

# Essential Oil & Plant Composition

# Research Article

# The chemical compositions of *Abies* foliar essential oils from the western United States

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# **Abstract**

The foliage of Abies species in western North America has been used by Native Americans in their traditional medicine for pulmonary problems, coughs, colds, and tuberculosis. As part of our interest in Native American traditional medicine as well as essential oils of aromatic medicinal plants, including gymnosperms, the foliar essential oils of A. amabilis, A. concolor, A. grandis, A. lasiocarpa, and A. magnifica were obtained by hydrodistillation and analyzed by gas chromatographic methods. The essential oils of two samples of A. amabilis from Mt. Hood, Oregon, were rich in  $\alpha$ -pinene (8.2%, 11.1%),  $\beta$ -pinene (12.1%, 20.9%), δ-3-carene (22.1%, 22.4%), limonene (4.2%, 14.3%), and β-phellandrene (10.6%, 16.1%). β-Pinene (40.5-50.7%) dominated the essential oils of A. concolor (four samples) from northern California, followed by  $\alpha$ -pinene (6.4-12.2%),  $\beta$ -phellandrene (7.0-21.3%), and  $\alpha$ -terpineol (7.1-9.9%). The major components in the essential oils of *A. grandis* from northern Idaho (three samples) were α-pinene (5.4-8.4%), camphene (10.3-12.1%),  $\beta$ pinene (10.2-20.7%), β-phellandrene (14.8-24.4%) and bornyl acetate (18.0-23.0%). Abies lasiocarpa was obtained from two locations, Mt. Hood, Oregon (two samples), and Mt. St. Helens, Washington (three samples). The essential oils showed notable differences in limonene (Mt. Hood, 1.8-2.1%; Mt. St. Helens, 32.5-60.0%) and  $\beta$ -phellandrene (Mt. Hood, 42.6-54.3%; Mt. St. Helens, 6.8-20.6%) concentrations.  $\beta$ -Phellandrene (41.2-44.6%) and  $\beta$ pinene (16.9-20.8%) dominated the essential oils of A. magnifica (three samples from Mt. Lassen, California). Enantioselective GC-MS revealed that the (-)-enantiomers predominated for  $\alpha$ -pinene, camphene, limonene,  $\beta$ -phellandrene, terpinen-4-ol, and  $\alpha$ terpineol. The relatively high concentrations of  $\alpha$ -pinene, camphene,  $\beta$ -pinene, limonene,  $\alpha$ -terpineol, and bornyl acetate in the *Abies* essential oils are consistent with the Native American traditional use of these species to treat pulmonary troubles (e.g., coughs, colds, and tuberculosis).

# 1. Introduction

Abies Mill. (Pinaceae) is a genus of 53 species of conifers distributed across the northern hemisphere [1]. The phytochemistry and biological activities of

Abies species were reviewed in 2018 by Kim and Park [2]. These authors concluded that at least 327 plant secondary metabolites have been identified in *Abies* 



species, with triterpenoids being the most abundant. In addition, *Abies* extracts have demonstrated various bioactivities, including antimicrobial, cytotoxic, anti-inflammatory, and antioxidant activities [2].

Abies amabilis (Douglas ex Loudon) J. Forbes (Pacific silver fir) is a large coniferous tree that grows up to 75 m tall. The leaves (needles) are 0.7 to 2.5 cm long, 1-3 mm wide, dark green above and lacking stomata with two white bands of stomata below, and prominently notched leaf apex (Fig. 1) [3].



**Figure 1.** *Abies amabilis* (Douglas ex Loudon) J. Forbes (Pacific silver fir). **A:** Leaves. **B:** Bark.

The bark is light gray and smooth with resin blisters when young, but reddish-gray with scaly plates on older trees (Fig. 1) [4]. The tree ranges naturally from subarctic Alaska and western British Columbia, south through the Cascades of Washington and Oregon to northern California (Fig. 2) [5]. The tree has also been introduced in Great Britain [1].



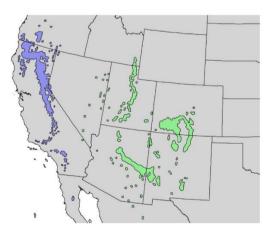
**Figure 2.** The native range of *Abies amabilis* (Pacific silver fir) [5].

Abies concolor (Gordon) Lindl. ex Hildebr. (white fir) is a large tree, 40-55 m tall, with thin gray bark on young trees that thickens and breaks into furrows on older trees (Fig. 3) [6, 7]. The leaves (needles) are 1.5-6 cm long and 2-3 mm wide, glaucous blue-green above with two glaucous bands of stomata below (Fig. 3) [8].



**Figure 3.** *Abies concolor* (Gordon) Lindl. ex Hildebr. (white fir). **A:** Leaves. **B:** Bark.

There are two recognized subspecies, *A. concolor* subsp. *concolor* (Rocky Mountain white fir) and *A. concolor* subsp. *lowiana* (Gordon) A.E. Murray (Sierra Nevada white fir). The *A. concolor* subsp. *lowiana* subspecies ranges from the southern Cascades of Oregon, south through the Sierra Nevada range of California, while *A. concolor* subsp. *concolor* is found in the mountainous areas of southern Idaho, Utah, Arizona, Colorado, and New Mexico (Fig. 4) [5].



**Figure 4.** The native range of *Abies concolor* subsp. *concolor* (Rocky Mountain white fir, green) and *Abies concolor* subsp. *lowiana* (Sierra Nevada white fir, blue) [5].

There have been several reports on the essential oils of *A. concolor* [9], including foliar essential oil samples from Arizona [10], California [11], Utah [11], Idaho [9],

Belarus [12], and Hungary [13]. In addition, bark [13] and wood [14] essential oils have been examined.

Abies grandis (Douglas ex D. Don) Lindl. (grand fir) is a large tree growing around 75-100 m tall. The bark is smooth and gray in young trees, but becomes brown and furrowed in older trees (Fig. 5). The leaves (needles) are 2-6 cm long, green above and silverywhite below. Stomatal rows are absent on the upper surface, but form rows on each side of the midrib on the lower surface (Fig. 5).

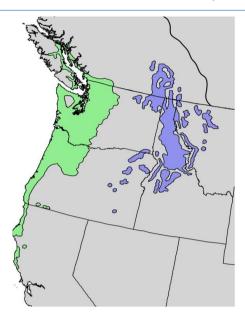




Figure 5. Abies grandis (Douglas ex D. Don) Lindl. subsp. idahoensis (grand fir). A: Leaves. B: Bark.

There are two recognized subspecies of A. grandis, namely A. grandis subsp. grandis, which is a coastal form of the species, and is found from southwestern British Columbia, through Washington and Oregon, and into northern California, and A. grandis subsp. idahoensis (Silba) Silba, an inland form that ranges from southeastern British Columbia to central Idaho (Fig. 6) [5,15,16]. The volatiles of A. grandis have been investigated. Adams and co-workers studied the leaf essential oils from several locations, including British Columbia. Idaho, Washington, Oregon, California [17]. The bark essential oils were examined in a chemotaxonomic investigation of A. grandis and A. grandis/A. concolor hybrids [18]. Ruggles et al. compared the wood essential oils of *A. grandis* and *A.* concolor [14].

Abies lasiocarpa (Hook.) Nutt. (subalpine fir) is native to the mountainous areas of western North America, from Alaska and the Yukon Territory of Canada, south through British Columbia and into the Cascade Range of Washington and Oregon, as well as the mountains of Idaho, Montana, Wyoming, Utah,



**Figure 6.** Natural distribution map for *Abies grandis* (grand fir). *Abies grandis* subsp. *grandis* in green. *Abies grandis* subsp. *idahoensis* in blue [5].

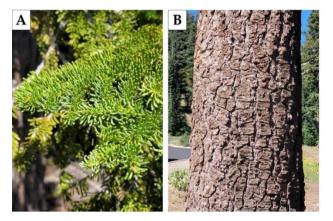
Colorado, Arizona, and New Mexico [19]. Three varieties of *A. lasiocarpa* have been described: *A. lasiocarpa* var. *lasiocarpa* (northern subalpine fir), which is found in the coastal region and the Cascades; *A. lasiocarpa* var. *bifolia* (western subalpine fir), which occurs in the Rocky Mountains; and *A. lasiocarpa* var. *arizonica* (corkbark fir), which is native to the southern Rocky Mountains [20, 21]. The trees are medium sized (around 20 m tall) with thin gray bark. The needles are 1.8-3.1 cm long and 1.5-2 mm wide, with stomata on both the upper and lower surfaces (Fig. 7) [21].

The essential oils of *A. lasiocarpa* have been investigated. The leaf essential oils of the Cascade and Rocky Mountain varieties were examined by Hunt and von Rudloff [22]. Adams and co-workers expanded the chemotaxonomic evaluation of the three varieties of *A. lasiocarpa* to include DNA sequences [23]. The foliar essential oil of *A. lasiocarpa* from Idaho was obtained and analyzed [19]. On the other hand, Zavarin et al. examined the volatile compositions of cortical monoterpenes of *A. lasiocarpa* throughout its range [24].

Abies magnifica A. Murray bis (California red fir) is a large tree species (60-70 m tall) with thin, gray bark on young trees, but with reddish-brown plates on older trees. The leaves are bluish-green, 2-3.7 cm long and 2 mm wide (Fig. 8) [25, 26]. The natural range of *A*.



**Figure 7.** *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir). **A:** Leaves. **B:** Bark of young tree.



**Figure 8.** *Abies magnifica* A. Murray bis (California red fir). **A:** Leaves. **B:** Bark.

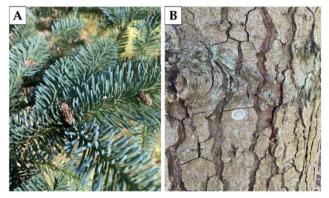
*magnifica* includes the Sierra Nevada range in California, the southern Cascades, the Klamath Mountains, and the coastal ranges of southwestern Oregon and northern California (Fig. 9) [5].



**Figure 9.** Natural range of *Abies magnifica* (California red fir) [5].

In the areas where the two species overlap in southern Oregon and northern California, *A. magnifica* can hybridize with *Abies procera* Rehder [26]. The intermediate forms have been called *A. magnifica* var. shastensis Lemmon, but it is more appropriate to label them *Abies* × shastensis Lemmon [25]. Another variety, *A. magnifica* var. critchfieldii Lanner, from the southern Sierra Nevada, has been described [27]. The volatiles from the cortical oleoresin from *A. magnifica* have been analyzed, which was particularly important in delineating the differences between *A. magnifica* and *A. procera* [28, 29].

Abies procera Rehder (noble fir) is a large tree, up to 80 m tall. The leaves are 1-3.5 cm long  $\times$  1.5-2 mm long and the bark is reddish brown with furrows and ridges (Fig. 10) [30, 31]. This tree is native to the Cascade Range of Oregon and Washington (Fig. 11) [5].



**Figure 10.** *Abies procera* Rehder (noble fir). **A:** Leaves. **B:** Bark [30].

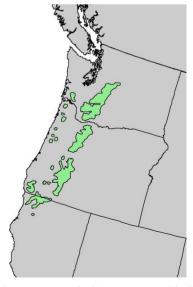


Figure 11. Native range of *Abies procera* (noble fir) [5].

Table 1. Collection and hydrodistillation details for Abies species.

Abies species	Collection date	Collection location	Voucher	Mass foliage (g)	Mass essential oil (g)	Yield (%)
Abies amabilis #1	25 June 2024	45°19'19" N, 121°42'20" W, 1667 m asl	WNS-Aama-286	143.73	6.8118	4.739
Abies amabilis #2	25 June 2024	45°19'17" N, 121°42'18" W, 1663 m asl		201.49	7.4581	3.701
Abies concolor subsp. lowiana #1	26 August 2024	40°06'49" N, 121°28'47" W, 1506 m asl	WNS-Acl-5376	152.56	4.8520	3.180
Abies concolor subsp. lowiana #2	26 August 2024	40°06'49" N, 121°28'47" W, 1506 m asl		172.25	4.6929	2.724
Abies concolor subsp. lowiana #3	26 August 2024	40°06'49" N, 121°28'47" W, 1506 m asl		145.98	4.8645	3.332
Abies concolor subsp. lowiana #4	26 August 2024	40°06'28" N, 121°35'03" W, 1281 m asl		136.91	4.6064	3.365
Abies grandis subsp. idahoensis #1	19 August 2024	48°33'41" N, 116°47'58" W, 1079 m asl	WNS-Agi-750	122.34	4.3174	3.529
Abies grandis subsp. idahoensis #2	19 August 2024	48°33'40" N, 116°47'59" W, 1083 m asl		156.49	4.5307	2.895
Abies grandis subsp. idahoensis #3	19 August 2024	48°33'38" N, 116°48'01" W, 1088 m asl		138.45	4.4461	3.211
Abies lasiocarpa #1	25 June 2024	45°19'19" N, 121°42'19" W, 1667 m asl	WNS-Alas-269	109.37	5.6068	5.126
Abies lasiocarpa #2	25 June 2024	45°19'18" N, 121°42'20" W, 1664 m asl		107.62	5.4512	5.065
Abies lasiocarpa #3	30 June, 2024	46°09'42" N, 122°05'53" W, 895 m asl	WNS-Alas-380	242.77	8.6773	3.574
Abies lasiocarpa #4	30 June, 2024	46°09'42" N, 122°05'53" W, 895 m asl		213.00	6.2231	2.922
Abies lasiocarpa #5	30 June, 2024	46°09'42" N, 122°05'51" W, 892 m asl		207.20	8.1484	3.933
Abies magnifica #1	28 August 2024	40°27'35" N, 121°31'35" W, 2356 m asl	WNS-Amag-5420	180.71	7.3536	4.069
Abies magnifica #2	28 August 2024	40°27'35" N, 121°31'35" W, 2356 m asl		164.55	5.8480	3.554
Abies magnifica #3	28 August 2024	40°27'35" N, 121°31'35" W, 2356 m asl		214.41	5.3626	2.501

Zavarin and co-workers had carried out a broad investigation of *A. procera* cortical oleoresin volatiles (see above) [29].

As part of our continuing interest in the characterization of essential oils of aromatic medicinal plants [32,33], gymnosperms of western North America [19, 34–36], and Native American traditional medicine [37], the purpose of this study is aimed to examine the volatile constituents of *Abies* species from California, Oregon, Washington, and Idaho.

# 2. Materials and methods

#### 2.1. Collection and identification

The details of the *Abies* collection are summarized in Table 1. The foliage was collected from individual trees at the locations indicated. Several branch tips from each individual tree were collected. Voucher specimens have been deposited at the University of Alabama in Huntsville herbarium. The identification in the field was carried out by W.N. Setzer and later verified by comparison with herbarium samples from the C.V. Starr Virtual Herbarium, New York Botanical Garden (https://sweetgum.nybg.org/science/vh/, accessed on 16 December 2024). The fresh foliage samples of each tree were stored frozen (–20 °C) until hydrodistillation.

### 2.2. Essential oils

For each sample of the *Abies* species, the foliage was chopped and the essential oils were obtained by hydrodistillation using a Likens-Nickerson apparatus

for four hours with continuous extraction of the distillate with dichloromethane [38–40] to give colorless essential oils. The hydrodistillation yields are summarized in Table 1.

# 2.3. Gas chromatographic analysis

The *Abies* foliar essential oils were subjected to gas chromatographic-mass spectral analyses (GC-MS) and enantioselective GC-MS, as previously described [19, 41]. The GC-MS conditions are summarized in *Supplementary Table S1*.

# 2.4. Statistical analyses

The agglomerative hierarchical cluster analysis (HCA) and principal component analysis (PCA) for *Abies concolor* were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The percentages of the major components ( $\alpha$ -pinene, camphene,  $\beta$ -pinene, myrcene,  $\delta$ -3-carene, limonene,  $\beta$ -phellandrene, terpinolene,  $\alpha$ -terpineol, piperitone, bornyl acetate, and  $\delta$ -cadinene) were used. Dissimilarity was used to define the clusters using the Euclidean distance, and Ward's method was used to define agglomeration. For the PCA, the Pearson correlation was carried out to verify the results of the HCA using the same major components.

# 3. Results

### 3.1. Abies amabilis

Two samples of *A. amabilis* were collected from the Mt. Hood area of Oregon, USA. Hydrodistillation yielded colorless essential oils with an orange-like odor at

**Table 2.** Compositions (%) and enantiomeric distributions (%) of the major components in the foliar essential oils of *Abies amabilis* from Mt. Hood, Oregon.

RIcalca	RIdbb		Chemical composition	1
IXI calc	IXIdb*	Compounds	A. amabilis #1	A. amabilis #2
933	933	$\alpha$ -Pinene	11.1	8.2
977	978	β-Pinene	20.9	12.1
989	989	Myrcene	3.0	3.3
1008	1006	lpha-Phellandrene	1.1	0.9
1010	1008	δ-3-Carene	22.4	22.1
1030	1030	Limonene	4.2	14.3
1032	1031	β-Phellandrene	16.1	10.6
1085	1086	Terpinolene	2.1	2.0
1176	1178	Benzoic acid	2.3	2.0
1196	1195	$\alpha$ -Terpineol	1.3	1.4
1960	1958	Palmitic acid	1.0	2.5
2139	2140	(Z)-Oleic acid	2.8	6.2
2147	2147	Abienol	0.4	1.6

RIcalc <sup>c</sup>	RIdbd	Ena	ntiomeric distributi	on
K1calc <sup>c</sup>	<b>K1</b> db"	Enantiomers	A. amabilis #1	A. amabilis #2
975	976	(–)-α-Pinene	40.3	50.6
982	982	(+)- $\alpha$ -Pinene	59.7	49.4
1001	998	(–)-Camphene	61.9	68.0
1006	1005	(+)-Camphene	38.1	32.0
1026	1027	(+)-β-Pinene	1.9	2.1
1028	1031	(–)-β-Pinene	98.1	97.9
1050	1052	(+)-δ-3-Carene	100.0	100.0
nd	na	(–)-δ-3-Carene	0.0	0.0
1074	1073	(–)-Limonene	88.3	95.4
1080	1081	(+)-Limonene	11.7	4.6
1081	1083	(–)-β-Phellandrene	95.3	98.6
1090	1089	(+)-β-Phellandrene	4.7	1.4
1344	1347	(–)-α-Terpineol	91.8	91.0
1355	1356	(+)- $\alpha$ -Terpineol	8.2	9.0

 ${}^{a}RI_{calc}$  = Retention index determined with respect to a homologous series of n-alkanes on a ZB-5ms column.  ${}^{b}RI_{db}$  = Reference retention index from the databases.  ${}^{c}RI_{calc}$  = Retention index determined with respect to a homologous series of n-alkanes on a Restek B-Dex 325 capillary column.  ${}^{d}RI_{db}$  = Retention index from our in-house database prepared using commercially available standards. nd = compound not detected. na = reference compound not available.

4.74% and 3.70% yields. A total of 93 compounds were identified in the two essential oils accounting for 100% of the composition in both samples (*Supplementary Table S2*). The major components are listed in Table 2. The essential oils were dominated by monoterpene hydrocarbons (83.4% and 75.9%), especially δ-3-carene (22.4% and 22.1%), β-pinene (20.9% and 12.1%), and β-phellandrene (16.1% and 10.6%), followed by α-pinene (11.1% and 8.2%) and limonene (4.2% and 14.3%). The (–)-enantiomers were dominant for camphene, β-pinene, limonene, β-phellandrene,

and  $\alpha$ -terpineol, while (+)- $\delta$ -3-carene was the only enantiomer observed in the essential oils of *A. amabilis.*  $\alpha$ -Pinene, however, was nearly racemic.

#### 3.2. Abies concolor

Four samples of *A. concolor* were collected from the Butte Meadows area of northern California. Based on the geographical area (Fig. 4), these are presumably *A. concolor* subsp. *lowiana*. Colorless essential oils were obtained with a yield of 2.72-3.37% yield. A total of 99 components were identified in the *A. concolor* essential oils, which accounted for 99.4-99.7% of the compo-

**Table 3.** Compositions (%) and enantiomeric distributions (%) of the major components in the foliar essential oils of *Abies concolor* subsp. *lowiana* from Butte Meadows, California.

			omposition			
$\mathbf{RI}_{\mathrm{calc}^{\mathbf{a}}}$	$RI_{db}{}^{b}$	Compounds	A. concolor	A. concolor	A. concolor	A. concolor
			lowiana #1	lowiana #2	lowiana #3	lowiana #4
933	933	α-Pinene	7.3	7.4	6.4	12.2
949	950	Camphene	3.9	3.4	2.0	2.2
978	978	β-Pinene	46.7	50.7	43.6	40.5
989	989	Myrcene	1.6	1.6	2.2	1.6
1029	1030	Limonene	2.6	3.3	2.1	2.2
1031	1031	β-Phellandrene	7.0	8.7	21.3	19.6
1085	1086	Terpinolene	0.8	0.9	1.3	1.1
1196	1195	α-Terpineol	9.9	8.8	9.4	7.1
1285	1285	Bornyl acetate	4.2	2.2	0.8	1.8

			Enantiomeric distribution					
$\mathbf{RI}_{calc^c}$	$RI_{db^d}$	Enantiomers	A. concolor	A. concolor	A. concolor	A. concolor		
			lowiana #1	lowiana #2	lowiana #3	lowiana #4		
975	976	(–)-α-Pinene	91.9	91.6	90.9	51.9		
982	982	(+)- $\alpha$ -Pinene	8.1	8.4	9.1	48.1		
1001	998	(–)-Camphene	93.9	94.4	93.8	91.5		
1006	1005	(+)-Camphene	6.1	5.6	6.2	8.5		
1026	1027	(+)-β-Pinene	1.1	1.0	1.2	1.3		
1028	1031	(–)-β-Pinene	98.9	99.0	98.8	98.7		
1074	1073	(–)-Limonene	89.6	93.1	92.4	90.3		
1080	1081	(+)-Limonene	10.4	6.9	7.6	9.7		
1081	1083	(–)-β-Phellandrene	100.0	100.0	100.0	100.0		
nd	1089	(+)-β-Phellandrene	0.0	0.0	0.0	0.0		
1344	1347	(–)-α-Terpineol	97.7	98.1	98.1	95.2		
1355	1356	(+)- $\alpha$ -Terpineol	2.2	1.9	1.9	4.8		

 $^{a}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a ZB-5ms column.  $^{b}$ RI<sub>db</sub> = Reference retention index from the databases.  $^{c}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a Restek B-Dex 325 capillary column.  $^{d}$ RI<sub>db</sub> = Retention index from our in-house database prepared using commercially available standards.  $^{d}$ R = compound not detected.

sitions (Supplementary Table S3). The A. concolor essential oils were dominated by  $\beta$ -pinene (40.5-50.7%) with lower concentrations of  $\beta$ -phellandrene (7.0-21.3%),  $\alpha$ -terpineol (7.1-9.9%), and  $\alpha$ -pinene (6.4-12.2%) (Table 3). In the essential oils of A. concolor subsp. lowiana, the (–)-enantiomers were dominant for each of the major monoterpenoid components.

#### 3.3. Abies grandis

Three *A. grandis* samples were collected from the Priest Lake area of northern Idaho. Based on the geographical location of this collection (Fig. 6), these are *A. grandis* subsp. *idahoensis*. The foliar essential oils of *A. grandis* were obtained in yields of 3.53%, 2.90%, and 3.21% as colorless essential oils with a citrus-like aroma. Gas chromatographic analysis of the essential oils revealed 102 identified compounds, accounting

for 100% of the composition in each sample (Supplementary Table S4). The major essential oil components of A. grandis subsp. idahoensis are listed in Table 4. The dominant components were bornyl acetate (18.0-23.0%),  $\beta$ -phellandrene (14.8-24.4%),  $\beta$ -pinene (20.2-20.7%), camphene (10.3-12.1%), and  $\alpha$ -pinene (5.4-8.4%). When detected, (+)- $\delta$ -carene was the exclusive enantiomer observed in A. grandis subsp. idahoensis. The (-)-enantiomers were predominant for  $\alpha$ -pinene, camphene,  $\beta$ -pinene, limonene, and  $\beta$ -phellandrene.

#### 3.4. Abies lasiocarpa

Five samples of *A. lasiocarpa* were collected, two from Mt. Hood, Oregon, and three from Mt. St. Helens, Washington, USA. The foliage was hydrodistilled to yield colorless essential oils with a citrus-like odor,

**Table 4.** Compositions (%) and enantiomeric distributions (%) of the major components in the foliar essential oils of *Abies grandis* subsp. *idahoensis* from Priest Lake, Idaho.

				Chemical composition	
$\mathbf{RI}_{calc^a}$	$RI_{db}{}^{b}$	Compounds	A. grandis idahoensis	A. grandis idahoensis	A. grandis idahoensis
			#1	#2	#3
923	923	Tricyclene	1.1	1.2	1.0
933	933	$\alpha$ -Pinene	6.8	8.4	5.4
949	950	Camphene	11.2	12.1	10.3
977	978	β-Pinene	14.6	10.2	20.7
989	989	Myrcene	1.3	1.3	1.3
1010	1008	δ-3-Carene	2.8	2.2	0.2
1030	1030	Limonene	1.9	1.2	2.2
1032	1031	β-Phellandrene	20.3	24.4	14.8
1085	1086	Terpinolene	1.0	0.8	0.6
1196	1195	$\alpha$ -Terpineol	3.7	1.2	2.4
1283	1282	Bornyl acetate	23.0	18.0	22.9
1518	1518	δ-Cadinene	1.1	3.6	3.2
1628	1628	1-epi-Cubenol	0.5	1.3	1.2

				Enantiomeric distributio	n
$RI_{calc}{}^{c}$	$RI_{db}{}^d$	Enantiomers	A. grandis idahoensis	A. grandis idahoensis	A. grandis idahoensis
			#1	#2	#3
975	976	(–)-α-Pinene	70.1	59.7	75.1
982	982	(+)- $\alpha$ -Pinene	29.9	40.3	24.9
1001	998	(-)-Camphene	96.1	97.3	96.8
1006	1005	(+)-Camphene	3.9	2.7	3.2
1026	1027	(+)-β-Pinene	1.4	1.8	1.2
1028	1031	(–)-β-Pinene	98.6	98.2	98.8
1050	1052	(+)-δ-3-Carene	100.0	100.0	n d
nd	na	(–)-δ-3-Carene	0.0	0.0	nd
1074	1073	(–)-Limonene	84.4	83.9	83.0
1080	1081	(+)-Limonene	15.6	16.1	17.0
1081	1083	(–)-β-Phellandrene	100.0	99.9	99.7
1090	1089	(+)-β-Phellandrene	0.0	0.1	0.3

 $^{a}RI_{calc}$  = Retention index determined with respect to a homologous series of n-alkanes on a ZB-5ms column.  $^{b}RI_{db}$  = Reference retention index from the databases.  $^{c}RI_{calc}$  = Retention index determined with respect to a homologous series of n-alkanes on a Restek B-Dex 325 capillary column.  $^{d}RI_{db}$  = Retention index from our in-house database prepared using commercially available standards.  $^{d}RI_{db}$  = reference compound not available.

with a yield of 2.92% to 5.13%. The complete essential oil compositions are presented in *Supplementary Table S5*. A total of 116 compounds were identified, which accounted for 99.7-100.0% of the composition. The major components (Table 5) in the essential oils were limonene (1.8% and 2.1% in the Mt. Hood samples, but 32.5-60.0% in the Mt. St. Helens samples),  $\beta$ -phellandrene (42.6% and 54.3% in the Mt. Hood samples, but 6.9-20.6% in the Mt. St. Helens samples),  $\beta$ -pinene (18.2% and 24.8%, Mt. Hood; 5.6-11.3%, Mt. St. Helens), and  $\alpha$ -pinene (4.2% and 4.8%, Mt. Hood; 6.9-10.5%, Mt. St. Helens). The (–)-enantiomers were

dominant for each of the major monoterpenoids in the *A. lasiocarpa* essential oils. The percentages of (–)- $\alpha$ -pinene were slightly higher for the Mt. Hood samples (85.7 ± 2.1%) than those for the Mt. St. Helens samples (62.4 ± 6.1%).

#### 3.5. Abies magnifica

Three samples of *A. magnifica* were collected from Mt. Lassen, California. Hydrodistillation of the samples yielded colorless essential oils with yields of 2.50-4.07%. Eighty-nine components were identified in the *A. magnifica* foliar essential oils (99.7-99.9% of the total composition (*Supplementary Table S6*). The major

**Table 5.** Compositions (%) and enantiomeric distributions (%) of the major components in the foliar essential oils of *Abies lasiocarpa* from Mt. Hood, Oregon (samples #1 and #2), and Mt. St. Helens, Washington (samples #3, #4, and #5).

			Chemical composition				
$RI_{calc^a}$	$RI_{db}{}^{b}$	Compounds	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa
			#1	#2	#3	#4	#5
933	933	α-Pinene	4.8	4.2	10.5	7.7	6.9
977	978	β-Pinene	24.8	18.2	11.3	5.6	6.9
989	989	Myrcene	2.3	1.7	2.6	3.4	3.0
1030	1030	Limonene	2.1	1.8	32.5	60.0	55.9
1032	1031	β-Phellandrene	42.6	54.3	20.6	6.8	9.6
1085	1086	Terpinolene	1.2	1.1	3.4	1.3	1.0
1196	1195	$\alpha$ -Terpineol	0.6	0.4	2.3	1.2	1.3
1254	1254	Piperitone	5.3	2.7	tr	tr	tr

		Enantiomeric d			ntiomeric distribi	ıtion	
$\mathbf{RI}_{\mathrm{calc}^{\mathbf{c}}}$	$RI_{db}{}^{d}$	Enantiomers	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa	A. lasiocarpa
			#1	#2	#3	#4	#5
975	976	(–)-α-Pinene	84.2	87.1	56.3	62.3	68.5
982	982	(+)- $\alpha$ -Pinene	15.8	12.9	43.7	37.7	31.5
1026	1027	(+)-β-Pinene	1.5	1.5	2.5	3.0	2.2
1028	1031	(–)-β-Pinene	98.5	98.5	97.5	97.0	97.8
1074	1073	(–)-Limonene	86.4	95.2	97.6	98.4	98.4
1080	1081	(+)-Limonene	13.6	4.8	2.4	1.6	1.6
1081	1083	(–)-β-Phellandrene	99.9	99.9	99.6	98.8	99.1
1090	1089	(+)-β-Phellandrene	0.1	0.1	0.5	1.2	0.9
1344	1347	(–)-α-Terpineol	91.9	96.9	90.4	88.9	91.6
1355	1356	(+)-α-Terpineol	8.1	3.1	9.6	11.1	8.4
1381	1380	(–)-Piperitone	91.4	91.2	m d	m d	n d
1386	1385	(+)-Piperitone	8.6	8.8	nd	nd	nd

 $^{a}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a ZB-5ms column.  $^{b}$ RI<sub>db</sub> = Reference retention index from the databases. tr = trace (< 0.05%).  $^{c}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a Restek B-Dex 325 capillary column.  $^{d}$ RI<sub>db</sub> = Retention index from our in-house database prepared using commercially available standards. nd = compound not detected.

components (Table 6) were  $\beta$ -phellandrene (41.2-44.6%),  $\beta$ -pinene (16.9-20.8%),  $\alpha$ -pinene (6.0-7.1%), and (E)- $\beta$ -caryophyllene (4.5-4.8%). As observed above, (–)- $\alpha$ -pinene, (–)- $\beta$ -pinene, (–)- $\beta$ -phellandrene, and (–)- $\alpha$ -terpineol were the dominant enantiomers in A. magnifica essential oils. The complete enantioselective GC-MS analysis of the Abies foliar essential oils is shown in  $Supplementary\ Table\ S7$ .

# 4. Discussion

# 4.1. Abies amabilis

As far as we are aware, this is the first report on the foliar essential oil of *A. amabilis*. The major components ( $\alpha$ -pinene,  $\beta$ -pinene,  $\delta$ -3-carene, and  $\beta$ -phellandrene) in the essential oil are consistent with the Native American use of this tree. The Nitinaht people of Vancouver Island, British Columbia,

bundled the fragrant boughs of A. amabilis and placed under their bedding as incense [42], while the Thompson people prepared a decoction of the boughs, to treat pulmonary troubles [43]. In addition to their fragrance qualities,  $\alpha$ -pinene has shown antibacterial activities against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* [44, 45] and as a treatment for allergic rhinitis in a mouse model [46]. Also,  $\beta$ -pinene,  $\delta$ -3-carene, and limonene reported to have antimycobacterial activity [47].

# 4.2. Abies concolor

Zavarin and co-authors examined the cortical oleoresins from 351 *A. concolor* trees from 43 locations and determined the monoterpenoid concentrations[48]. Based on their analyses, these authors were able to define four groups based on monoterpenoid

**Table 6.** Compositions (%) and enantiomeric distributions (%) of the major components in the foliar essential oils of *Abies magnifica* from Mt. Lassen, northern California.

RIcalca	RIdbb	Commoundo	Chemical composition				
<b>K1</b> calc <sup>a</sup>	Kldb	Compounds	A. magnifica #1	A. magnifica #2	A. magnifica #3		
933	933	$\alpha$ -Pinene	6.0	7.1	6.5		
977	978	β-Pinene	16.9	20.8	17.3		
989	989	Myrcene	1.7	2.1	2.7		
1031	1031	β-Phellandrene	41.2	44.6	42.1		
1085	1086	Terpinolene	1.6	1.0	0.9		
1195	1195	α-Terpineol	2.7	2.6	4.2		
1334	1335	δ-Elemene	1.2	1.1	0.8		
1418	1417	( $E$ )- $β$ -Caryophyllene	4.8	4.5	4.8		
1832	1832	(2 <i>E</i> ,6 <i>E</i> )-Farnesyl acetate	2.0	1.5	0.4		
2053	2053	Manool	1.2	0.8	1.5		

RI <sub>calc</sub> c	RIdbd	Compounds	Enantiomeric distribution				
KIcalc	KIdb"	Compounds	A. magnifica #1	A. magnifica #2	A. magnifica #3		
975	976	(–)-α-Pinene	86.4	89.8	94.2		
982	982	(+)- $\alpha$ -Pinene	13.6	10.2	5.8		
1026	1027	(+)-β-Pinene	1.8	1.6	1.9		
1028	1031	(–)-β-Pinene	98.2	98.4	98.1		
1081	1083	(–)-β-Phellandrene	99.9	99.9	99.9		
1090	1089	(+)-β-Phellandrene	0.1	0.1	0.1		
1344	1347	(–)-α-Terpineol	94.9	97.2	96.7		
1355	1356	(+)-α-Terpineol	5.1	2.8	3.3		

 ${}^{a}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a ZB-5ms column.  ${}^{b}$ RI<sub>db</sub> = Reference retention index from the databases.  ${}^{c}$ RI<sub>calc</sub> = Retention index determined with respect to a homologous series of n-alkanes on a Restek B-Dex 325 capillary column.  ${}^{d}$ RI<sub>db</sub> = Retention index from our in-house database prepared using commercially available standards.

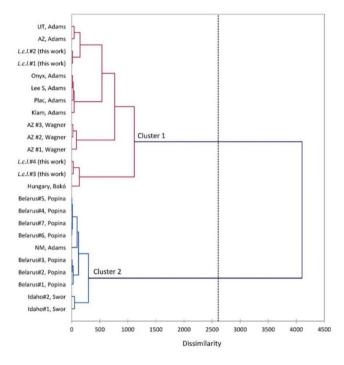
compositions: *A. concolor* subsp. *lowiana* from California, and three subgroups of *A. concolor* subsp. *concolor* (a group from Nevada and Utah, a group from central Arizona, and a group from Colorado, New Mexico, and southern Arizona). Adams and coauthors had carried out a subsequent investigation of the leaf essential oils of *A. concolor* from northern and southern California, Utah, Arizona, and New Mexico [11]. Their work largely corroborated the conclusions of the Zavarin study. Samples from northern California constitute *A. concolor* subsp. *lowiana*, while the samples from Utah, Arizona, and New Mexico constitute *A. concolor* subsp. *concolor*.

The samples of *A. concolor* in this study, all from northern California, are presumably *A. concolor* subsp. *lowiana* based on geographical location (Fig. 4). However, there are notable differences in the concentrations limonene and  $\beta$ -phellandrene between this study and that of Adams et al. In the Adams study, the limonene concentrations were relatively

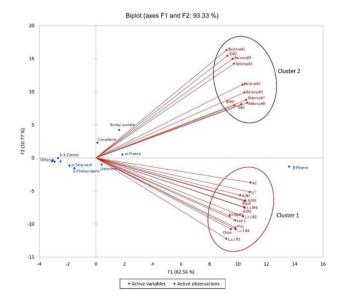
high for *A. concolor* subsp. *lowiana* (average 21.2%) whereas the β-phellandrene concentrations were relatively low (average 2.4%). In contrast, in the present work, β-phellandrene concentrations were greater (average 14.2%) than limonene concentrations (2.6%). Adams et al. found that both limonene and β-phellandrene concentrations were relatively low (average 6.6% and 2.9%, respectively) for *A. concolor* subsp. *concolor*. Limonene and β-phellandrene concentrations were similarly low for *A. concolor* subsp. *concolor* from southern Idaho (average 6.2% and 2.8%, respectively) [9]. A cultivated sample of *Abies concolor* from Hungary had 0.0% limonene and 15.9% β-phellandrene [13].

Samples of *A. concolor* from Belarus showed relatively high concentrations of camphene (average 14.7%), and bornyl acetate (average 20.9%) [12]. Samples of *A. concolor* subsp. *concolor* from Idaho [9] and New Mexico [11] were also rich in bornyl acetate (18.4% and 20.2%, respectively). To statistically compare the

volatile compositions of *Abies concolor*, multivariate analyses (hierarchical cluster and principal component) were performed (Figs. 12 and 13). There are two well-defined clusters, both of which were high in  $\alpha$ - and  $\beta$ -pinene.



**Figure 12.** Dendrogram obtained from agglomerative hierarchical cluster analysis (HCA) based on the major components in the essential oils of *Abies concolor*. Adams [11], Wagner [10], Bakó [13], Popina [12], Swor [9].



**Figure 13.** Biplot obtained from principal component analysis (PCA) based on the major components in the essential oils of *Abies concolor*.

The components defining the clusters are the relative concentrations of limonene (high in cluster 1) and camphene and bornyl acetate (high in cluster 2). It is also apparent that the two subspecies, *lowiana* and *concolor*, are not readily separated based on the major components. The samples from Arizona and Utah are both found in the cluster populated by the California samples. The cultivated samples from Belarus are more closely aligned with *A. concolor* subsp. *concolor*, whereas the cultivated sample from Hungary is more closely aligned with *A. concolor* subsp. *lowiana*.

Abies concolor is also important in Native American traditional medicine. The Paiute and Shoshoni people took a decoction of the needles and bark resin for pulmonary troubles [49]. The Western Keres people prepared a bath of a decoction of the foliage to treat for rheumatism [50]. Both α-pinene and β-pinene have shown activity against respiratory pathogens [44, 45,47]. α-Terpineol has also shown antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [51], and has also shown selective inhibition of cyclooxygenase 2 (COX-2) [52]. Thus, the major components in the essential of *A. concolor* are consistent with the traditional use of this tree.

#### 4.3. Abies grandis

There have been two subspecies suggested for A. grandis, A. grandis subsp. grandis (the coastal form) and A. grandis subsp. idahoensis (the inland form) [16, 53]. However, these subspecies are not recognized in the Flora of North America [15]. Furthermore, Zavarin et al., using bark essential oils [18], and von Rudloff [54] and Adams et al. [17], using leaf essential oils, concluded that the chemosystematic analyses do not differentiate between the coastal and inland forms of A. grandis. The chemical composition of A. grandis in this current work from northern Idaho corroborates the similarities observed in the essential oils. The major components of A. grandis (this study and previously published works [13, 17, 54]) are camphene (11.4  $\pm$  1.7%),  $\beta$ -pinene (22.1  $\pm$  6.6%),  $\beta$ phellandrene (17.1  $\pm$  5.0%), and bornyl acetate (19.1  $\pm$ 3.9%).

The Chehalis tribe and the Green River Group of Native Americans prepared and consumed a decoction of *A. grandis* needles as a treatment for colds [55]. The high concentrations of biologically active

volatile components, camphene,  $\beta$ -pinene, and bornyl acetate, support the traditional use of this tree in treating colds (see above).

# 4.4. Abies lasiocarpa

There has been some disagreement regarding the infraspecific taxa of A. lasiocarpa [19, 23]. As many as three varieties have been suggested, including Abies lasiocarpa (Hook.) Nutt. var. lasiocarpa (coastal subalpine fir, ranging from British Columbia south through the Cascade Mountains of Washington and Oregon), Abies lasiocarpa var. bifolia (A. Murray bis) Eckenw. (Rocky Mountain subalpine fir, ranging from British Columbia south through the Rocky Mountains of Idaho, Montana and Colorado), and Abies lasiocarpa var. arizonica (Merriam) Lemmon (corkbark fir, found in high mountains of Arizona and New Mexico) based on morphological and monoterpenoid profiles [23, 56]. The foliar essential oil compositions of the three varieties have been investigated previously by Hunt and von Rudloff [22] and by Adams et al. [23]. The essential oil of the coastal variety has been characterized by relatively high concentrations of βphellandrene (36.8-58.8%), while the essential oil of the Rocky Mountain variety is relatively abundant in camphene (7.3-16.2%) and bornyl acetate (13.0-31.6%) [22]. The Arizona variety also has high concentrations of camphene (15.2%) and bornyl acetate (34.4%) [23]. Adams and co-workers have carried out DNA studies and concluded that A. lasiocarpa var. bifolia should not be considered a distinct variety, but rather a chemotype of A. lasiocarpa var. lasiocarpa [23]. The World Flora Online currently recognizes only A. lasiocarpa var. arizonica and A. lasiocarpa var. lasiocarpa [1].

The two samples from Mt. Hood (Oregon Cascade Range) in this study were dominated by  $\beta$ -phellandrene (42.6% and 54.3%) with only small concentrations of limonene (2.1% and 1.8%, respectively). These data are consistent with the coastal variety described by Hunt and von Rudloff and Adams et al. However, two samples (#4 and #5) collected from Mt. St. Helens (Washington Cascade Range) were rich in limonene (60.0% and 55.9%), but low in  $\beta$ -phellandrene (6.8% and 9.6%), which is more consistent with the Rocky Mountain variety. However, the *A. lasiocarpa* samples in this study all

had relatively low concentrations of camphene and bornyl acetate (< 0.5%). Interestingly, sample #3 (from Mt. St. Helens) had intermediate concentrations of limonene (32.5%) and  $\beta$ -phellandrene (20.6%). In contrast, samples of A. lasiocarpa from southern Idaho were rich in limonene (20.3% and 34.6%), bornyl acetate (24.7% and 18.5%), and camphene (10.9% and 7.4%), with relatively low concentrations of  $\beta$ phellandrene (6.7% and 7.1%) [19]. The Idaho samples are clearly representative of the Rocky Mountain variety of A. lasiocarpa. The ranges of A. lasiocarpa and A. procera overlap (Fig. 11), indicating the potential for hybridization [21], which could affect their chemical composition. The leaf essential oil of A. procera from Oregon showed limonene (44.0%), β-phellandrene (19.9%),  $\beta$ -pinene (9.2%), and  $\alpha$ -pinene (6.2%) as the major components, with lower concentrations of camphene (1.1%) and bornyl acetate (1.6%) [30].

# 4.5. Abies magnifica

Based on the bark essential oil compositions, Zavarin and co-workers have concluded that *A. magnifica* in the northern part of its range (Figure 9) can hybridize with *A. procera* from the southern part of its range (Fig. 11). There are notable differences in the compositions of the major components between the *A. magnifica* samples from Mt. Lassen (this study) and *A. procera* from Oregon [30]. For example,  $\beta$ -pinene averaged 18.3% for *A. magnifica* compared to 9.1% for *A. procera*; limonene averaged 0.7% for *A. magnifica*, but 43.8% for *A. procera*;  $\beta$ -phellandrene averaged 42.6% for *A. magnifica*, but 19.8% for *A. procera*; and (*E*)- $\beta$ -caryophyllene averaged 4.7% for *A. magnifica*, but only 0.1% for *A. procera*.

# 4.6. Enantiomeric distribution

The enantiomeric distributions of the chiral monoterpenoid components of the *Abies* foliar essential oils are compiled in *Supplementary Table S7*. Notable trends were observed in the enantiomeric ratios. (–)- $\alpha$ -Pinene generally predominated, but the ratio was variable. *Abies amabilis* #1 had only 40.3% (–)- $\alpha$ -pinene, but *A. magnifica* #3 showed 94.3% (–)- $\alpha$ -pinene. The enantiomeric distribution of  $\alpha$ -pinene in *Pinus* species is also variable, both between and within species [35, 41, 57]. In contrast, (+)- $\alpha$ -pinene predominates in members of the Cupressaceae, including *Chamaecyparis*, *Juniperus*, and *Thuja* [36]. The

**Table 7.** Native American use of *Abies* species.

Abies species	Native-American Ethnopharmacology	Major components (> 5%, average)
Abies amabilis (Pacific silver fir, Mt. Hood, Oregon)	Nitinaht – Boughs bundled up and used as home air fresheners [42].  Thompson – Decoction of boughs and/or bark taken for tuberculosis [43].	$\alpha$ -Pinene (9.6%), $\beta$ -pinene (16.5%), $\delta$ -3-carene (22.2%), limonene (9.2%), $\beta$ -phellandrene (13.4%) [this study]
Abies concolor (white fir, Kuna, Idaho)	Paiute & Shoshoni – Decoction of needles and bark resin taken for pulmonary troubles [49].	$\alpha$ -Pinene (17.9%), camphene (8.8%), $\beta$ -pinene (24.9%), δ-3-carene (6.0%), limonene (6.2%), bornyl acetate (18.4%) [9]. $\alpha$ -Pinene (8.3%), $\beta$ -pinene (45.4%), $\beta$ -
Abies concolor (white fir, Butte Meadows, California)	pullionary troubles [42].	phellandrene (14.2.2%), $\alpha$ -terpineol (8.8%) [this study]
Abies grandis (grand fir, Priest Lake, Idaho)	Chehali & Green River Group – Decoction of needles taken for colds [55].	$\alpha$ -Pinene (6.9%), camphene (11.2%), $\beta$ -pinene (15.2%), $\beta$ -phellandrene (19.8%), bornyl acetate (21.3%) [this work]
Abies lasiocarpa (subalpine fir, Boise Foothills, Idaho)	Blackfoot – Needle smudge smoke inhaled for headaches [58]; poultice of	Camphene (9.2%), $\beta$ -pinene (11.5%), limonene (27.5%), $\beta$ -phellandrene (6.9%), bornyl acetate (21.6%) [19]. $\alpha$ -Pinene (8.4%), $\beta$ -pinene (7.9%),
Abies lasiocarpa (subalpine fir, Mt. St. Helens, Washington)	leaves applied for chest colds [59]. Crow – Infusion of crushed needles	limonene (49.5%), $\beta$ -phellandrene (12.3%) [this study]
Abies lasiocarpa (subalpine fir, Mt. Hood, Oregon)	used for coughs and colds [60].	$\beta$ -Pinene (21.5%), $\beta$ -phellandrene (48.5%) [this study]
Abies magnifica (California red fir, Mt. Lassen, California)	To our knowledge, there are no published ethnobotanical uses of California red fir by Native Americans.	$\alpha$ -Pinene (6.5%), $\beta$ -pinene (18.3%), $\beta$ -phellandrene (42.6%) [this study]
Abies procera (noble fir, Portland, Oregon)	Paiute – Crumbled leaves smoked for colds; decoction of leaves taken as cough medicine [61].	$\alpha$ -Pinene (6.2%), $\beta$ -pinene (9.1%), limonene (43.8%), $\beta$ -phellandrene (19.8%) [30].

major camphene enantiomer in *Abies* was the (–)-enantiomer. Other members of the Pinaceae also show this predominance. However, members of the Cupressaceae have (+)-camphene predominating [36]. The (–)-enantiomers were the major stereoisomers in *Abies* essential oils for limonene (91.6  $\pm$  5.0%),  $\beta$ -phellandrene (99.4  $\pm$  1.2%), terpinen-4-ol (64.0  $\pm$  5.5%), and  $\alpha$ -terpineol (94.3  $\pm$  3.2%).

# 4.7. Ethnopharmacological consideration

The foliage of *Abies* species has been used in Native American traditional medicine for several ailments, including pulmonary troubles, coughs, and colds (see above). The *Abies* species, their traditional uses, and their major components are summarized in Table 7. The relevant biological activities of the major components of *Abies* foliar essential oils are

summarized in Table 8. The relatively high concentrations and biological activities of the major components are consistent with the traditional medicinal uses of *Abies* species by Native Americans.

#### 5. Conclusions

The foliar essential oils of five *Abies* species were obtained by hydrodistillation and analyzed by gas chromatographic methods. The essential oils were generally dominated by monoterpene hydrocarbons, especially  $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\delta$ -3-carene, limonene, and  $\beta$ -phellandrene. Oxygenated monoterpenoids were also abundant in *A. concolor* ( $\alpha$ -terpineol) and *A. grandis* (bornyl acetate). The essential oil analyses of *A. concolor*, *A. grandis*, *A. lasiocarpa*, and *A. magnifica* complement earlier works

Table 8. Relevant biological activities of major components of Abies essential oils.

Compound	Relevant biological activity	Ref.
lpha-Pinene	Antibacterial (Streptococcus pneumoniae, MIC 172 µg/mL; Klebsiella pneumoniae, MIC	
	178 $\mu g/mL$ ) anti-inflammatory (inhibition of inflammatory mediators NF- $\kappa B$ , TNF- $\alpha$	[44]
	and IL-6)	
	Anti-inflammatory (suppression of MAPKs and the NF-κB pathway)	[62]
	Allergic rhinitis treatment (mouse model, decrease in the number of nasal, eye, and	[46]
	ear rubs, and spleen weight)	[46]
	Antinociceptive activity (inhibition of COX-2)	[63]
	Antibacterial, antibiofilm (Acinetobacter baumannii, MIC 0.625 µL/mL; 89.36% biofilm	FC 41
	inhibition at 0.625 μL/mL)	[64]
	Antibacterial (Klebsiella pneumoniae, MIC 8-64 μg/mL, depending on strain)	[45]
	Antibacterial (Streptococcus pneumoniae, MIC 78.1 µg/mL)	APRO
Camphene	Antinociceptive (mouse model, significant reduction in acetic acid-induced writhing	
	and formalin-induced pain)	[65]
	Cytoprotective, rat alveolar macrophages (significantly increased SOD activity, GSH	
	content; significantly decreased NO release and ROS generation); may be useful in	[66]
	lung inflammatory diseases	-
	Anti-inflammatory, antinociceptive (mouse model, significant reduction in formalin-	F / P7
	induced pain and thermal hyperalgesia)	[67]
	Inhalation expectorant (rabbit model, significant increase in respiratory tract fluid	F ( 0 )
	volume)	[68]
	Antibacterial (Streptococcus pneumoniae, MIC 78.1 μg/mL)	APRO
β-Pinene	Antibacterial (Streptococcus pneumoniae, MIC 20 μL/mL)	[69]
	Antibacterial (Streptococcus pneumoniae, MIC 39.1 µg/mL)	APRO
	Antibacterial (Klebsiella pneumoniae, MIC 8-64 µg/mL, depending on strain)	[45]
	Antimycobacterial (Mycobacterium tuberculosis, MIC 10.4 µg/mL; Mycobacterium bovis,	F 4 1773
	MIC 41.7 μg/mL)	[47]
δ-3-Carene	Antimycobacterial (Mycobacterium tuberculosis, MIC 16.7 µg/mL; Mycobacterium bovis,	[47]
	MIC 33.3 μg/mL)	[47]
Limonene	Antibacterial (Klebsiella pneumoniae, MIC 8-64 µg/mL, depending on strain)	[45]
	Antimycobacterial (Mycobacterium tuberculosis, MIC 25.0 µg/mL; Mycobacterium bovis,	F 4 1773
	MIC 41.7 μg/mL)	[47]
	Antibacterial (Streptococcus pneumoniae, MIC 78.1 μg/mL)	APRO
	Anti-inflammatory (inhibition of NF- $\kappa$ B, TNF- $\alpha$ , iNOS, IL-1 $\beta$ , and IL-6)	[70]
β-Phellandrene	To the best of our knowledge, there are no published studies on the biological	
	activities related to coughs, colds, or other respiratory problems.	
lpha-Terpineol	Antibacterial (Pseudomonas aeruginosa, Klebsiella pneumoniae, zone-of-inhibition	[E1]
	assays)	[51]
	Anti-inflammatory (inhibition of NF- $\kappa$ B, COX-2, TNF- $\alpha$ , IL-1 $\beta$ , IL-8, IL-10, and PGE2).	[52]
Bornyl acetate	Anti-inflammatory (downregulated the levels of proinflammatory cytokines in vitro,	[71]
	RAW 264.7 cells, and in vivo, lung, mouse model)	[71]
	Anti-inflammatory (inhibition of IL-6, IL-8, MMP-1, and MMP-13)	[70]
	Anti-inflammatory (significant elevation of anti-inflammatory cytokine IL-11)	[72]
	Anti-inflammatory (mitigates expression of pro-inflammatory cytokines)	[73]
	Anti-inflammatory (inhibition of the NF-κB signal pathway, down-regulates pro-	[7/1]
	inflammatory cytokines), immune modulation (up-regulation of CD86+)	[74]
	Antibacterial (Klebsiella pneumoniae, MIC 8-64 µg/mL, depending on strain)	[45]
	Antibacterial (Streptococcus pneumoniae, MIC 312.5 µg/mL)	APRC

APRC = Aromatic Plant Research Center, unpublished.

on these species and provide additional chemotaxonomic data delineating infraspecific taxa within the species and possible hybridization between species. The differences in the essential oil compositions of each species can be attributed to geography, genetics, climate, seasonal variation and herbivory, however, this study cannot address these effects. Future research on each Abies species could address these issues. As far as we are aware, this study represents the first analysis of the foliar essential oil of A. amabilis and the first reports on the enantiomeric distributions of chiral monoterpenoids in the essential oils of A. amabilis, A. concolor, A. grandis, A. lasiocarpa, and A. magnifica. The enantiomeric trends in Abies essential oils were (–)- $\alpha$ -pinene and (–)-camphene which generally predominated, while the (-)enantiomers were dominant in each of the Abies essential oils for limonene, β-phellandrene, terpinen-4-ol, and α-terpineol. (–)-β-Phellandrene was the exclusive enantiomer observed in A. grandis, A. lasiocarpa, and A. magnifica. (+)-δ-3-Carene was the only enantiomer observed in the Abies essential oils. Native Americans have used Abies foliage to treat several ailments, particularly pulmonary troubles including coughs, colds, and tuberculosis. The biological activities of the major essential oil components are consistent with Native American traditional uses of Abies.

# Supplementary materials

The following supporting information can be obtained from the corresponding author:

Table S1. Instrument details for the gas chromatographic analyses of Abies species.

Table S2. Foliar essential oil composition of Abies amabilis from Mt. Hood, Oregon.

Table S3. Foliar essential oil composition of Abies concolor subsp. lowiana from Butte Meadows, California.

Table S4. Foliar essential oil composition of Abies grandis subsp. idahoensis from Priest Lake, Idaho.

Table S5. Foliar essential oil composition of Abies lasiocarpa from Mt. Hood, Oregon, and Mt. St. Helens, Washington.

Table S6. Foliar essential oil composition of Abies magnifica from Mt. Lassen, northern California;

Table S7. Enantiomeric distributions (%) of chiral monoterpenoid components in Abies foliar essential oils.

Fig. S1. Gas chromatograms of Abies amabilis foliar essential oils.

Fig. S2. Gas chromatograms of Abies concolor subsp. lowiana foliar essential oils.

Fig. S3. Gas chromatograms of Abies grandis subsp. idahoensis foliar essential oils.

Fig. S4. Gas chromatograms of Abies lasiocarpa foliar essential oils.

Fig. S5. Gas chromatograms of Abies magnifica foliar essential oils.

Supplementary material to this article can be found online at <a href="https://www.currentsci.com/images/">https://www.currentsci.com/images/</a> articlesFile/supplementary.1752851831.pdf

# Authors' contributions

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

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# Availability of data and materials

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

# **Conflicts of interest**

The authors declare no conflict of interest.

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