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Research Article

Essential oil from naturally exuded *Pseudotsuga menziesii* var. *glauca* (Pinaceae) resin

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Abstract

Pseudotsuga menziesii (Douglas fir) is an essential oil-bearing tree in the Pinaceae family. Essential oil was produced by hydrodistillation of naturally exuded resin and was analyzed by GC/MS and GC/FID to establish the essential oil profile. The resulting essential oils (n = 3) were largely composed of (average values) α-pinene (57.7%), camphene (1.5%), sabinene (3.1%), β-pinene (5.9%), δ-3-carene (17.6%), and limonene (6.4%). The essential oil yield and profile (monoterpenoids, sesquiterpenoids, diterpenoids) share many similarities with frankincense (*Boswellia spp.*) resin essential oils, particularly *B. sacra*. The current study, to the author's knowledge, is the first investigation of the essential oil obtained by hydrodistilled Douglas fir resin and provides the foundation for future research on the production and application of a novel and sustainable product.

1. Introduction

Pseudotsuga menziesii (Mirb.) Franco (Douglas fir) is an essential oil-bearing plant in the Pinaceae family. This coniferous species, which is native to the western region of North America, is distinguished further into two groups, var. *menziesii* (Pacific variety) and var. *glauca* (Rocky Mountain variety) [1, 2]. Populations native to Utah are of the Rocky Mountain variety [2, 3]. While native to North America, Douglas fir is cultivated throughout the world [4, 5].

Previous research on the essential oil of Douglas fir has been primarily focused on extractions from the limbs and leaves, with relatively little research on extractions from other plant parts or plant exudates. Prominent compounds of limbs and leaves, from both native populations in North America and non-native populations in Europe, include α-pinene (3.9-9.2%), sabinene (14.0-31.7%), β-pinene (7.2-30.3%), α-terpinene (3.1-5.6%), β-phellandrene (4.8-8.1%), γ-terpinene (5.5-5.8%), terpinolene (12.7-23.2%), terpinen-4-ol (7.5-14.8%), and citronellyl acetate (5.5%) [6-8]. Stark variation in essential oil profiles have also been observed between the Pacific and Rocky Mountain varieties in North America [9]. Prominent compounds in essential oil extracted from young shoots of Douglas fir included α-pinene (18.4%), β-pinene (49.8%), and germacrene D (5.5%) [10]. Prominent compounds in essential oil extracted from barks are primarily composed of sabinene (14.9%), β-pinene (15.1%), (*Z*)-β-ocimene (19.3%), (*E*)-β-ocimene (15.4%), terpinolene (11.3) [11]. Essential oils extracted from woody and leaf material of Douglas fir is primarily composed of monoterpenoids and, to a lesser extent, sesquiterpenoids. Previous research on evergreen species in the Pinaceae family has shown that essential oil extracted from different plant parts of the same tree (trunk, limbs, leaves, resinous cones) often contains unique compounds and profiles [12-14].

Terpenoid resin production is viewed as a plant defense mechanism that is controlled by plant hormones, specifically methyl jasmonate induced ethylene production [15,16]. Previous authors have provided different names for the resin that is naturally exuded from Douglas fir trees, depending on the source, cause of excretion, and description. Previously established names include oleoresin, callus resin, pocket resin, and cortical oleoresin [17-19]. This resin is typically yellow in color when excreted and darkens (amber, brown, black) with oxidation and age [18]. Analysis of Douglas fir resin solvent extractions has shown the presence of both volatile (monoterpenoids, sesquiterpenoids, diterpenoids) and non-volatile (resin acids, lignans, etc.) fractions [17-19]. Volatile fraction profiles have been shown to be predominantly composed of α-pinene, limonene, α-terpineol, bornyl acetate, citronellyl acetate, longifolene, α-muurolene, and isocembrol [17, 19, 20]. Like essential oil profiles of Douglas fir limb and leaf, these profiles vary greatly based on species variety (Pacific vs. Rocky Mountain) [19]. A distinguishing characteristic of the limb/leaf and resin volatile profile is the presence of diterpenes in the latter, such as cembrene and isocembrol [17, 20].

Erdtman and associates [17] discuss the collection of Douglas fir resin by an Oregon-based company for a commercial "pocket resin" product. To the author's knowledge, this product is no longer produced. Additionally, the essential oil of hydrodistilled resin from Douglas fir has never been fully analyzed. The current study investigates the essential oil produced from hydrodistillation of naturally exuded Douglas fir resin (Rocky Mountain variety, Utah). A thorough literature review of other commercial resin essential oils is discussed to demonstrate the potential commercial production and application of Douglas fir resin essential oil.

2. Materials and methods

Pseudotsuga menziesii resin was collected on November 5, 2022, and November 15, 2022, from native populations located on public lands (Bureau of Land Management) in Tooele County, Utah, USA (40°28'3" N 112°10'26" W; 2805 m elevation). Naturally exuded resin (Fig. 1) was collected, bagged, and stored at -20 ± 2 °C. The research was performed to determine the weight, yield, and composition of the extracted essential oil. For simplicity and consistency, each sample is referred to by a letter, A-C. A representative voucher sample is held in the Young Living Aromatic Herbarium (YLAH): *P. menziesii* (Mirb.) Franco, Wilson 2022-01.

Figure 1. Photograph showing an example of collected *Pseudotsuga menziesii* resin that was exuded through natural means. Resin was found in various conditions; semi-liquid and viscous vs. hard and dry, colors ranging from amber to yellow to brown.

Plant material (resin) was prepared for laboratory-scale distillation as follows: frozen resin was meticulously cleaned from bark, branch fragments, leaves, insects, etc. as best possible (Fig. 2), separated into 3 groups, and stored at -20 ± 2 °C until steam distilled. Steam distillation was performed in triplicate, resulting in 3 distillations over the course of this project.

Laboratory-scale distillation was as follows (custom distillation unit): 1.5 L of water was added to a 2 L distillation chamber, plant material accurately weighed and added to the distillation chamber, distillation for 2 h from pass-over by hydrodistillation, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were each

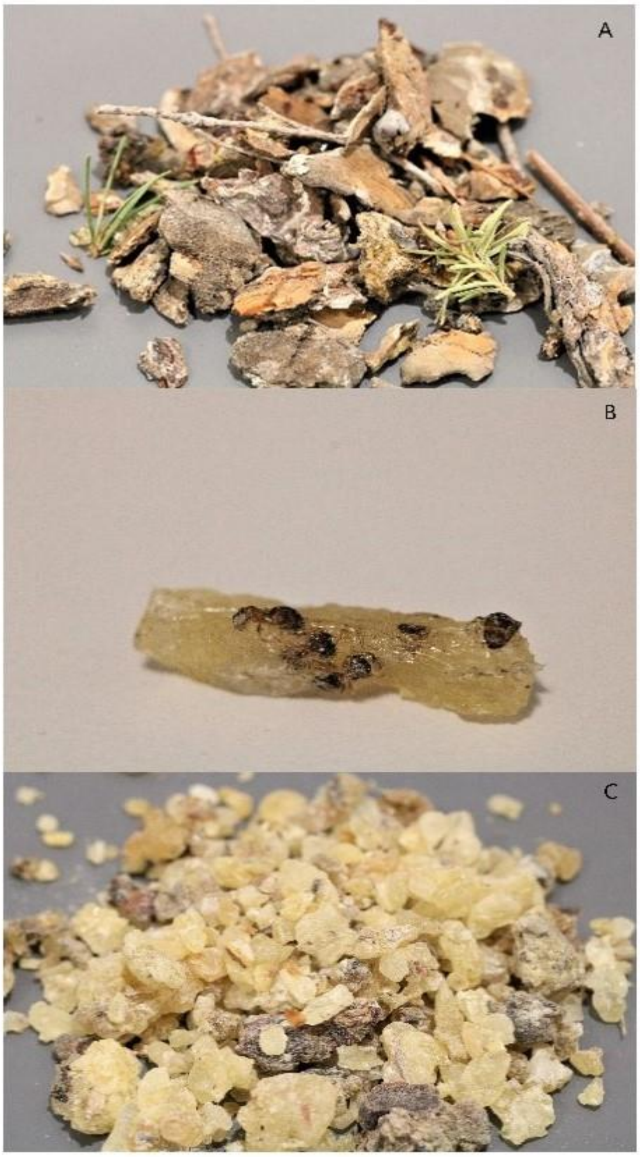

Figure 2. Photographs showing the resin cleaning process: (A) material that was separated from the resin, (B) material (insects) that could not be reasonably separated from the resin, (C) cleaned resin prior to distillation.

filtered and stored at room temperature in a sealed amber glass bottle until analysis. The percent yield was calculated as the ratio of the mass of processed plant material immediately before distillation to the mass of essential oil produced, multiplied by 100.

Essential oil samples were analyzed, and volatile compounds identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample (20% soln. for essential oils in ethanol), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min., oven ramp rate of 4.5 °C per min. to 310 °C with a hold time of 5 min. The electron ionization energy was 70 eV, scan range 35-650 amu, scan rate 2.4 scans per sec., source temp. 230 °C, and quadrupole temp. 150 °C. Volatile compounds were identified using the Adams volatile oil library (version 4) [21] using Chemstation library search in conjunction with retention indices. Note that trans-pinocarveol/cis-verbenol and terpinen-4-ol/m-cymen-8-ol elutes as single peaks. Their amounts were determined by the ratio of masses 70 and 92 (trans-pinocarveol), 79 and 94 (cis-verbenol), 71 and 93 (terpinen-4-ol), 91 and 134 (m-cymen-8-ol). Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B GC and Agilent J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample (20% soln. for essential oils in ethanol, 1% for reference compounds in ethanol, 0.1% soln. for C7-C30 alkanes in hexane), 25:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 2 min., oven ramp rate of 3.0 °C per min. to 250 °C with a hold time of 3 min. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation ≤ 0.5 for all compounds). Compounds were identified using retention indices coupled with retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

3. Results and discussion

The essential oil yield (w/w) is on average 8.39%. Distillation details and yield data are provided in Table 1. The distilled essential oil yield (w/w) was identical for samples B and C. The difference in yield for sample A is currently under investigation. We observed that the resin in sample A appeared to contain a larger amount of darker colored, or aged, resin than samples B and C. Future research on *Pseudotsuga menziesii* (Douglas fir) and other plant exudates will further investigate this observation.

Prominent volatile compounds (defined as values > 1%) in the distilled essential oil include α-pinene (avg. 57.7%), camphene (avg. 1.5%), sabinene (avg. 3.1%), β-pinene (avg. 5.9%), δ-3-carene (avg. 17.6%), and limonene (avg. 6.4%) (Table2). Comparing the three samples, essential oil profiles are relatively consistent. Standard deviations for values of the same compounds are typically < 0.1, except for α-pinene(0.6), sabinene (0.3) and δ-3-carene (0.4).

Table 1. Yield data, including mass of resin distilled (g), essential oil yield (g), and calculated yield (%) from *Pseudotsuga menziesii* samples (*n* = 3).

Samples	Resin Mass Distilled (g)	Yield EO (g)	Yield EO (%)
A	117.69	8.24	7.00
B	118.88	10.80	9.08
C	122.01	11.08	9.08
Avg:	119.53	10.04	8.39
Standard deviation (*n* = 3)			0.98

A thorough literature review has revealed many similarities between Douglas fir resin and some frankincense (*Boswellia spp.*) essential oils. Hydrodistillations of *B. carteri* and *B. sacra* resin have reported essential oil yields with upper ranges of 5.5% and 8.16% (w/w), respectively (vs. 8.39% in Douglas fir) [22,23]. The essential oil profiles also share many similarities, with the lightest fractions (monoterpenoids) comprising most of the essential oil profiles of both Douglas fir and *Boswellia spp*. Prominent monoterpenoids in *B. carteri* essential oil include α-thujene (1.6-52.4%), α-pinene (4.8-40.4%), sabinene (3.2-5.6%), myrcene (3.2-9.9%), p-cymene (3.4-6.2%), and limonene (2.6-20.4%) [24-26]. Prominent monoterpenoids in *B. sacra* essential oil include α-thujene (0.6-11.2%), α-pinene (5.3-68.2%), sabinene (2.9-6.9%), myrcene (0.7-6.9%), p-cymene (0.2-5.9%), limonene (6.2-33.5%), and and (E)-β-ocimene (nd-32.3%) [23, 25, 26].

Table 2. Aromatic profile of *Pseudotsuga menziesii* resin essential oil samples A-C.

KI	Compound Name	***Pseudotsuga menziesii* area %**		
		A	**B**	**C**
764*	Toluene	0.3	0.3	0.3
889*	Unknown compound	0.6	0.6	0.6
921	Tricyclene	0.9	0.9	0.9
924	α-Thujene	0.2	0.3	0.3
932	α-Pinene	58.5	57.4	57.3
946	Camphene	1.5	1.5	1.5
953	2,4,(10)-Thujadiene	0.1	0.1	0.1
966*	3,7,7-Trimethyl-1,3,5-cycloheptatriene	0.7	0.7	0.7
969	Sabinene	2.7	3.4	3.1
974	β-Pinene	5.9	6.0	5.9
988	Myrcene	0.2	0.2	0.2
1001	δ-2-Carene	0.1	0.1	0.1
1002	α-Phellandrene	0.1	0.1	0.1
1008	δ-3-Carene	17.0	17.9	18.0
1014	α-Terpinene	0.1	0.1	0.1
1020	p-Cymene	0.1	0.1	0.1
1022	o-Cymene	0.5	0.5	0.5
1024	Limonene	6.4	6.4	6.4
1032	(*Z*)-β-Ocimene	0.1	0.1	0.1
1044	(*E*)-β-Ocimene	t	t	t
1054	γ-Terpinene	0.1	0.1	0.1
1065	*cis*-Sabinene hydrate	t	t	t
1086	Terpinolene	0.4	0.5	0.5
1095	Linalool	0.2	0.2	0.2
1122	α-Campholenal	0.1	0.1	0.1

Table 2. *(Continued)*

KI	Compound Name	***Pseudotsuga menziesii* area %**		
		A	**B**	**C**
1135	trans-Pinocarveol	0.2	0.1	0.1
1137	cis-Verbenol	0.1	0.1	0.1
1140	trans-Verbenol	0.3	0.3	0.3
1165	Borneol	0.1	0.1	0.1
1166	p-Mentha-1,5-dien-8-ol	0.1	0.1	0.1
1174	Terpinen-4-ol	0.2	0.1	0.1
1176	m-Cymen-8-ol	0.1	t	t
1179	p-Cymen-8-ol	0.1	0.1	0.1
1186	α-Terpineol	0.1	0.1	0.1
1194	Myrtenol	0.1	0.1	0.1
1204	Verbenone	t	t	t
1226	cis-Carveol	t	t	t
1232	Thymol methyl ether	t	t	t
1284	Bornyl acetate	0.4	0.3	0.4
1350	Citronellyl acetate	t	t	t
1350	α-Longipinene	t	t	t
1359	Neryl acetate	t	t	t
1389	β-Elemene	t	t	0.1
1407	Longifolene	0.1	0.1	0.1
1489	β-Selinene	t	t	t
1492	α-Selinene	t	t	t
1493	epi-Cubebol	t	t	t
1937	Cembrene	0.1	0.1	0.1
2036*	18-Norabieta-8,11,13-triene	t	t	t
2072*	Isocembrol	0.2	0.1	0.1
Total Identified		**98.5**	**98.7**	**98.6**

The reported values (Table2) represent averages from samples analyzed in triplicate, which was done to ensure repeatability (standard deviation ≤ 0.5 for all compounds). Values less than 0.1% are denoted as trace (t). Unidentified compounds less than 0.5% are not included. KI is the Kovat's Index value and was previously calculated by Robert Adams using a linear calculation on a DB-5 column [21]. Relative area percent was determined by GC/FID. *KI not previously calculated [21]. Manual calculation performed using alkane standards.

Table 3. Summary of the aromatic profile of *Pseudotsuga menziesii* resin (average values from samples A-C), *Pseudotsuga menziesii* var. *glauca* leaf [6], and two *Boswellia spp.* (*B. sacra* and *B. carteri*) [26] essential oils.

Compound Name	*Pseudotsuga menziesii* resin area (%)	*Pseudotsuga menziesii* leaf area (%)	*Boswellia sacra* area (%)	*Boswellia carteri* area (%)
α-Pinene	57.7	15-20	68.2	37.3
Camphene	1.5	20-30	2.1	0.8
Sabinene	3.1	0.1-0.5	2.9	4.9
β-Pinene	5.9	5-10	2.0	1.8
δ-3-Carene	17.6	nd	3.9	0.9
Limonene	6.4	5-10	6.2*	14.4*
Total Identified	**92.2**	**45.1-70.5**	**85.3**	**60.1**

*Research by Woolley et al. 2012 reported this value as a co-eluted peak (limonene + β-phellandrene). The compound β-phellandrene was not detected in any *Pseudotsuga menziesii* samples.

Woolley and associates [26] distinguished *B. sacra* from *B. carteri* by, among other analytical techniques, GC profiles. Looking at average values from their study (while focusing on 6 prominent compounds in Douglas fir samples from the current study), resin essential oils from Douglas fir shares many similarities with *B. sacra* (Table 3). The major difference between Douglas fir resin and *Boswellia spp.* resin is in the values of δ-3-carene. Additionally, to demonstrate the similarities between Douglas fir resin and *Boswellia sacra* resin, Douglas fir leaf [6] essential oil profiles is also included. While many similarities can be found with Douglas fir and some frankincense species (*B. sacra*, *B. carteri*), other frankinense oils of economic importance are not comparable. The volatile profile of *B. papyrifera* oil is predominantly composed of octyl acetate (37.0%) and that of *B. serrata* is predominantly composed of α-thujene (22.7-69.8%) [27-29].

Given the extraction limitations of hydrodistillation at atmospheric pressure, most compounds comprising resin essential oils are comprised of lighter volatile compounds (monoterpenoids and sesquiterpenoids). However, relatively small amounts of diterpenoids and related compounds have also been found in both tree resins and resin essential oils. Cembrene ($C_{20}H_{32}$), which is present in samples from the current study, and cembrene diterpenoids have been found in *Boswellia spp.* resin and resin extracts [30-32]. While 18-norabieta-8,11,13-triene ($C_{19}H_{28}$) and isocembrol ($C_{20}H_{34}O$) have not been previously detected in resin from *Boswellia spp.*, they have been previously detected in resin from trees in the Pinaceae family [20, 31]. Additionally, other diterpenes have been detected in *Boswellia spp.* that were not detected in Douglas fir samples from the current study (cembrenol, incensole, cembrene isomers, etc.) [30-32].

4. Conclusions

Resins and resin essential oils, particularly those from the Burseraceae family, are important cultural and economic products. While Douglas fir resin has historically been used for various applications, it does not appear to currently be in use. The current study established the yield and GC profile of hydrodistilled Douglas fir resin essential oil. Additionally, the current study investigates the similarities of Douglas fir resin essential oil to similar products from *Boswellia spp.* Findings suggest that Douglas fir, due to the widespread distribution and similarities in the terpenoid profiles, could be a reliable and sustainable substitute for resin and resin extracts from the *Boswellia* genus, particularly *B. sacra.* Additional research is needed to investigate yields and profiles of hydrodistilled resin from different Douglas fir populations and varieties and should also focus on other extraction techniques to investigate profiles of diterpenes and triterpenes that may be present. Future research should also investigate applications of Douglas fir resin essential oil in the flavor and fragrance, cosmetic, and pharmaceutical industries.

Authors' contributions

Conceptualization, data curation, formal analysis (GC/MS, GC/FID), methodology, sample procurement, software, validation, writing – original draft, T.M.W.; Conceptualization, writing – original draft, writing – review and editing, E.A.Z.; Funding

acquisition, validation, writing – review and editing, R.E.C.

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Conflicts of interest

The authors declare no conflict of interest. The funding entity had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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