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Enantioselective gas chromatographic analysis of Cupressaceae foliar essential oils: *Callitropsis nootkatensis, Calocedrus decurrens, Sequoia sempervirens,* and *Thuja plicata*

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Abstract

Members of the Cupressaceae have historically held importance in traditional medicine as well as sources of essential oils. As part of our interest in medicinal and aromatic plants, we obtained essential oils from the foliage of *Callitropsis nootkatensis*, *Calocedrus decurrens*, *Sequoia sempervirens*, and *Thuja plicata* growing in the western United States. The essential oils were analyzed by gas chromatography particularly enantioselective gas chromatography to ascertain whether there are trends in the enantiomeric distribution of chiral monoterpenoids in this family. The major components in *C. nootkatensis* were α-pinene (33.5%, 16.4%; 81.8% (−)-α-pinene), (+)-δ-3-carene (28.6-11.0%), and limonene (34.4%, 4.2%; 89.7% (+)-limonene). The major components in *C. decurrens* were (+)-δ-3-carene (28.6-11.0%), limonene (44.4-14.7%, 92.4% (+)-limonene, and terpinolene (10.0-5.4%). The major components in *S. sempervirens* were α-pinene (18.8-10.1%, 91.6% (−)-α-pinene), limonene (8.7-6.2%, 87.9% (+)-limonene), and germacrene B (8.2-5.4%). (−)-α-Thujone (65.9%, 62.5%) and (+)-β-thujone (12.1%, 10.2%) dominated the essential oil of *T. plicata*. The chemical compositions of the essential oils are comparable to those reported previously. However, this is the first investigation of the enantiomeric distribution of chiral monoterpenoids in *C. nootkatensis*, *C. decurrens*, and *S. sempervirens*. (+)-Sabinene is the major enantiomer in the Cupressaceae, whereas (−)-sabinene is the major enantiomer in *Pinus* spp. (+)-Limonene is the major enantiomer in the Cupressaceae, while (−)-limonene predominates in the Pinaceae. In contrast to members of the Pinaceae (e.g., *Abies* and *Pinus* spp.), in which (−)-terpinen-4-ol and (−)-α-terpineol were dominant, these two monoterpene alcohols showed variable enantiomeric distributions in the Cupressaceae. The enantioselective gas chromatographic analysis of the Cupressaceae adds to our understanding of the phytochemistry of this family.

1. Introduction

The Cupressaceae is made up of 29 genera, which include *Cupressus* L., *Juniperus* L., and *Thuja* L., as well as *Callitropsis* Oerst., *Calocedrus* Kurz, and *Sequoia* Endl. Most members of this family are resinous, and their foliage generally emits strong aromas when rubbed or bruised [1]. As such, several members of the family are commercially important sources of essential oils, including Mediterranean cypress

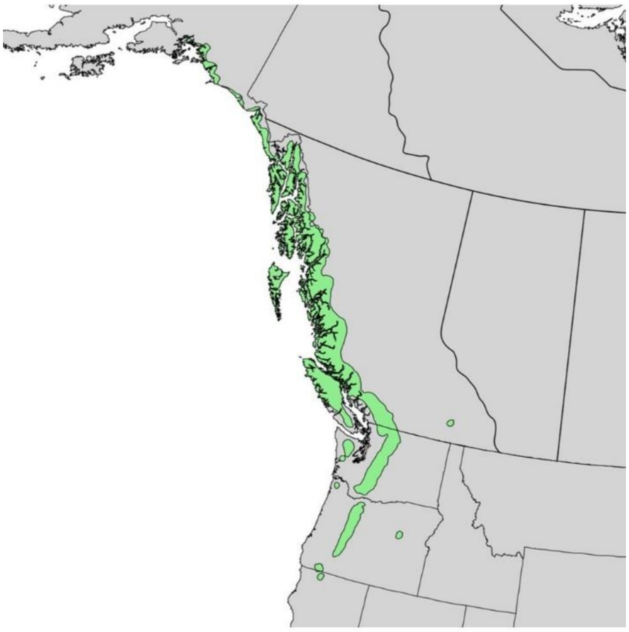

Figure 1. Natural range of *Callitropsis nootkatensis* (D. Don) Oerst. [3].

(*Cupressus sempervirens* L.) foliar essential oil, juniper (*Juniperus communis* L.) "berry" essential oil, eastern red cedar (*Juniperus virginiana* L.) wood essential oil, and eastern white cedar (*Thuja occidentalis* L.) foliar essential oil [2].

Callitropsis nootkatensis (D. Don) Oerst. (syn. *Chamaecyparis nootkatensis* (D. Don) Spach, *Xanthocyparis nootkatensis* (D. Don) Farjon & D.K. Harder, *Cupressus nootkatensis* D. Don), Cupressaceae (Nootka cypress, Alaska yellow-cedar), ranges naturally in coastal northwestern North America, from southern Alaska, through British Columbia and into Washington, including the Cascade Ranges of Washington and Oregon to the Oregon-California border (Fig. 1) [3]. Nootka cypress is an evergreen tree that grows up to around 40 m in height. The foliage forms flat sprays with green scale-like leaves, 1.5-2.5 mm long (Fig. 2) [4].

The Kwakiutl tribe of British Columbia used *C. nootkatensis* in their traditional medicine [5]. The foliage was used in a sweat bath to treat arthritis and rheumatism, an infusion of the foliage was used externally to treat sores and swellings and it was taken internally as a panacea. Previous studies of the foliar essential oils of *C. nootkatensis* were carried out by Andersen and Syrdal [6], Cheng and von Rudloff, who were able to isolate and characterize individual enantiomers (–)-α-pinene, (+)-δ-3-carene, and (+)-limonene [7], and Adams et al. [8, 9]. In addition, volatiles from the heartwood extracts of *C. nootkatensis* have been characterized [10].

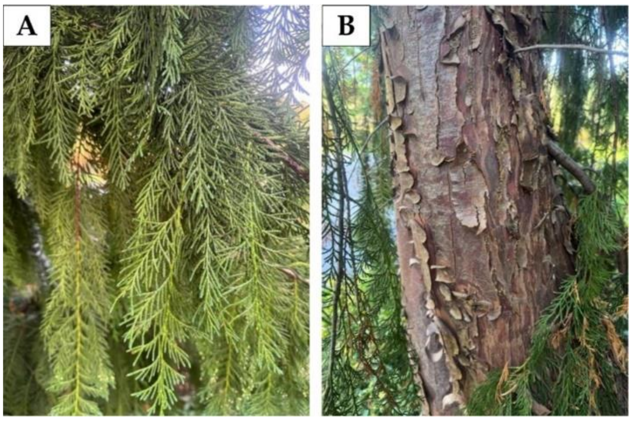

Figure 2. *Callitropsis nootkatensis* (D. Don) Oerst. A: Foliage. B: Bark.

Calocedrus decurrens (Torr.) Florin (incense cedar) is a tree growing up to 57 m tall, with cinnamon brown, fibrous bark, and leaves 3-14 mm long (Fig. 3) [11].

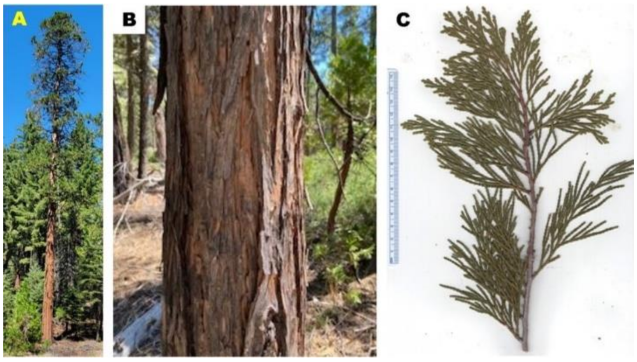

Figure 3. *Calocedrus decurrens* (Torr.) Florin (incense cedar). A: Habit. B: Bark, C: Foliage.

The tree is native to montane forests from Oregon south through California to northern Baja California, Mexico, and east to western Nevada (Fig. 4) [12]. The Mendocino Indian Tribe used *C. decurrens* as a gastrointestinal aid and a decoction of foliage was taken for stomach troubles [13]. The Paiute inhaled an infusion of the foliage to treat colds [14]. The Klamath people used the foliage of *C. decurrens* to prepare an herbal steam bath [15]. The essential oils of *C. decurrens* have been studied, including heartwood [16], bark [17], branch [18], and resin [17] essential oils, as well as foliar essential oils [17–22].

Sequoia sempervirens (D. Don) Endl. (California redwood) is a very large tree, probably the tallest tree

Figure 4. Native range of *Calocedrus decurrens* [23].

species in the world, growing up to around 110 m tall. The bark is reddish-brown, thick and fibrous, and deeply furrowed; the leaves are 1-30 mm long with stomata on both surfaces (Fig. 5). The natural range of the tree is confined to the coastal areas of California, from the Oregon border, south to Big Sur State Park [24]. In addition, the tree is a popular ornamental as well as an agroforestry resource. The Pomo people of California applied a poultice of heated leaves to treat earaches [25]. The bark [17], wood [26], and leaf [19, 27–30] essential oils of *S. sempervirens* have been analyzed. The major components in the leaf essential oils were generally sabinene (8.5-16.5%), limonene (8.7-10.4%), β-phellandrene (3.1-13.7%), α-pinene (6.3-10.4%), and myrcene (3.5-7.0%), while the wood was rich in α-pinene (27.4% and 21.0%), abietadiene (10.0% and 9.6%), *trans*-totarol (16.8% and 7.8%), 1-dodecanol (6.6% and 6.8%), and 1-tetradecanol (8.6% and 17.0%) [26].

There are two populations of *Thuja plicata* Donn ex. D. Don (western red cedar), a Coast Range-Cascade Range population from southeastern Alaska to northwestern California, and a Rocky Mountain

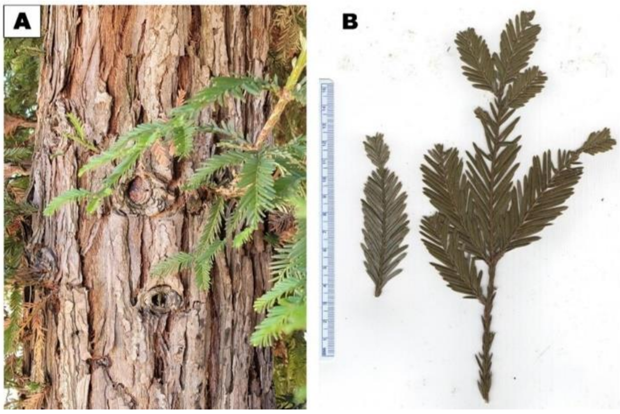

Figure 5. *Sequoia sempervirens* (D. Don) Endl. A: Leaves and bark. B: Scan of leaves.

Figure 6. Native range of *Thuja plicata* [3].

population ranging from British Columbia to Idaho and Montana (Fig. 6) [3, 31].

Western red cedar is a tree up to 50 m tall. The bark is reddish-brown or grayish-brown and fibrous with longitudinal fissures; the foliage is in sprays, 15-50 cm long and 5-15 cm wide (Fig. 7) [31].

There have been several investigations on the foliar essential oil compositions of *T. plicata*, which has been reviewed [32]. The essential oils were dominated by α-thujone (70.2 ± 8.0%), with lower concentrations of β-thujone (6.8 ± 1.8%), sabinene (3.5 ± 1.5%), and terpinen-4-ol (3.0 ± 0.8%).

Figure 7. *Thuja plicata* Donn ex. D. Don. A: Bark. B: Foliage.

As part of our ongoing interest in the essential oils of gymnosperms and aromatic and medicinal plants of the western United States, this study aimed to obtain and analyze the essential oils of four members of the Cupressaceae, *Callitropsis nootkatensis*, *Calocedrus decurrens*, *Sequoia sempervirens*, and *Thuja plicata*, using gas chromatographic methods. Although the foliar essential oil compositions of these four tree species have been previously reported, this study confirms and complements the previous investigations. Furthermore, to the best of our knowledge, this is the first report on the enantioselective gas chromatographic analyses of *C. nootkatensis*, *C. decurrens*, and *S. sempervirens*. Chiral GC-MS not only provides additional phytochemical characterization of these plants, but also serves to establish a baseline for the comparison of essential oils that may be commercialized and subject to adulteration.

2. Materials and methods

2.1. Plant identification and collection

Foliage from individual *C. nootkatensis* trees was collected from Mt. Hood, Oregon, USA, and Pine Lake, Washington, USA; *C. decurrens* samples were collected from Mt. Lassen, California, USA; *S. sempervirens* samples were collected from Paradise, California, USA; and *T. plicata* samples were collected from Tillamook State Forest, Oregon, USA. The collection details are summarized in Table 1. The trees were tentatively identified in the field by W.N. Setzer and 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 12 December 2024). Voucher specimens (Table 1) were deposited at the University of Alabama in Huntsville herbarium. Plant materials were stored frozen (–20 °C) until processed.

2.2. Hydrodistillation

The foliar essential oils of *C. nootkatensis*, *C. decurrens*, *S. sempervirens*, and *T. plicata* were obtained by hydrodistillation of the chopped fresh/frozen samples using a Likens-Nickerson apparatus with continuous extraction of the distillate with dichloromethane. The hydrodistillation details are summarized in Table 1.

2.3. Gas chromatographic analysis

The essential oils were analyzed by gas chromatography (GC-MS, and enantioselective GC-MS) as previously described [33, 34]. The gas chromatographic instrumentation and protocols are summarized in *Supplementary Table S1.* Retention indices were determined using the method of van den Dool and Kratz [35]. Essential oil components were determined by comparing with mass spectral fragmentation patterns and retention index values with those reported in the databases of Adams [36], Mondello [37], NIST20 [38], and Satyal [39].

2.4. Statistical analyses

Agglomerative hierarchical cluster analyses (HCA) were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). In each case, the major components were used for the analysis, dissimilarity was used to determine clustering based on the Euclidean distance, and Ward's method was used to define agglomeration. Analysis of variance was conducted by one-way ANOVA followed by Tukey's post hoc test using Minitab® 18 (Minitab Inc., State College, PA, USA). Differences at $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Callitropsis nootkatensis

Hydrodistillation of *C. nootkatensis* foliage from Oregon and Washington, USA, gave pale yellow essential oils in yields of 5.35% and 7.09% (w/w), respectively. The essential oils were analyzed by gas chromatography. A total of 115 compounds were identified in the two essential oil samples, which accounted for 99.2% and 99.8% of the total composition (*Supplementary Table S2, Supplementary Fig. S1*).

Table 1. Collection and hydrodistillation details of Cupressaceae foliar essential oils.

Plant sample	Voucher	Collection Date	Collection location	Mass foliage (g)	Mass essential oil (g)
Callitropsis nootkatensis, Oregon	WNS-Cnoot-0287	25 June 2024	45°18'18" N, 121°32'19" W, 1149 m asl	138.33	5.345
Callitropsis nootkatensis, Washington	WNS-Cnoot-1004	25 October 2024	47°35'13" N, 122°01'34" W, 116 m asl	141.10	7.085
Calocedrus decurrens #1	WNS-Cdec-5408	28 August 2024	40°21'04" N, 121°32'19" W, 1216 m asl	176.11	4.718
Calocedrus decurrens #2		28 August 2024	40°21'04" N, 121°32'18" W, 1213 m asl	210.02	4.796
Calocedrus decurrens #3		28 August 2024	40°21'04" N, 121°32'18" W, 1214 m asl	155.49	4.619
Sequoia sempervirens #1	WNS-Ssemp-1247	29 August 2024	39°46'06" N, 121°34'36" W, 603 m asl	112.86	3.0740
Sequoia sempervirens #2		29 August 2024	39°46'06" N, 121°34'36" W, 603 m asl	100.70	2.5025
Sequoia sempervirens #3		29 August 2024	39°46'06" N, 121°34'36" W, 603 m asl	126.14	3.6446
Thuja plicata #1	WNS-ThPl-0306	28 June 2024	45°18'51" N, 123°26'33" W, 580 m asl	110.77	3.539
Thuja plicata #2		28 June 2024	45°18'52" N, 123°26'32" W, 582 m asl	77.37	3.469

The major essential oil components are listed in Table 2. The major components in the Oregon and Washington samples were *α*-pinene (33.5% and 16.4%, predominantly (–)-*α*-pinene), (+)-δ-3-carene (28.3% and 30.2%), limonene (4.2% and 34.4%, predominantly (+)-limonene), and β-phellandrene (6.1% and 0.7%).

Cheng and von Rudloff analyzed five samples of *C. nootkatensis* foliage from Vancouver Island, British Columbia, Canada, and found *α*-pinene (34.1 ± 2.7%), δ-3-carene (30.0 ± 4.9%), and limonene (17.3 ± 6.5%) to be the most abundant components [7]. Furthermore, the enantiomers (–)-*α*-pinene, (+)-δ-3-carene, and (+)-limonene were identified [7]. Adams and co-workers [9] examined foliar essential oils collected from several locations in Alaska, British Columbia, Washington, and Oregon, and used the compositions to describe several chemotypes. Regardless of chemotype, each of the essential oil samples described by Adams et al. showed that *α*-pinene, δ-3-carene, and limonene were the abundant constituents. In order to place the compositions of this current study into the context of previous reports [7–9], an agglomerative hierarchical cluster analysis (HCA) was carried out (Fig. 8).

Four well-defined clusters were identified based on the HCA. Cluster 1 has relatively high, comparable concentrations of δ-3-carene (17.7 ± 2.8%), limonene (18.5 ± 4.0%), and β-phellandrene (18.5 ± 4.0%), but relatively low *α*-pinene concentration (10.6 ± 3.4%). Cluster 2 is made up of the *C. nootkatensis* sample from Washington in this study, as well as samples from Washington and Vancouver Island, British Columbia, from Adams et al. (2007) [8]. These samples are from geographically comparable locations, therefore, the similarities are not surprising. Cluster 2 is characterized by high limonene (37.2 ± 3.6%), but relatively low *α*-pinene (12.8 ± 4.1%) and δ-3-carene (19.3 ± 9.1%) concentrations. The sample of *C. nootkatensis* from Oregon in this study is found in Cluster 3, along with the samples from Cheng and von Rudloff [7] and Adams et al. [9]. High *α*-pinene (32.0 ± 3.9%) and δ-3-carene (28.9 ± 5.1%) concentrations describe this chemotype, along with relatively low limonene (3.4 ± 1.6%) and β-phellandrene (3.2 ± 0.7%) concentrations. Finally, Cluster 4 shows moderate *α*-pinene (18.2 ± 5.5%) and δ-3-carene (20.9 ± 4.9%) concentrations, as well as increased concentrations of limonene (8.5 ± 3.8%) and β-phellandrene (7.4 ± 4.7%). An ANOVA treatment based on the four major components in the four clusters was carried out. The differences between the four clusters are illustrated in

Table 2. Major chemical components of the foliar essential oils (percentages) and monoterpene enantiomeric distribution (percentages) of *Callitropsis nootkatensis*.

RIcalc[a]	RIdb[b]	Compounds	Major chemical components (%)	
			Oregon	Washington
933	933	α-Pinene	33.5	16.4
948	948	α-Fenchene	1.1	1.3
978	978	β-Pinene	3.7	1.5
989	989	Myrcene	4.2	2.7
1009	1008	δ-3-Carene	28.3	30.2
1030	1030	Limonene	4.2	34.4
1032	1031	β-Phellandrene	6.1	0.7
1086	1086	Terpinolene	2.7	2.9
RIcalc[c]	**RIdb[d]**	**Enantiomers**	**Enantiomeric distribution (%)**	
			Oregon	**Washington**
974	976	(−)-α-Pinene	84.5	79.1
982	982	(+)-α-Pinene	15.5	20.9
1027	1027	(+)-β-Pinene	2.6	7.1
1031	1031	(−)-β-Pinene	97.4	92.9
1046	1052	(+)-δ-3-Carene	100.0	100.0
nd[e]	na[f]	(−)-δ-3-Carene	0.0	0.0
1075	1073	(−)-Limonene	19.5	1.2
1079	1081	(+)-Limonene	80.5	98.8
1085	1083	(−)-β-Phellandrene	22.7	88.1
1089	1089	(+)-β-Phellandrene	77.3	11.9

a RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. b RIdb = Reference retention index values from the databases [36–39]. c RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. d RIdb = Retention index from our in-house database prepared using commercially available standards. [e] nd = compound not detected. [f] na = standard compound not available.

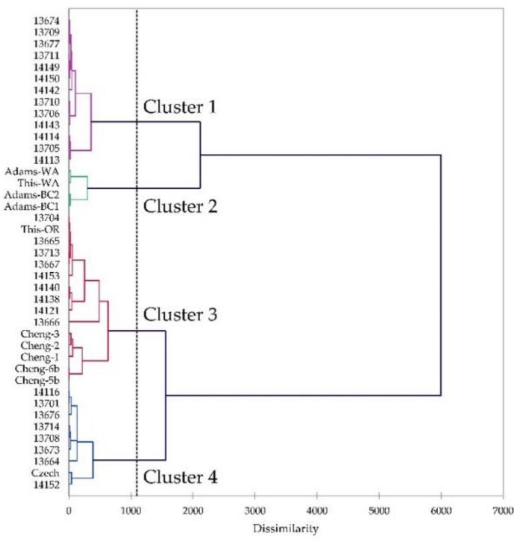

Figure 8. Dendrogram obtained from an agglomerative hierarchical cluster analysis (HCA) of *Callitropsis nootkatensis* essential oil samples. Samples with five numbers are from Adams et al. 2014 [9]; samples Adams-WA, Adams-BC1, and Adams-BC2 are from Adams et al. 2007 [8]; samples This-WA and This-OR are from this current study; samples Cheng-1, -2, -3, 5b, and 6b are from Cheng and von Rudloff 1970 [7]; the sample Czech is a cultivated sample from the Czech Republic [40].

Fig. 9. The ANOVA comparison supports the dissimilarities observed in the HCA.

3.2. Calocedrus decurrens

The foliar essential oils of *C. decurrens* were obtained as colorless liquids with a yield of 2.28-2.97% (w/w). The major components of the essential oils by gas chromatographic analyses are summarized in Table 3. A total of 74 compounds were identified in the essential oils, which accounted for 91.6%, 95.9%, and 93.9% of the total composition (*Supplementary Table S3, Supplementary Fig. S2*).

The essential oils were dominated by monoterpene hydrocarbons, including limonene (14.7-44.4%, predominantly (+)-limonene), δ-3-carene (11.0-28.6%, exclusively (+)-δ-3-carene), terpinolene (5.4-10.0%), and myrcene (5.5-6.7%), followed by the oxygenated monoterpenoid α-terpinyl acetate (4.9-6.8%).

The foliar essential oils of *C. decurrens* have been previously investigated. Samples of *C. decurrens* from northern California were analyzed by von Rudloff

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Table 3. Major chemical components of the foliar essential oils (percentages) and monoterpene enantiomeric distribution (percentages) of *Calocedrus decurrens*.

RI_{calc} [a]	RI_{db} [b]	Compounds	Major chemical components (%)		
			C.d. #1	*C.d.* #2	*C.d.* #3
934	933	α-Pinene	7.9	4.1	5.0
989	991	Myrcene	6.7	5.9	5.5
1011	1009	δ-3-Carene	28.6	14.3	11.0
1030	1030	Limonene	14.7	38.7	44.4
1086	1086	Terpinolene	10.0	6.4	5.4
1219	---	Methyl pin-2-en-8-oate [c]	2.1	1.9	3.6
1326	---	Pin-2-en-8-yl acetate [c]	3.2	-	-
1345	1346	α-Terpinyl acetate	5.0	6.8	4.9
RI_{calc} [d]	**RI_{db} [e]**	**Enantiomers**	**Enantiomeric distribution (%)**		
			C.d. #1	*C.d.* #2	*C.d.* #3
976	976	(−)-α-Pinene	82.8	53.1	48.0
982	982	(+)-α-Pinene	17.2	46.9	52.0
1049	1052	(+)-δ-3-Carene	100.0	100.0	100.0
nd [f]	na [g]	(−)-δ-3-Carene	0.0	0.0	0.0
1074	1073	(−)-Limonene	16.5	3.7	2.6
1078	1081	(+)-Limonene	83.5	96.3	97.4

[a] RI_{calc} = Retention index values determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. [b] RI_{db} = Reference retention index values from the databases [36–39]. [c] Identification tentative; reference RI value not available. [d] RI_{calc} = Retention index values determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. [e] RI_{db} = Retention index from our in-house database prepared using commercially available standards. [f] nd = compound not detected. [g] na = standard compound not available.

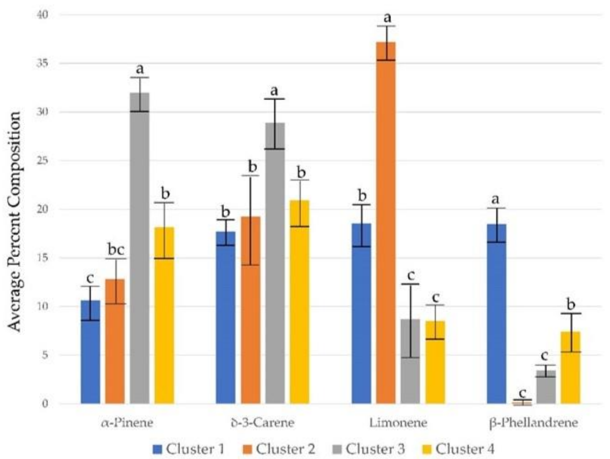

Figure 9. Average percentage compositions of the major components in *Callitropsis nootkatensis* essential oil clusters. For each component, bars with the same numbers are not significantly different ($p > 0.05$, ANOVA followed by Tukey's test).

[19] while Adams and co-workers analyzed samples of *C. decurrens* from southern Oregon, northern Oregon, and southern California [20]. Essential oils from cultivated samples of *C. decurrens* were reported from Poland [21], Corsica [18], Serbia [22], and Hungary [17]. The major components in these essential oils were consistently δ-3-carene, α-pinene, limonene, myrcene, terpinolene, and α-terpinyl acetate, but the variations in the major components can be visualized in a hierarchical cluster analysis (HCA, Fig. 10).

There are three obvious clusters observed in the HCA dendrogram. Cluster 1 has α-pinene (32.0 ± 9.0%) and δ-3-carene (24.4 ± 9.8%) as the major components, Cluster 2 is dominated by δ-3-carene (35.5 ± 7.0%), and Cluster 3 is dominated by limonene (30.4 ± 10.3%). ANOVA analysis further illustrates the differences in these three clusters (Fig. 11). It is apparent that the chemical compositions are not necessarily influenced by geographical location. Samples #2 and #3 from this study are grouped with the samples from Adams et al. and von Rudloff, which is not surprising; they were all from native populations. However, *C. decurrens* sample #1 is found in Cluster 2, which is also populated by samples from Poland, Hungary, Corsica, and Serbia.

3.3. *Sequoia sempervirens*

Foliage from *S. sempervirens* was obtained from three individual trees growing in Paradise, California, USA.

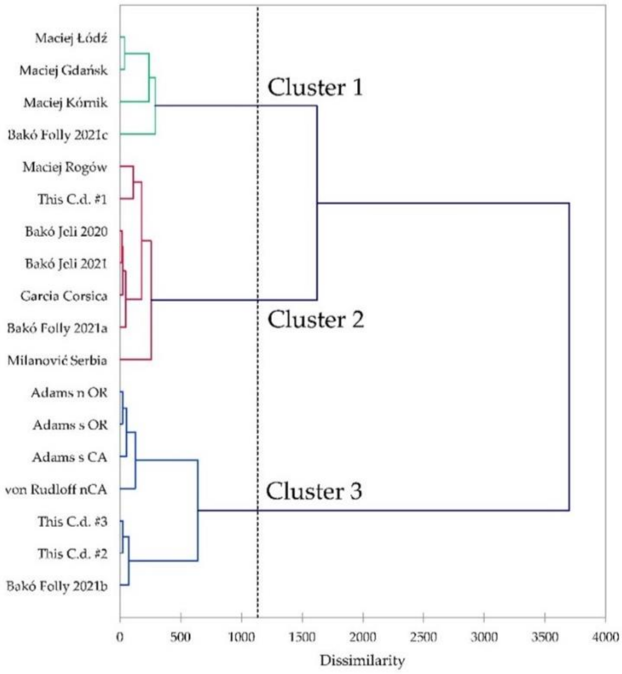

Figure 10. Dendrogram obtained by agglomerative hierarchical cluster analysis (HCA) of the major chemical components of *Calocedrus decurrens* foliar essential oils. Maciej = samples from Poland [21], Bakó = samples from Hungary [17], This = samples from this study, Garcia = sample from Corsica [18], Milanović = sample from Serbia [22], Adams = samples from Oregon and California [20], von Rudloff = sample from northern California [19].

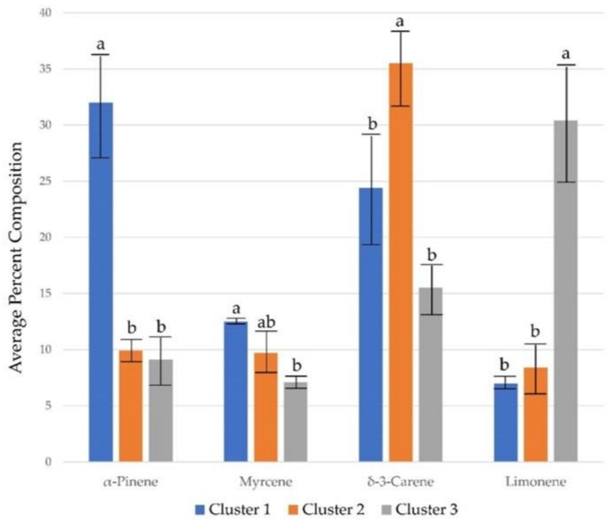

Figure 11. Average percentage compositions of the major components in *Calocedrus decurrens* essential oils. For each constituent, bars with the same numbers are not significantly different ($p > 0.05$, ANOVA followed by Tukey's test).

Hydrodistillation of the foliage gave colorless essential oils in yields of 2.72%, 2.49%, and 2.89% (w/w). Gas chromatographic analysis of the three essential oils identified 87 compounds, which accounted for 99.9%, 99.8%, and 98.5% of the total essential oil composition (*Supplementary Table S4, Supplementary Fig. S3*). The major components in the essential oils were α-pinene (10.1-18.8%), myrcene (3.4-6.7%), limonene (6.2-8.7%), β-phellandrene (3.5-7.0%), γ-terpinene (2.5-7.2%), terpinen-4-ol (3.9-5.3%), germacrene B (5.4-8.2%), γ-eudesmol (3.6-6.5%), and α-eudesmol (4.1-8.7%) (Table 4).

The foliar essential oils of *S. sempervirens* have been examined previously. Gregonis and co-workers analyzed *S. sempervirens* foliar essential oil from Oregon, USA, and found α-pinene (19.7%), limonene (10.1%), γ-terpinene (9.8%), and terpinen-4-ol (8.8%) to be the major components [27]. von Rudloff found a similar composition in a sample from northern California, USA, with α-pinene (24.9%) dominating, followed by β-phellandrene (9.9%), limonene (8.6%), γ-terpinene (6.2%), and terpinen-4-ol (5.6%), along with germacrene D (5.5%) [19]. Okamoto et al. carried out a seasonal foliage maturation study of *S. sempervirens* [28]. The major components in the late season (August) old foliage essential oil were α-pinene (23.2%), γ-terpinene (10.1%), β-phellandrene (10.0%), myrcene (6.8%), and germacrene D (4.9%). Bakó and co-authors analyzed the foliar essential oils from three collections from Hungary [17]. There was some variation in the composition of these samples, but the major components were α-pinene (7.7-22.0%), γ-terpinene (3.8-15.6%), terpinen-4-ol (4.4-9.8%), β-phellandrene (0.0-11.3%), α-terpinene (0.0-13.7%), sabinene (3.2-8.0%), and myrcene (2.6-6.5%). Thus, the foliar essential oil compositions of *S. sempervirens* are relatively similar, with the compositions composed largely of α-pinene (18.0 ± 5.8%), myrcene(5.2 ± 1.5%), limonene (6.6 ± 3.4%), β-phellandrene (7.1 ± 3.5%), γ-terpinene (7.7 ± 3.9%), and terpinen-4-ol (5.5 ± 2.4%).

As far as we are aware, this is the first report on the enantioselective analysis of *S. sempervirens* leaf essential oil. (–)-α-Pinene was the dominant enantiomer (91.6 ± 0.6%) in contrast to the wood essential oils [26] where the (+)-enantiomer was dominant (70.1% and 73.3%). (+)-Sabinene (96.1 ± 0.2%), (–)-β-pinene (88.9 ± 0.2%), and (+)-limonene (87.9 ± 2.1%) were also predominant. (+)-Limonene (90.0% and 97.3%) also dominated the wood essential oil. Interestingly, the dominant enantiomer of

Table 4. Major components (% of total) and enantiomeric distribution (enantiomer %) of chiral monoterpenoids in the foliar essential oils of *Sequoia sempervirens*.

RIcalc[a]	RIdb[b]	Compounds	Major chemical components (%)		
			S.s. #1	*S.s.* #2	*S.s.* #3
934	933	α-Pinene	18.8	16.4	10.1
972	972	Sabinene	4.4	3.5	2.2
977	978	β-Pinene	1.5	1.3	0.9
989	989	Myrcene	6.7	6.0	3.4
1017	1018	α-Terpinene	1.2	1.2	0.3
1030	1030	Limonene	8.5	8.7	6.2
1032	1031	β-Phellandrene	7.0	6.8	3.5
1058	1057	γ-Terpinene	7.2	6.3	2.5
1087	1087	Terpinolene	2.3	2.2	1.1
1183	1180	Terpinen-4-ol	4.1	3.9	5.3
1197	1195	α-Terpineol	0.9	1.0	1.4
1347	1346	α-Terpinyl acetate	2.4	2.5	3.8
1390	1390	*trans*-β-Elemene	1.4	1.6	2.1
1420	1418	(*E*)-β-Caryophyllene	1.1	1.3	1.7
1430	1430	γ-Elemene	2.2	2.7	3.2
1482	1483	Germacrene D	2.6	3.6	3.3
1549	1549	α-Elemol	1.6	1.7	2.9
1560	1557	Germacrene B	5.4	6.2	8.2
1632	1632	γ-Eudesmol	3.6	4.2	6.5
1655	1656	β-Eudesmol	1.6	1.8	5.5
1656	1655	α-Eudesmol	2.6	2.6	3.1
RIcalc[c]	**RIdb[d]**	**Enantiomers**	**Enantiomeric distribution (%)**		
			S.s. #1	*S.s.* #2	*S.s.* #3
972	976	(–)-α-Pinene	92.2	91.6	91.0
981	982	(+)-α-Pinene	7.8	8.4	9.0
1020	1021	(+)-Sabinene	95.9	96.4	96.0
1030	1030	(–)-Sabinene	4.1	3.6	4.0
1027	1027	(+)-β-Pinene	11.2	10.9	11.3
1031	1031	(–)-β-Pinene	88.8	89.1	88.7
1075	1073	(–)-Limonene	13.8	12.8	9.7
1079	1081	(+)-Limonene	86.2	87.2	90.3
1083	1083	(–)-β-Phellandrene	71.6	71.2	63.4
1088	1089	(+)-β-Phellandrene	28.4	28.8	36.6
1297	1297	(+)-Terpinen-4-ol	69.9	70.6	70.8
1301	1300	(–)-Terpinen-4-ol	30.1	29.4	29.2
1347	1347	(–)-α-Terpineol	66.4	67.8	68.2
1355	1356	(+)-α-Terpineol	33.6	32.2	31.8

[a] RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. [b] RIdb = Reference retention index values from the databases [36–39]. [c] RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. [d] RIdb = Retention index from our in-house database prepared using commercially available standards.

α-terpineol was the (–)-enantiomer in the leaf essential oil (67.5 ± 0.9%), whereas the (+)-enantiomer predominated in the wood essential oil (74.7% and 72.2%).

3.4. Thuja plicata

Two samples of *T. plicata* were collected from the Coastal Range of Oregon, USA. Hydrodistillation of the foliage gave pale-yellow essential oils in 3.20% and 4.48% (w/w) yield.

The major components in the essential oil are

Table 5. Major components (% of total) and enantiomeric distribution (enantiomer %) of chiral monoterpenoids in the foliar essential oils of *Thuja plicata*.

RIcalc[a]	RIdb[b]	Compounds	Major chemical components (%)	
			T.p. #1	*T.p.* #2
933	933	α-Pinene	1.1	1.4
972	971	Sabinene	3.2	2.6
989	989	Myrcene	1.5	1.8
1028	1030	Limonene	0.8	0.9
1056	1057	γ-Terpinene	1.1	1.1
1107	1105	α-Thujone	65.9	62.5
1120	1118	β-Thujone	10.2	12.1
1181	1180	Terpinen-4-ol	3.4	3.1
2319	---	15-Beyeren-19-yl acetate [c]	1.8	3.7
RIcalc[d]	**RIdb[e]**	**Enantiomers**	**Enantiomeric distribution (%)**	
			T.p. #1	*T.p.* #2
977	976	(–)-α-Pinene	2.8	1.8
981	982	(+)-α-Pinene	97.2	98.2
1018	1021	(+)-Sabinene	100.0	100.0
nd [f]	1030	(–)-Sabinene	0.0	0.0
1076	1073	(–)-Limonene	3.8	3.2
1082	1081	(+)-Limonene	96.2	96.8
nd [f]	1213	(+)-α-Thujone	0.0	0.0
1220	1222	(–)-α-Thujone	100.0	100.0
1229	1230	(+)-β-Thujone	100.0	100.0
nd [f]	na [g]	(–)-β-Thujone	0.0	0.0
1296	1297	(+)-Terpinen-4-ol	73.4	73.9
1300	1300	(–)-Terpinen-4-ol	26.6	26.1

[a] RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. [b] RIdb = Reference retention index values from the databases [36–39]. [c] Identification tentative; reference RI value not available. [d] RIcalc = Retention index values determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. [e] RIdb = Retention index from our in-house database prepared using commercially available standards. [f] nd = compound not detected. [g] na = standard compound not available.

listed in Table 5. A total of 92 compounds were identified in the essential oils accounting for 99.5% of the total composition (*Supplementary Table S5, Supplementary Fig. S4*).

The major components of the *T. plicata* foliar essential oils in this study are comparable to those in previous investigations [32, 34, 41–44]. The major components in this study were (–)-α-thujone (65.9% and 62.5%), (+)-β-thujone (10.2% and 12.1%), (+)-sabinene (3.2% and 2.6%), terpinen-4-ol (3.4% and 3.1%, predominantly (+)-enantiomer), and 15-beyeren-19-yl acetate (1.8% and 2.7%).

3.5. Enantiomeric distribution

In addition to this study, enantioselective GC-MS analyses have been carried out on other members of the Cupressaceae, including *Chamaecyparis lawsoniana* (A. Murray bis) Parl [32], *Juniperus horizontalis* Moench [45], *Juniperus occidentalis* Hook. [46], *Juniperus osteosperma* (Torr.) Little [47], *Juniperus scopulorum* Sarg. [45], as well as a previous study of *T. plicata* [32]. The enantiomeric distributions of Cupressaceae species are summarized in *Supplementary Table S6*.

With these data on the Cupressaceae, the distributions of chiral monoterpenoids in the family can be generalized and compared those of the Pinaceae [32–34, 48–53]. When observed, (–)-α-thujene is the exclusive enantiomer in both the Cupressaceae and Pinaceae. (–)-α-Pinene is variable in the Pinaceae as well as in the Cupressaceae; it depends on the genus (e.g., the (–)-enantiomer is major in *C. nootkatensis* and *S. sempervirens*, but the (+)-enantiomer is major in

Juniperus spp. and *T. plicata*). When observed, (–)-camphene is the major enantiomer in the Pinaceae, but depends on the genus in the Cupressaceae (e.g., (–)-camphene in *C. nootkatensis* and *S. sempervirens*, but (+)-camphene in *Juniperus* spp. and *T. plicata*). (–)-Sabinene is the major enantiomer in *Pinus* spp. [52], whereas (+)-sabinene dominates in *Tsuga heterophylla* [32] and (+)-sabinene seems to dominate in the Cupressaceae. (–)-β-Pinene is the major enantiomer in the Pinaceae, but varies in the Cupressaceae, depending on the genus, (+)-enantiomer in *Juniperus* spp. and *T. plicata*, but (–)-enantiomer in *C. nootkatensis*, and *C. decurrens*. When observed, (+)-δ-3-carene was the exclusive enantiomer in both the Cupressaceae and the Pinaceae. *α*-Phellandrene was nearly racemic in *S. sempervirens*, but variable in *Abies* spp. [53]. (+)-*α*-Phellandrene was the dominant enantiomer in *Pinus monticola* [52] and *Tsuga heterophylla* [32]. (+)-Limonene is the major enantiomer in the Cupressaceae, while (–)-limonene predominates in the Pinaceae. Although variable, (–)-β-phellandrene generally predominates in both the Cupressaceae and Pinaceae. When observed (–)-*α*-thujone and (+)-β-thujone were the only enantiomers observed. (–)-Linalool was the major enantiomer in *C. decurrens* (Cupressaceae) as well as in *P. edulis* (Pinaceae) [51], but (+)-linalool was major in *J. horizontalis* and *J. scopulorum*. When observed, (+)-*cis*-sabinene hydrate and (+)-*trans*-sabinene hydrate were the major enantiomers in the Cupressaceae. Only (+)-camphor was observed in *Juniperus* spp., while (–)-camphor was observed in *Abies grandis* [53]. (–)-Terpinen-4-ol was the major enantiomer in *Abies* spp. [53] and *Pinus* spp. [52], but (+)-terpinen-4-ol is generally predominant in most species of the Cupressaceae. Likewise, (–)-*α*-terpineol predominated in *Abies* spp. [53] and *Pinus* spp. [52], but was variable in the Cupressaceae, with the (–)-enantiomer predominating in *C. nootkatensis* and *S. sempervirens*, and (+) in other genera of the family. (–)-Borneol seems to predominate in both the Cupressaceae and Pinaceae, but note that the retention index values are very similar. When observed, (+)-verbenone (*Juniperus* spp.) and (–)-piperitone (*C. nootkatensis* and *C. decurrens*) were the dominant enantiomers in the Cupressaceae.

4. Conclusions

In this study, the foliar essential oils of *Callitropsis nootkatensis*, *Calocedrus decurrens*, *Sequoia sempervirens*, and *Thuja plicata* have been obtained and analyzed by GC-MS and chiral GC-MS. The compositions of these Cupressaceae members are comparable to previously published works and serve to corroborate the volatile phytochemistry of these species. Based on the essential oil compositions, four chemotypes of *C. nootkatensis* and three chemotypes of *C. decurrens* have been identified. In addition, the enantiomeric distributions of chiral monoterpenoids in these species have been determined and identified some trends in the family. (+)-Sabinene (97.0 ± 9.2%) seems to dominate in the Cupressaceae while (–)-sabinene is dominant in the Pinaceae. When observed, (+)-δ-3-carene is the exclusive enantiomer in both the Cupressaceae and the Pinaceae. (+)-Limonene (95.0 ± 4.8%) was the major enantiomer in the Cupressaceae, while (–)-limonene predominated in the Pinaceae. (–)-β-Phellandrene (87.6 ± 16.3%) generally predominates in both the Cupressaceae and the Pinaceae. (+)-Terpinen-4-ol (68.1 ± 7.7%) is generally predominant in most species of the Cupressaceae. (–)-Borneol seems to predominate in both the Cupressaceae (100%) and Pinaceae (99.6%). Several monoterpenes show variability in their enantiomeric distribution depending on the genus. The enantioselective analyses of members of the Cupressaceae serve as additional phytochemical characterizations of members of the family. Furthermore, enantiomeric distribution information may be useful if essential oils are commercialized (e.g., biological activities of the essential oils, detection of contamination or adulteration). However, additional research on these and other members of the Cupressaceae is necessary to confirm the trends observed in the present study.

Supplementary materials

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

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

Table S2. Chemical composition of the foliar essential oils of Callitropsis nootkatensis.

Table S3. Chemical composition of the foliar essential oils of Calocedrus decurrens.

Table S4. Chemical composition of the foliar essential oil of Sequoia sempervirens.
Table S5. Chemical composition of the foliar essential oil of Thuja plicata.
Table S6. Enantiomeric distributions (averages) of chiral monoterpenoids in members of the Cupressaceae.
Figure S1. Gas chromatograms of Callitropsis nootkatensis foliar essential oils.
Figure S2. Gas chromatograms of Calocedrus decurrens foliar essential oils.
Figure S3. Gas chromatograms of Sequoia sempervirens foliar essential oils.
Figure S4. Gas chromatograms of Thuja plicata foliar essential oils.

Supplementary material to this article can be found online at https://www.currentsci.com/images/articlesFile/supplementary.1757952346.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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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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