed bright blue-white fluorescence under UV light, indicating the presence of coumarins.

Plants were identified by Setzer by consulting the botanical descriptions,[21,39] and by comparison with samples from the New York Botanical Garden Virtual Herbarium (https://sweetgum.nybg.org/science/vh/, accessed on August 1, 2022). Voucher specimens (WNS-Atw-5745, WNS-Atv-5680, and WNS-Atv-5714) have been deposited in the University of Alabama in Huntsville herbarium. For each specimen, the aerial parts were fresh frozen and stored at −20 °C until distilled. The plant material was hydrodistilled using a Likens-Nickerson apparatus for 4 h to give the essential oils (Table 5).

Table 5. *Artemisia tridentata* Collection and Hydrodistillation Details.

Plant	Sample number	Location	Mass aerial parts (g)	Yield essential oil (g)
A. tridentata subsp. *wyomingensis*	Atw#1	43°18′52″ N, 116°25′26″ W, 853 m elevation	49.37	1.8361
	Atw#2	43°18′58″ N, 116°27′12″ W, 845 m elevation	43.81	1.1579
	Atw#3	43°18′58″ N, 116°27′14″ W, 844 m elevation	37.33	1.6610
	Atw#4	43°18′5″ N, 116°23′19″ W, 869 m elevation	28.02	1.1661
	Atw#5	43°18′5″ N, 116°23′19″ W, 869 m elevation	35.10	0.8203
	Atw#6	43°18′21″ N, 116°22′50″ W, 875 m elevation	29.44	0.8195
A. tridentata subsp. *vaseyana*	Atv#1	43°24′21″ N, 115°17′33″ W, 1423 m elevation	106.35	1.8424
	Atv#2	43°24′21″ N, 115°17′33″ W, 1423 m elevation	157.36	2.4966
	Atv#3	43°43′58″ N, 116°7′42″ W, 1673 m elevation	81.11	0.7190
	Atv#4	43°43′34" N, 116°9′28" W, 1481 m elevation	61.57	0.5896

Gas Chromatography Coupled with Flame Ionization Detection and Gas Chromatography–Mass Spectrometry Analyses

The essential oils of *A. tridentata* subsp. *wyomingensis* and *A. tridentata* subsp. *vaseyana* were analyzed by gas chromatography coupled with flame ionization detection, gas chromatography–mass spectrometry (GC/MS), and chiral GC/MS as previously described.[6] The essential oil components were identified by comparison of the mass spectral fragmentation patters and by comparison of RI values available in the Adams,[25] FFNSC 3,[26] NIST20,[27] and our own in-house database.[28] The identification of enantiomers was determined by the comparison of retention times with authentic samples obtained from Sigma-Aldrich (Milwaukee, WI, USA).

Statistical Analysis

For the HCA, the 13 *A. tridentata* essential oil compositions were treated as operational taxonomic units, and the percentages of the 22 most abundant essential oil components, namely, santolina triene, 4,4-dimethyl-2-buten-4-olide, camphene, artemiseole, yomogi alcohol, 1,8-cineole, santolina epoxide, *cis*-arbusculone, artemisia ketone, *trans*-arbusculone, α-santolina alcohol, artemisia alcohol, α-thujone, methyl santolinate, camphor, (*Z*)-tagetone, β-artemisyl acetate, unidentified (1234), davana ether 2, davana ether 4, unidentified (1554), and davanone D, were used to delineate the chemical associations between the *A. tridentata* essential oil samples. Pearson correlation was used to measure similarity, and the unweighted pair group method with arithmetic average was used for cluster definition. PCA was performed for the visual verification of the essential oil interrelationships of the different *A. tridentata* subspecies using the 13 major components as variables with a Pearson correlation matrix. The HCA and PCA analyses were performed using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). ANOVA was conducted by one-way ANOVA followed by the Tukey test using Minitab® 18 (Minitab Inc., State College, PA, USA). Differences at $P < 0.05$ were considered to be statistically significant.

Acknowledgement

This work was carried out as a part of the activities of the Aromatic Plant Research Center (https://aromaticplant.org/).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

Ethical approval is not applicable for this article.

Data Availability Statement

The data presented in this study are available upon reasonable request from the corresponding author.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

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

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Supplemental Material

Supplemental material for this article is available online.

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