JEOPC, 3(2), 95-104, 2025
https://doi.org/10.58985/jeopc.2025.v03i02.69
ISSN (Print): 2958-9630

Journal of Essential Oil & Plant Composition

Research Article

Comparison of *Abies grandis* hydrosol samples extracted with different organic solvents employing gas chromatography/mass spectrometry

Lincoln Vance Mitchell[1], **Ryland Randall Anderson**[1], **Christopher Ryan Bowerbank**[2] and **David Clark Collins**[1*]

1. Department of Chemistry and Biochemistry, Brigham Young University—Idaho, Rexburg, Idaho 83460, United States.
2. Young Living Essential Oils, D. Gary Young Research Institute, Lehi, Utah, 84043, United States.

Article Information
Received: 05 June 2025
Revised: 24 June 2025
Accepted: 27 June 2025
Published: 10 July 2025

Academic Editor
Prof. Dr. Radosław Kowalski

Corresponding Author
Prof. Dr. David Clark Collins
E-mail: collinsd@byui.edu
Tel.: +1-208-496-7707

Keywords
Hydrosol, hydrolat, hydrolate, floral water, solvent extraction, grand fir, *Abies grandis*, gas chromatography/ mass spectrometry.

Abstract

The chemical profiles of the hydrosol of *Abies grandis* (i.e., grand fir) produced via steam distillation were compared using extractions with various organic solvents. This study evaluated the variation in the hydrosol volatile composition profiles due to the solvent employed for the isolation of these components. Extractions were performed using petroleum ether, hexane, ethyl acetate, chloroform, and dichloromethane, resulting in the identification of 14, 15, 13, 20 and 33 compounds, respectively. A total of 10 compounds were common to all extracts. For all extracts, >80% of all hydrosol signals were associated with α-terpineol (~63%), borneol (~13%) and terpinen-4-ol (~4.5%). The aqueous hydrosol was also directly analyzed (13 compounds) and its chemical profile was compared with the profiles of the hydrosol produced by various organic solvents. The aqueous hydrosol resulted in >13% of its total signal associated with the polar compounds hexanoic acid, *p*-menthan-1,8-diol, and *p*-menthane-3,8-diol, which was significantly greater than that of any hydrosol extract. Dichloromethane resulted in the greatest number of compounds identified and more than 6 times the total signal of any other solvent. The results suggest that no single solvent produces a complete profile of all components present in the *Abies grandis* hydrosol and strongly suggest the extraction with only one solvent can be ineffective at determining a comprehensive constituent profile of an aqueous hydrosol solution.

1. Introduction

Hydrosols have gained in popularity as the essential-oils market has continued to expand, resulting in significant investigations into their composition, biological properties, and uses [1]. Hydrosol also referred to as herbal waters, floral waters, or hydrolates are byproducts of essential oil steam distillation and contain many of the same compounds found in essential oils. A significantly greater amount of hydrosol is produced during steam distillation than the essential oil. Historically, hydrosols have been used as herbal remedies [2]. Modern hydrosol usage is largely focused on the cosmetics industry due to the presence of bioactive compounds (i.e., terpenes and phenols), which help soften the skin and reduce inflammation [3]. Additional research has been conducted to investigate their potential as antioxidants [4], natural food sanitizers [5], and for other uses [6-8]. The analysis of hydrosols and their compounds has been the subject of many studies that have analyzed the hydrosols of various essential-oil-

bearing plants [1, 9-12]. Although hydrosols share compounds with their corresponding essential oils, they also contain compounds not found in the essential oil [13] due to the contrasting aqueous environment of the hydrosol compared to the relatively nonpolar, organic essential oil containing primarily terpenes and terpenoids. The hydrosol used in this study was derived from the plant *Abies grandis* (i.e., grand fir). *Abies grandis* is native to North America and has been used in the cosmetics industry and papermaking. Previous studies on *Abies grandis* have been conducted due to its ability to increase the synthesis of monoterpenes in response to wounding [14, 15]. Analytical studies have been conducted on the essential oils produced from other species within the *Abies* genus [3, 16]. There are few studies analyzing the composition of *Abies grandis* essential oil [17] and no known studies have assessed the composition of *Abies grandis* hydrosol. As expected, the *Abies grandis* hydrosol primarily contained terpenes and terpenoids (e.g., α-terpineol, borneol, limonene, verbenone, camphor, and terpinen-4-ol). This is consistent with analyses performed on other trees within the *Abies* genus [3, 17, 18]. In this study, the hydrosol was analyzed via gas chromatography/mass spectrometry (GC-MS). A common precaution when employing GC-MS is to limit the direct injection of aqueous samples onto a GC column to prevent potential oxidation and/or physical damage to the GC column and sample flashback due to the greater expansion of the aqueous sample compared to mostly organic-based samples. Hence, liquid-liquid extraction techniques with an organic solvent are often used to liberate the organic compounds from the hydrosol to analyze their content. The solvent reduces the concern associated with injecting aqueous samples onto the GC column and can also be used to concentrate the hydrosol components prior to analysis, increasing the ability to characterize the hydrosol. Most studies employ only one organic solvent to isolate the hydrosol components, assuming that the extracted components are representative of the actual hydrosol composition [19-21]. The solvent used varies significantly among the literature (e.g., cyclohexane, *n*-hexane, petroleum ether, benzene, ethyl acetate, chloroform, diethyl ether, methylene chloride, and dichloromethane) [1]. This variability raises concerns regarding the "true" hydrosol composition. The primary focus of this study was to provide insights into this specific concern using *Abies grandis* hydrosol as a test case. The chemical profiles from each solvent extraction were compared with each other and with a direct analysis of the aqueous hydrosol.

2. Materials and methods

2.1. Reagent and solutions

Chloroform (99.8% purity), dichloromethane (99.5% purity), ethyl acetate (99.5% purity), hexane (98.5% purity), and petroleum ether were obtained from Fisher Chemical (Waltham, MA USA). The *Abies grandis* hydrosol was obtained from the Young Living Highland Flats Tree Farm and Distillery (Naples, ID, USA), where all required good practices were followed for the harvesting and distillation of wood chips using a proprietary steam distillation process.

2.2. Instrumentation and conditions

GC-MS data were collected using a Hewlett Packard 5890 Series II gas chromatograph employing a Hewlett Packard 5972 Series A mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Samples were injected into the gas chromatograph using an Agilent 6890 Series autoinjector.

The following gas chromatographic conditions were employed for all separations: Restek (Bellefonte, PA, USA) 60-m Rxi-1ms column, 0.25 mm i.d., 1 μm film thickness, helium carrier gas, split ratio 5:1, injection volume 0.5 μL, injector temperature 285 °C, 1 mL/min flow rate, initial temperature 40 °C, hold 0.5 min, ramp rate of 22.0 °C/min to 75 °C, ramp rate of 2.3 °C/min to 190 °C, then 12 °C/min to 285 °C, hold 3 min, ion source and transfer line 280 °C, and total time of 70 min.

The following mass spectrometric conditions were employed for all detections: solvent delay of 17 min, mass range from 25.0 to 400.0 *m/z*, sampling threshold of 2, and two scans per second. These conditions resulted in a conservative detection limit of ~400 fg for the GC-MS when analyzing essential oil compounds.

2.3. Solvent extraction and blanks

Extractions were performed using a standard liquid-liquid extraction procedure employing a 125 mL separatory funnel with a solvent/hydrosol ratio of 1:25

(v/v). The more polar solvent, ethyl acetate, had a ratio of 1:20 (v/v). Small volumes of organic solvents were used with larger volumes of hydrosols to increase the concentrations of the hydrosol components within the extracts. Blanks of each solvent were analyzed using the same chromatographic conditions to ensure that potential contaminants in the organic solvents were not misidentified as components of the hydrosol. Although most contaminants eluted during the solvent delay, i.e., before any significant hydrosol components, others eluted with significant intensity within the separation window (e.g., bromobenzene is found to varying extents in most solvents at 19.75 min and is most prominent in dichloromethane).

2.4. Data collection and analysis

The area-percent reports from each GC-MS chromatogram were downloaded and placed into a spreadsheet. Notable peaks were verified manually using the mass spectra of each compound and searched individually against the NIST mass spectral library 2014 (National Institute of Standards and Technology, Gaithersburg, MD, USA). Mass spectral backgrounds were subtracted when necessary from small chromatographic peak signals to improve the library match quality. Peaks were compared across the extraction profiles. After verification of the most prominent peaks, the data from the area percent reports were used to determine the relative abundance, which resulted in the removal of contaminant and solvent peaks. This was accomplished by taking the area of each prominent peak and dividing it by the summed total area of all prominent peaks, i.e., all small peak areas of insignificance were removed. The resulting percentages were then indicative of the relative abundance of each compound within the specific hydrosol extract.

3. Results and discussion

The ten compounds that were common to all solvent extractions are listed in Table 1. These compounds were required to have at least a quality match of 80 against the NIST library to be included in the list. The percentages were calculated as a function of the individual peak areas divided by the total area of all compounds. Multiple replicates of each extraction were performed (n = 3-5), and the values were averaged for each solvent type. A moderately consistent % RSD of <5% was assessed for percent relative abundances. The most abundant of these compounds in every extraction was α-terpineol (~63%). The next most abundant compounds were borneol (~13%) and terpinen-4-ol (~4.5%). These three compounds comprise the majority of hydrosol signals (i.e., >80%). Other common compounds include benzaldehyde, *p*-cymene, limonene, fenchol, camphor, verbenone, and bornyl acetate.

3.1. Petroleum ether

The extraction obtained using petroleum ether, with a polarity index of 0.1, contained 14 compounds with a quality match of at least 80. These compounds are listed in Table 2. Compounds extracted by petroleum ether but not by other solvents included: α-campholenal, isoborneol, citronellol, and *p*-cymen-7-ol with relative abundances of 1.63%, 1.77%, 0.82%, 0.19%, respectively. There were also differences in the peak areas of the same compounds when extracted by different solvents. As expected, the relative values of peak area and abundance shared the same trend, i.e., the compound α-terpineol had the highest peak area of 1.38E8 followed by borneol (3.04E7) and terpinen-4-ol (1.04E7). These three compounds had the highest relative abundance within every extraction profile, but the differences in the absolute peak areas for these compounds (and others) between extraction profiles should be noted. Although all compounds extracted by petroleum ether were also extracted with chloroform and dichloromethane, the resulting absolute peak areas for the compounds in chloroform and dichloromethane were approximately 3 and 18 times greater than those in petroleum ether, respectively.

3.2. Hexane

Hexane, similar to petroleum ether, is the most nonpolar of the solvents used with a polarity index of 0.1. When hexane was used to extract the organic compounds from the hydrosol, 15 distinguishable compounds were found. These compounds are listed in Table 3. The compounds with the highest abundance were once again α-terpineol (64.45%), borneol (13.99%), and terpinen-4-ol (4.96%).

Many of the other compounds extracted are nonpolar and not observed in extractions performed with more

Table 1. Compounds universally extracted from the hydrosol by all solvents investigated (peak area % of total).

Compounds	Ret. index	CAS #	Chloroform (%)	DCM (%)	Ethyl acetate (%)	Hexane (%)	Petroleum ether (%)
Benzaldehyde	952	100-52-7	0.34	0.30	0.47	0.23	0.29
p-Cymene	1025	99-87-6	0.65	0.31	0.33	0.68	0.73
Limonene	1034	138-86-3	1.10	1.15	1.33	1.04	1.58
Fenchol	1110	1632-73-1	2.87	2.71	3.06	3.54	3.36
Camphor	1142	464-48-2	1.52	1.40	1.55	1.65	1.62
Borneol	1160	10385-78-1	13.10	12.26	13.74	13.92	14.17
Terpinen-4-ol	1173	562-74-3	4.36	4.77	4.37	4.94	4.85
α-Terpineol	1183	8000-41-7	63.66	60.97	61.69	64.12	64.57
Verbenone	1204	80-57-9	1.28	1.30	1.23	0.88	1.00
Bornyl acetate	1275	5655-61-8	2.14	2.51	2.39	3.94	2.41

Table 2. Compound extraction profile of petroleum ether.

Compounds	Ret. index	Ret. time (min)	Total area (%)
Benzaldehyde	952	20.60	0.29
p-Cymene	1025	25.70	0.73
Limonene	1034	26.40	1.58
α-Campholenal	1098	30.53	1.63
Fenchol	1110	31.63	3.37
Camphor	1142	33.30	1.63
Isoborneol	1154	24.59	1.77
Borneol	1160	35.10	14.19
Terpinen-4-ol	1173	35.78	4.86
α-Terpineol	1183	36.48	64.67
Verbenone	1204	37.24	1.00
Citronellol	1212	38.24	0.82
p-Cymen-7-ol	1275	42.14	0.19
Bornyl acetate	1275	42.74	2.41

Table 3. Compound extraction profile of hexane.

Compounds	Ret. index	Ret. time (min)	Total area (%)
Benzaldehyde	952	20.53	0.23
p-Cymene	1025	25.65	0.68
Limonene	1034	26.29	1.05
Acetophenone	1038	27.12	0.23
Fenchol	1110	31.56	3.55
Camphor	1142	33.22	1.66
Isoborneol	1154	34.51	1.76
Borneol	1160	35.02	13.99
Terpinen-4-ol	1173	35.71	4.96
α-Terpineol	1183	36.40	64.45
Verbenone	1204	37.10	0.88
Citronellol	1212	38.13	0.87
Piperitone	1256	39.98	0.64
Bornyl acetate	1275	42.66	3.96
Cubenol	1610	59.50	0.88

polar solvents, such as ethyl acetate, which will be assessed in the next section. The compounds in hexane included acetophenone (0.23%), piperitone (0.64%), and cubenol (0.88%). Interestingly, the absolute peak areas of the universally extracted compounds (in addition to the percent abundance) were similar among hexane, petroleum ether, and ethyl acetate. For example, α-terpineol had absolute peak areas of 1.38E8, 1.45E8, and 1.30E8 for petroleum ether, hexane, and ethyl acetate, respectively. This implies that these solvents have similar extraction affinities for the most abundant hydrosol compounds. Although hexane had a similar polarity index to that of petroleum ether, the only extracted compounds in common with petroleum ether (beyond those listed in Table 1) were isoborneol and citronellol. Both compounds were found in similar abundances in each non-polar solvent extraction.

3.3. Ethyl acetate

Ethyl acetate, with a polarity index of 4.4, is the most polar solvent used for extraction. Ethyl acetate extracted 13 identifiable compounds, as shown in Table 4. Compounds of note include acetophenone (0.35%), α-campholenal (1.38%), *p*-menthane-1,8-diol (5.1%). Ethyl acetate showed a preference for extracting more polar compounds, such as the alcohol *p*-menthane-1.8-diol. Furthermore, *p*-menthane-1,8-diol showed a relatively high abundance (5.10%) in ethyl acetate, but was completely absent from hexane and petroleum ether extracts and was found only at lower percentages in chloroform (1.51%) and dichloromethane (0.57%). In addition, piperitone extracted by hexane, dichloromethane, and

Table 4. Compound extraction profile of ethyl acetate.

Compounds	Ret. index	Ret. time (min)	Total area (%)
Benzaldehyde	952	20.57	0.47
p-Cymene	1025	25.64	0.33
Limonene	1034	26.29	1.33
Acetophenone	1038	27.14	0.35
α-Campholenal	1098	30.45	1.38
Fenchol	1110	31.55	3.06
Camphor	1142	33.21	1.55
Borneol	1160	35.01	13.74
Terpinen-4-ol	1173	35.69	4.37
α-Terpineol	1183	36.39	61.69
Verbenone	1204	37.10	1.23
Bornyl acetate	1275	42.65	2.39
p-menthane-1,8-diol	1279	43.08	5.10

chloroform was undetectable in ethyl acetate.

This high abundance of *p*-menthane-1,8-diol found in ethyl acetate suggests that the compound is a significant component of the *Abies grandis* hydrosol, however it is unable to be extracted, in representation of the hydrosol composition, employing nonpolar solvents such as hexane and petroleum ether.

Because ethyl acetate was the most polar solvent used, it proved troublesome in the liquid-liquid extraction. When mixed with the hydrosol, it was observed that much of the solvent was miscible with the aqueous hydrosol and did not result in a distinct layer that could be separated. This resulted in only half of a milliliter of organic extract produced. All other solvents produced 2 mL of extract upon addition of 2 mL of the solvent. Based on the theory of liquid-liquid extraction at the relatively small volumes of organic solvent employed, this could have resulted in a slight (e.g., 10-20%) decrease in the absolute concentrations of the extracted compounds. However, it likely had little effect on the percent relative abundances, as demonstrated by the similar values for α-terpineol (61.69%), borneol (13.74%), and terpinen-4-ol (4.37%) in all other solvents. Small volumes of organic solvents were used with larger volumes of hydrosols to increase the concentrations of the hydrosol components within the extracts.

3.4. Chloroform

Chloroform, with a polarity index of 4.1, is the second most polar solvent used and extracted 20 identifiable compounds from the hydrosol, as shown in Table 5.

Chloroform had the second most diverse compound extraction profile among all the solvents used in this study; however, none of the compounds extracted by chloroform were exclusive to chloroform. The relative abundance of the most prevalent compounds is consistent with that of other solvent extractions. The compound α-terpineol had the highest relative abundance (63.66%) followed by borneol (13.10%) and then terpinen-4-ol (4.36%). Although the relative abundance of these compounds is similar to the other solvent extractions, as mentioned previously, the peak areas of the most abundant compounds are much larger in chloroform than in ethyl acetate, petroleum ether, and hexane. The peak area of α-terpineol when extracted by chloroform was 4.32E8, which was approximately three times greater than that of α-terpineol in ethyl acetate, despite the similarity in the polarity indices of the two solvents. This trend was also observed for borneol and terpinen-4-ol, with peak areas of 8.89E7 and 2.96E7, respectively, when extracted with chloroform. This suggests that chloroform, is more efficient and a better solvent for extracting these and other compounds from *Abies grandis* hydrosol for improved sensitivity than petroleum ether, hexane, and ethyl acetate.

Table 5. Compound extraction profile of chloroform.

Compounds	Ret. index	Ret. time (min)	Total area (%)
2-Acetylfuran	886	20.33	0.17
Benzaldehyde	952	20.60	0.34
Lavender lactone	997	24.72	0.43
p-Cymene	1025	25.67	0.65
Limonene	1034	26.32	1.10
Acetophenone	1038	27.15	0.33
α-Campholenal	1098	30.48	1.41
Fenchol	1110	31.58	2.87
Camphor	1142	33.24	1.52
Isoborneol	1154	34.52	1.51
Borneol	1160	35.06	13.10
Terpinen-4-ol	1173	35.73	4.36
α-Terpineol	1183	36.51	63.66
Verbenone	1204	37.15	1.28
Citronellol	1212	38.17	0.97
Piperitone	1256	39.99	0.78
p-Cymen-7-ol	1275	42.05	0.29
Bornyl acetate	1275	42.66	2.14
p-Menthane-1,8-diol	1279	43.06	1.51
Cubenol	1610	59.48	0.48

3.5. Dichloromethane

Dichloromethane (DCM), with a polarity index of 3.1 had the greatest number of extracted and identifiable compounds from the hydrosol compared to all other solvents. A total of 33 compounds were identified and listed in Table 6. While the most abundant compound was still α-terpineol, its relative abundance was slightly lower than that of all other solvents at 60.97%. This is likely due to the significantly larger number of compounds found in DCM, which added to the total summed peak area and reduced the percent

Table 6. Compound extraction profile of dichloromethane.

Compounds	Ret. index	Ret. time (min)	Total area (%)
2-Acetylfuran	886	17.56	0.13
1-Methoxy-1-buten-3-yne	905	18.02	0.02
5-Methyl furfural	934	20.32	0.15
Benzaldehyde	952	20.60	0.30
Lavender lactone	997	24.75	0.36
p-Cymene	1025	25.70	0.31
Limonene	1034	26.35	1.15
Acetophenone	1038	27.18	0.31
γ-Terpinene	1045	28.05	0.14
p-Cymenene	1066	29.66	0.13
2-Carene	1010	30.07	0.55
α-Campholenal	1098	30.52	1.25
cis-Thujone	1102	30.73	0.19
Fenchol	1110	31.65	2.71
Nopinone	1129	32.33	0.16
Camphor	1142	33.29	1.40
Cyclohexanol, 5-methyl-2-(1-methyl)	1157	33.64	0.25
4-Ethyl-phenol	1160	33.90	0.19
Camphene Hydrate	1148	34.08	0.57
Isoborneol	1154	34.59	1.50
Borneol	1160	35.24	12.26
Terpinen-4-ol	1173	35.85	4.77
α-Terpineol	1183	36.87	60.97
Verbenone	1204	37.39	1.30
Citronellol	1212	38.26	1.04
Piperitone	1256	40.09	0.62
Phellandral	1252	41.68	0.23
Thymol	1269	41.97	0.20
p-Cymen-7-ol	1275	42.15	0.31
Bornyl Acetate	1275	42.74	2.51
p-Menthane-1,8-diol	1279	43.15	0.57
trans-Calamenene	1529	56.23	0.01
Cubenol	1610	59.51	0.60

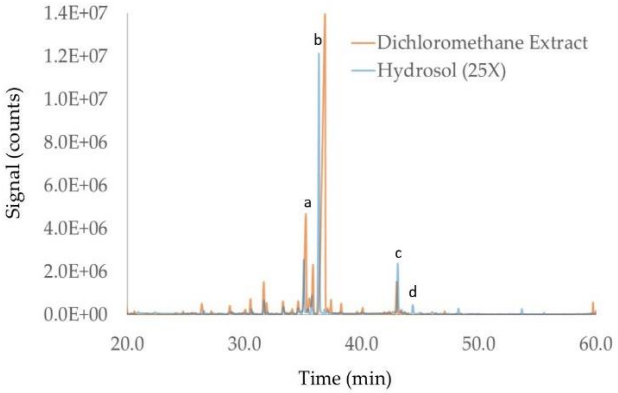

Figure 1. Overlaid chromatograms of hydrosol (25X for direct comparison) and dichlormethane extract. Selected peak identities are as follows: a) borneol, b) α-terpineol, c) *p*-menthane-1,8-diol, and d) *p*-menthane-3,8-diol.

abundance. This was also observed for borneol at its lowest abundance of 12.26%. The DCM extract also contained *p*-menthane-1,8-diol, albeit at the abundance of only 0.57%. This is most likely due to the polarity of DCM being less than chloroform and ethyl acetate, which both showed higher abundance of the diol. Not only did DCM have the largest diversity of compounds (with 13 of them being identifiable only in the hydrosol when extracted with DCM), but the absolute peak areas of the extracted compounds were also significantly larger than those of any other solvent extractions. This is most easily seen when observing the absolute peak area of α-terpineol, which was found to be 2.49E9. This was six times larger than the next closest α-terpineol peak found in chloroform.

3.6. Direct analysis of aqueous hydrosol

A direct injection of the aqueous hydrosol (i.e., 5 µL instead of 0.5 µL) was also performed resulting in the identification of 13 compounds (Table 7). The compounds and their relative abundances found in the hydrosol closely mirrored the solvent extraction profiles. One significant deviation from the extraction profiles was the presence of two diols, i.e., *p*-menthane-1,8-diol and *p*-menthane-3,8-diol; with relative abundances of 11.50 and 2.02%, respectively, and hexanoic acid (0.26%). The compound *p*-menthane-1,8-diol was only found in the more polar extracts of dichloromethane, chloroform, and ethyl acetate with relative abundances of 0.57, 1.51 and5.10%, respectively. The compounds *p*-menthane-3,8-diol and hexanoic acid were exclusively found in

Table 7. Compound profile of hydrosol.

Compounds	Ret. index	Ret. time (min)	Total area (%)
Hexanoic acid	975	21.23	0.26%
p-Cymene	1025	26.17	0.21%
Limonene	1034	26.83	0.80%
Fenchol	1110	31.96	2.79%
Camphor	1142	33.63	1.79%
Isoborneol	1154	34.88	1.41%
Borneol	1160	35.36	12.76%
Terpinen-4-ol	1173	36.05	3.92%
α-Terpineol	1183	36.65	60.20%
Verbenone	1204	37.39	0.72%
Citronellol	1212	38.45	0.64%
p-Menthane-1,8-diol	1279	43.39	11.50%
p-Menthane-3,8-diol	1301	44.67	2.02%

The hydrosol. Fig. 1 shows the overlaid chromatograms of dichloromethane solvent extraction and the hydrosol, visually highlighting the differences in composition.

3.7. Summary

Table 8 summarizes all solvents employed in this study, the compounds extracted with each, and their relative percent abundances. The results corroborate the published findings for the chemicals common to *Abies grandis* and other related species [3, 5, 8, 17]. As mentioned earlier, dichloromethane not only resulted in the greatest number of extracted compounds but also had the highest recovery and absolute peak area. It is surmised that dichloromethane would be the best solvent to use for both qualitative and quantitative extractions of *Abies grandis* hydrosol. In addition, the relative abundance of many compounds in the other organic solvent extracts varied significantly compared to that in the dichloromethane extract (e.g., bornyl acetate and *p*-menthane-1,8-diol).

When comparing all solvent extracts to the direct analysis of the hydrosol, the hydrosol showed a significantly higher relative percent abundance for *p*-menthane-1,8-diol, i.e., 11.50% compared to 0.57, 1.51, and 5.10% for dichloromethane, chloroform, and ethyl acetate, respectively. It is expected that due to the polar nature of water, the more polar organic compounds would inherently partition more into the water than into the essential oil during steam distillation. This suggests that the use of less polar organic solvents may not result in the adequate extraction of organic constituents from hydrosols. Although logically apparent, such direct acknowledgement is not common to hydrosol analyses [1, 4, 6, 7, 9-13]. This was observed to a limited degree in this study, with the significantly increased percent abundance of *p*-menthane-1,8-diol in the direct analysis of the hydrosol and the complete absence of *p*-menthane-3,8-diol and hexanoic acid from all solvent extracts, despite these compounds being found with a significant percent abundance in the hydrosol. The use of more polar solvents, such as ethyl acetate, results in considerable dissolution with the hydrosol making the extraction more difficult. Similarly, more polar solvents than ethyl acetate, such as ethanol and methanol, are fully miscible with the hydrosol and thus cannot be used as extraction solvents.

4. Conclusions

Although a more complete and true profile of compounds found within the hydrosol may require a direct injection of a potential nonselective, concentrated form of the aqueous hydrosol, there are concerns associated with injecting aqueous samples into a GC-MS. Future work would include attempts to concentrate the hydrosol, while retaining its aqueous form and associated compounds. Some solvents (e.g., dichloromethane) may provide a more representative profile of the hydrosol components if limitations (i.e., potential absence or reduced abundances of more polar compounds) are recognized. The preferential use of dichloromethane with other hydrosols, was not evaluated and could be further elucidated in future studies.

As delineated in this manuscript, using *Abies grandis* hydrosol as a test case, a single-solvent extraction is not sufficient to fully characterize the composition of a hydrosol, as is typically performed in conventional analyses. Although, additional work with other hydrosols employing multiple organic solvents for the extraction of components is required to validate the universality of the results, this studies highlights the definitive differences found in the characterization of *Abies grandis* hydrosol when using solvents with varying properties for characterization. The authors recommend that when employing solvent extraction for the determination of hydrosol composition, a

Table 8. Compound extraction profile comparison between every solvent.

Compounds	CAS #	Ret. index	DCM (%)	Chloroform (%)	Ethyl acetate (%)	Hexane (%)	Petroleum ether (%)
2-Acetylfuran	1192-62-7	886	0.13	-	-	-	-
1-Methoxy-1-buten-3-yne	2798-73-4	905	0.02	-	-	-	-
5-Methylfurfural	620-02-0	934	0.15	0.17	-	-	-
Benzaldehyde	100-52-7	952	0.30	0.34	0.47	0.23	0.29
Lavender lactone	1073-11-6	997	0.36	0.43	-	-	-
p-Cymene	99-87-6	1025	0.31	0.65	0.33	0.68	0.73
Limonene	138-86-3	1034	1.15	1.10	1.33	1.04	1.58
Acetophenone	98-86-2	1038	0.31	0.33	0.35	0.23	-
γ-Terpinene	99-85-4	1045	0.14	-	-	-	-
p-Cymenene	1195-32-0	1066	0.13	-	-	-	-
2-Carene	554-61-0	1010	0.55	-	-	-	-
α-Campholenal	91819-58-8	1098	1.25	1.41	1.38	-	1.63
Cis-thujone	546-80-5	1102	0.19	-	-	-	-
Fenchol	1632-73-1	1110	2.71	2.87	3.06	3.54	3.36
Nopinone	24903-95-5	1129	0.16	-	-	-	-
Camphor	464-48-2	1142	1.40	1.52	1.55	1.65	1.62
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2α,5β)-	491-01-0	1157	0.25	-	-	-	-
4-Ethylphenol	123-07-9	1160	0.19	-	-	-	-
Camphene hydrate	465-31-6	1148	0.57	-	-	-	-
Isoborneol	124-76-5	1154	1.50	1.51	-	1.75	1.77
Borneol	507-70-0	1160	12.26	13.10	13.74	13.92	14.17
Terpinen-4-ol	562-74-3	1173	4.77	4.36	4.37	4.94	4.85
α-Terpineol	8000-41-7	1183	60.97	63.66	61.69	64.12	64.57
Verbenone	80-57-9	1204	1.30	1.28	1.23	0.88	1.00
Citronellol	106-22-9	1212	1.04	0.97	-	0.87	0.82
Piperitone	89-81-6	1256	0.62	0.78	-	0.64	-
Phellandral	21391-98-0	1252	0.23	-	-	-	-
Thymol	89-83-8	1269	0.20	-	-	-	-
p-Cymen-7-ol	536-60-7	1275	0.31	0.29	-	-	0.19
Bornyl Acetate	76-49-3	1275	2.51	2.14	2.39	3.94	2.41
P-Menthane-1,8-diol	80-53-5	1279	0.57	1.51	5.10	-	-
trans-Calamenene	73209-42-4	1529	0.01	-	-	-	-
Cubenol	21284-22-0	1610	0.60	0.48	-	0.87	-

minimum of both a polar and nonpolar solvents should be used with recognition of the potential limitations associated with liquid-liquid solvent extraction.

Authors' contributions

Conceptualization, C.B.; methodology, D.C.; formal analyses, R.A., L.M; resources, C.B.; writing—original draft preparation, R.A., L.M; writing—review, D.C., C.B.; supervision, D.C., C.B.

Acknowledgements

We graciously acknowledge the contribution of Zabrina Ruggles and Michael Carter at Young Living Highland Flats Tree Farm and Distillery for the procurement of *Abies grandis* hydrosol. We also

acknowledge Tyler Wilson from Young Living Essential Oils, D. Gary Young Research Institute for his review and contributions to this manuscript. Lastly, we thank AmandaMueller from Young Living Essential Oils for the illustration of the distillation apparatus.

Funding

The research received no external funding.

Availability of data and materials

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

Conflicts of interest

The authors declare no conflict of interest.

References

1. Tavares, C.S.; Gameiro, J.A.; Roseiro, L.B.; Figueiredo, A.C. Hydrolates: a review on their volatiles composition, biological properties and potential uses. Phytochem. Rev. 2022, 21, 1661-1737. https://doi.org/10.1007/s11101-022-09803-6
2. Sağdıç, O. Sensitivity of four pathogenic bacteria to Turkish thyme and oregano hydrosols. Lebensm.-Wiss. U.-Technol., 2003, 36(5), 467–473. https://doi.org/10.1016/s0023-6438(03)00037-9
3. Swor, K.; Poude, A.; Rokaya, A.; Satyal, P.; Setzer, W.N. Foliar essential oil of White Fir (*Abies concolor* (Gordon) Lindl. ex Hildebr.): Chemical composition and enantiomeric distribution. Nat. Prod. Commun., 2022, 17(3). https://doi.org/10.1177/1934578x221086901
4. Aazza, S.; Lyoussi, B.; Miguel, M.G. Antioxidant activity of eight hydrosols from morocco. Asian J. Plant Sci., 2012, 11(3), 137–142. https://doi.org/10.3923/ajps.2012.137.142
5. Gijzen, M.; Lewinsohn, E.; Croteau, R. Characterization of the constitutive and wound-inducible monoterpene cyclases of grand fir (*Abies grandis*). Arch. Biochem. Biophys.1991, 289(1), 267–273. https://doi.org/10.1016/0003-9861(91)90471-t
6. Hamedi, A.; Afifi, M.; Etemadfard, H. Investigating chemical composition and indications of hydrosol soft drinks (Aromatic waters) used in Persian folk medicine for women's hormonal and reproductive health conditions. J. Evid. Based Complement. Altern. Med. 2017, 22(4), 824–839. https://doi.org/10.1177/2156587217717413
7. Inouye, S.; Takahashi, M.; Abe, S. A comparative study on the composition of forty-four hydrosols and their essential oils. Int. J. Essent. Oils. 2008, (2), 89-104. https://eurekamag.com/research/029/621/029621204.php
8. Katoh, S.; Croteau, R. Individual variation in constitutive and induced monoterpene biosynthesis in grand fir. Phytochem.1998, 47(4), 577–582. https://doi.org/10.1016/s0031-9422(97)00422-6
9. Komnenić, A.; Lazarević, Z.; Jovović, Z.; Šavikin, K.; Pljevljakušić, D. Multiple liquid-liquid extraction of dissolved compounds in immortelle hydrosol with four different solvents. Chem. Biodivers. 2023, 21(2). https://doi.org/10.1002/cbdv.202301528
10. Kunc, N.; Frlan, A.; Baričevič, D.; Glavač, N.K.; Ladan, M.K. Essential oil and hydrosol composition of immortelle (*Helichrysum italicum*). Plants. 2022, 11(19), 2573. https://doi.org/10.3390/plants11192573
11. Ladan, M.K.; Glavač, N.K. GC–MS analysis of a *Helichrysum italicum* hydrosol: Sensitivity, repeatability and reliability of solvent extraction versus direct hydrosol analysis. Appl. Sci., 2022, 12(19), 10040. https://doi.org/10.3390/app121910040
12. Lante, A.; Tinello, F. Citrus hydrosols as useful by-products for tyrosinase inhibition. Innov. Food Sci. Emerg. Technol. 2015, 27, 154–159. https://doi.org/10.1016/j.ifset.2014.11.001
13. Almeida, H.H.S.; Fernandes, I.P.; Amaral, J.S.; Rodrigues, A.E.; Barreiro, M. Unlocking the potential of hydrosols: Transforming essential oil byproducts into valuable resources. Molecules. 29(19), 2024, 4660. https://doi.org/10.3390/molecules29194660
14. Lei, G., Wang, L.; Liu, X.; Zhang, A. Chemical composition of essential oils and hydrosols from fresh flowers of *Cerasus subhirtella* and *Cerasus serrulata* from East China. Nat. Prod. Res. 2014, 28(21), 1923–1925. https://doi.org/10.1080/14786419.2014.948437
15. Petrakis, E.A.; Kimbaris, A.C.; Lykouressis, D.P.; Polissiou, M.G.; Perdikis, D.C. Hydrosols evaluation in pest control: Insecticidal and settling inhibition potential against *Myzus persicae* (Sulzer). J. Appl. Entomol. 2014, 139(4), 260–267. https://doi.org/10.1111/jen.12176
16. Pulatoğlu, A.Ö.; Güney, K., Çeter, T.; Yilmaz, E.S. Chemical composition of essential oils obtained from Abies taxa in Türkiye and investigation of antimicrobial activities. Kastamonu Univ. J. For. Fac. 2023, 23(1), 31–46. https://doi.org/10.17475/kastorman.1269485
17. Ruggles, Z.; Wilson, T.N.; Abad, A.; Packer, C. Trunk wood essential oil profile comparison of Abies concolor (Pinaceae) and *Abies grandis* (Pinaceae) from northern Idaho (USA). Phytolog. 2023, 105, 18-24.

https://www.phytologia.org/uploads/2/3/4/2/23422706/105_1_18-24rugglespiceapungens21-02-2023.pdf

18. Sakai, T.; Maarse, H.; Kepner, R. E.; Jennings, W.G.; Longhurst, W.M. Volatile components of Douglas fir needles. J. Agric. Food Chem., 1967, 15(6), 1070–1072. https://doi.org/10.1021/jf60154a027
19. Shen, X.; Chen, W.; Zheng, Y.; Lei, X.; Tang, M.; Wang, H.; Song, F. Chemical composition, antibacterial and antioxidant activities of hydrosols from different parts of *Areca catechu* L. and *Cocos nucifera* L. Ind. Crops Prod. 2016, 96, 110–119. https://doi.org/10.1016/j.indcrop.2016.11.053
20. Tornuk, F.; Cankurt, H.; Ozturk, I.; Sagdic, O.; Bayram, O.; Yetim, H. Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella typhimurium* on fresh cut carrots and apples. Int. J. Food Microbiol. 2011, 148(1), 30–35. https://doi.org/10.1016/j.ijfoodmicro.2011.04.022
21. Traka, C.K.; Petrakis, E.A.; Kimbaris, A.C.; Polissiou, M.G.; Perdikis, D.C. Effects of *Ocimum basilicum* and *Ruta chalepensis* hydrosols on *Aphis gossypii* and *Tetranychus urticae*. J. Appl. Entomol., 2018, 142(4), 413–420. https://doi.org/10.1111/jen.12486