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Research Article

Essential oil from naturally exuded *Pinus contorta* var. *latifolia* (Pinaceae) resin

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Abstract

Pinus contorta var. *latifolia* (Rocky Mountain lodgepole pine) is an essential oil-bearing tree in the family Pinaceae. Essential oil was extracted via hydrodistillation of naturally exuded resin and was analyzed by GC/MS and GC/FID to determine the essential oil profile. The resulting essential oils (n = 4) were largely composed of δ-3-carene (avg. 40.3%), β-pinene (avg. 19.3%), β-phellandrene (avg. 14.9%), α-pinene (avg. 9.3%), limonene (avg. 4.8%), o-cymene (avg. 1.2%), and camphene (avg. 1.1%). The essential oil from naturally exuded resin shares similar constituents (monoterpenes, sesquiterpenes) with internal resin. However, some prominent constituents (myrcene and terpinolene) differ in their relative abundance. The current study is, to the authors' knowledge, the first investigation of the essential oil obtained by hydrodistillation of Rocky Mountain lodgepole pine resin and provides a groundwork for future research on essential oils from both naturally exuded and internal resins. Findings provide an approach to evaluate forest health as it relates to resin essential oil production and composition.

1. Introduction

Pinus contorta var. *latifolia* Engelm (Rocky Mountain lodgepole pine) is an evergreen tree belonging to the family Pinaceae [1]. Currently, four varieties are recognized within the genus, including var. *latifolia*, var. *contorta*, var. *murrayana*, and var. *bolanderi* [2]. This species represents one of the largest native conifer distributions in North America, from the Pacific Northwest eastward to South Dakota and as far south as regions in Mexico [3, 4]. Specific to the state of Utah, *P. contorta* var. *latifolia* is most common and comprises large stands in the higher altitudes of the Uintah Mountains [5]. Among the varieties, *P. contorta* var. *latifolia* touts incredible fire adaptation culminating in highly successful succession following wildfires [2].

Ethnobotanical importance and current importance of Rocky Mountain lodgepole pines are recorded in various uses. While remarked as having weak wood, uses still included construction lumber for railroad ties and mine shaft supports. Beyond the wood, resin

was also sought after for use as caulking in ship construction [4, 6]. Resin from these trees was used by indigenous peoples as adhesive, sealant, and in a medical capacity in salves and rubs for the treatment of wounds and respiratory illness [7, 8].

Like other Pinaceae, Rocky Mountain lodgepole pine is an essential oil-bearing plant. A large component of these essential oils (EO) includes terpenoids found in various tissues and plant exudates, like resin. Throughout this manuscript, 'internal resin' refers to resin held within plant tissue and has been studied previously by other researchers, while 'exuded resin' refers to resin that is naturally exuded to the outer surface of the tree and is the current topic of focus. Production and storage of resin and terpenoid constituents occurs within specialized resin ducts. In Rocky Mountain lodgepole pine, these terpenoids are important components in the constitutive and induced defenses against pests like *Dendroctnus ponderosae* (mountain pine beetle) which act as a vector for life threating fungi such as *Grossmannia clavigera* [9, 10]. Following attack from mountain pine beetles, ruptured resin ducts and canals will cause an efflux of resin to the wound site, attempting to remove, trap, or eliminate the pest, however, the amount of resin can vary [11].

Previous research on volatiles found in Rocky Mountain lodgepole pine highlights extractions of leaves and cones with extensive work done on stem and bark extractions. Evaluation of compounds found in the leaves of *P. contorta* var *latifolia* include β-phellandrene (45.1%), δ-3-carene (11.5%), β-pinene (10.3%), α -pinene (3.4%), myrcene (2.1%), terpinolene (2.0%), α-phellandrene (1.7%), and α-terpinene (1.6%) [12]. Interestingly, variations in the compounds in leaf extractions were very similar between var. *latifolia* and var. *controta*, but those in var. *murrayana* were strikingly different [12]. Another researcher found the following prominent compounds in leaves, β-phellandrene (53.1-78.7%), δ-3-carene (5.6-28.6%), β-pinene (3.2-15.1%), α-pinene (3.6-8.7%), myrcene (1.8-4.7%), limonene (0.9-3.1%), sabinene (0.9-2.1%), α-phellandrene (0.8-1.6%), terpinolene (0.2-2.6%), and camphene (0.4-0.9%) [13]. When samples taken from higher elevations were compared in the same study, there was no significant difference in EO composition, although the relative amounts of δ-3-carene appeared to be higher. Most recently, evaluation of leaf essential oil included β-pinene (27.0%), β-phellandrene (21.8%) δ-3-carene (3.6%) (2*E*)-hexenal (7.1%), α-pinene (5.0%), and α-terpineol (6.7%) accounting for 71.2% of total identified compounds [14]. Essential oil derived from conifer needles is commercially relevant because of its aroma and use in products such as fragrance [9, 14], however to the authors knowledge, external resin essential oil has no current commercial use.

Internal resin analyzed via GC identified β-phellandrene (57-62%), β-pinene (15-22%), α-pinene (7-10%), δ-3-carene (7-17%), myrcene (3%), limonene (1-2%) with variation in average percent composition between both sample individuals and populations [15]. Steam distillation of resin saturated wood following bark beetle attack corroborated with the expected constituents of the volatile oils in Smith's work [13]. It is important to note that the amount of resin present post bark beetle attack is variable, with some having no resin afforded to wounded tissues [16]. Relative amounts of almost all constituents were similar among groups of non-resinous, resinous, and healthy tissues. However, β-phellandrene was much higher in the resinous response group (51%), moderate in the healthy group (25.6%), and low in the non-resinous response group (10.1%) [16]. In a different study, evaluation of internal resin included β-phellandrene (36.16%), limonene (16.74%), α-pinene (12.71%), δ-3-carene (8.56%), β-pinene (7.88%), and myrcene (4.82%); with these 6 compounds representing 86.87% of total monoterpenes in the internal resin [17]

Further studies involving change in bark terpenoid composition following attack in Rocky Mountain lodgepole pine substantiated the idea of an increase of resin constituents found in phloem tissue. Following a simulated attack and subsequent inoculation of fungus, seven terpenoids comprising 95% of total constituents were observed to have increased. These terpenoids included β-phellandrene, β-pinene, δ-3-carene, α-pinene, limonene, myrcene, and terpinolene [10]. Importantly, many of these compounds help promote defense against herbivory and fungal infection [10]. Additional studies on constitutive and induced terpenoids found in bark resin agreed with the presence of important bioactive defense monoterpenes, including δ-3-carene, myrcene, and

terpinolene [18]. In a separate study, evaluation of internal resin from surviving trees correlated survival with increased levels of limonene and δ-3-carene, as well as decreased α-pinene, myrcene, and terpinolene, which are used as precursors and synergistic compounds for beetle aggregation hormones [19]. These studies, important for understanding the forest ecology of their respective systems, have focused mostly on the evaluation of internal resins taken with samples of bark and phloem tissue [18].

Much of the related previous work has evaluated terpenoid constituents occurring in tissues and internal resins, along with their role in plant defense. However, understanding the composition of exuded resin from Rocky Mountain lodgepole pine has not been fully studied. The current study investigates the chemical composition of the essential oil obtained through hydrodistillation of naturally expelled Rocky Mountain lodgepole pine resin. Exploration of resin essential oil composition from both individual and population samples is discussed along with a literature review to explore forest health as it may relate to resin essential oil composition.

2. Materials and methods

2.1 Plant material

Pinus contorta var. *latifolia* resin was collected on July 3, 2024, from native populations located on public lands (Bureau of Land Management) in Rich County, Utah, USA (41°42'03.5" N 111°22'01.0" W; 2268 m elevation). Naturally exuded resin was collected, bagged, and stored at -20 ± 2 °C until it was ready for processing. A representative voucher sample is held in the Young Living Aromatic Herbarium (YLAH): *P. contorta* var. *latifolia* Engelm, Wilson 2024-01.

Three samples (A-C) were each obtained from individual trees, and sample D was obtained from an aggregate sampling of 10+ trees. Plant material (resin) of each sample was prepared for laboratory-scale distillation as follows: frozen resin was meticulously cleaned from bark, branch fragments, leaves, insects, etc. (Fig. 1) and stored at -20 ± 2 °C until hydrodistilled. Hydrodistillation was performed for each sample (A-D) individually.

2.2 Extraction of the essential oil

Laboratory-scale distillation was performed using a

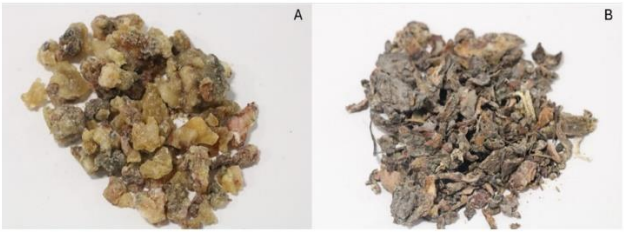

Figure 1. (A) Collected resin cleaned of plant and other organic debris, representing an aggregate from several trees. **(B)** Organic debris cleaned and removed from the resin sample.

custom hydrodistillation apparatus (Fig. 2) as discussed in previously published work [20]. 1.5L of water was added to a 2 L distillation chamber, then plant material was accurately weighed and added to the distillation chamber. Distillation was conducted for 2 hours following pass-over, with the essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were each filtered and stored at room temperature in sealed amber glass bottles until analysis. The percent yield was calculated as the ratio of the mass of essential oil produced to the mass of processed plant material immediately before distillation, multiplied by 100.

Figure 2. Custom stainless steel hydrodistillation apparatus and water-cooled condenser column. Illustrated by Rick Simonson, Science Lab Studios, Inc. (Kearney, NE, USA).

2.3 Essential oil analysis

Essential oil samples were analyzed, and volatile compounds were identified, by gas chromatography/mass spectroscopy (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% solution of essential oils in ethanol), 100:1 split ratio, initial oven temp. of 40 °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 was 230 °C, and quadrupole temp was 150 °C. Volatile compounds were identified using Adams volatile oil library (version 4) [21] using Chemstation library search in conjunction with retention indices. Note that the β-phellandrene and limonene elute as a single peak. Their amounts were determined by the ratio of masses 77 and 93 (β-phellandrene), 68 and 79 (limonene).

Volatile compounds were then quantified and reported as a relative area percentage by gas-chromatography with flame ionization detection (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 and 0.1 µL of reference standard (20% solution for essential oils in ethanol, and 0.1% solution for C7-C30 alkanes standards 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. Compounds were identified using retention indices coupled with retention time data of reference compounds (α-pinene, β-pinene, α-phellandrene, δ-3-carene, limonene, terpinolene, methyl chavicol and eugenol) (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

Analysis of each sample in triplicate (repeat injection) indicated standard deviations < 0.1 for all compounds in samples A, B and D. Sample C displayed similar consistency in standard deviation, < 0.1 apart from α-pinene (0.6) and β-pinene (0.5).

3. Results and discussion

Following hydrodistillation, the average essential oil yield (w/w) was 2.49%. Details and data for each distillation are included in Table 1. All resin samples were uniform in color and texture in their respective samples. Sample C had the lowest resin mass and displayed the lowest yield (w/w) of 1.30%. Between all groups, the standard deviation for percent yield was 0.89. Variables such as age, color, and consistency of the resin collected may help with further predictions for percent yield and should be the focus of future studies.

Table 1. Yield data, including mass of resin distilled (g), essential oil yield (g), and calculated yield (%) from *Pinus contorta* var. *latifolia*

Samples	Resin Mass Distilled (g)	Yield EO (g)	Yield EO (%)
A	46.79	0.94	2.01
B	252.35	9.1	3.61
C	21.53	0.28	1.30
D	119.13	3.61	3.03
Avg:	109.95	3.48	2.49
Standard deviation (n = 4)			0.89
samples (n = 4).			

The prominent volatile compounds (> 1%) identified in the current study included δ-3-carene (avg. 40.3%), β-pinene (avg. 19.3%), β-phellandrene (avg. 14.9%), α-pinene (avg. 9.3%), limonene (avg. 4.8%), o-cymene (avg. 1.2%), and camphene (avg. 1.1%). Table 2 provides detailed data specific to the aromatic profile of each sample. Averages for prominent constituents display large variation between individual trees (samples A-C), specifically for alpha-pinene (σ = 6.2), beta-pinene (σ = 14.2), delta-3-carene (σ = 5.8), and beta-phellandrene (σ = 17.0) (Table 3).

Ott et al. [10] observed changes in prominent terpenes of internal resin in response to attack from mountain bark beetles and fungal inoculation following attack, including increases in α-pinene, β-pinene, δ-3-carene, limonene, and β-phellandrene. No quantifiable measurements regarding bark beetle attack status were recorded for the current study, however, the large variation (Table 3) of these same terpenes between individual samples may be explained by this phenomenon. Higher levels of α- and β-phellandrene observed in Rocky Mountain lodgepole pine have also been linked to host identification by mountain bark beetles [17, 22]. Many of the samples herein displayed large variations in the percents of both these compounds, along with increases in other terpenes, including β-pinene. Other studies which focused on trees that resisted mountain pine beetle infestation have correlated tree survival with favorable

Table 2. Aromatic profile of *Pinus contorta* var. *latifolia* resin essential oil samples A-D.

KI	Compounds	*Pinus contorta* var. *latifolia* area (%)			
		A	B	C	D
764	Toluene	t	0.3	0.1	0.2
890	Styrene	t	0.1	t	0.1
921	Tricyclene	0.3	0.6	0.9	0.3
924	α-Thujene	0.5	0.9	0.9	0.7
932	α-Pinene	**9.2**	**15.9**	**24.3**	**9.3**
946	Camphene	1.4	1.9	2.8	1.1
953	Thuja-2,4,(10)-diene	t	0.1	0.1	t
966	3,7,7-Trimethyl-1,3,5-cycloheptatriene	0.4	0.9	0.4	1.5
969	Sabinene	0.5	0.5	0.8	0.7
974	β-pinene	**6.2**	**17.8**	**40.4**	**19.3**
988	Myrcene	1.7	1.1	0.4	0.7
1002	α-Phellandrene	2.2	1.6	0.1	0.7
1008	δ-3-Carene	**16.3**	**22.0**	**7.8**	**40.3**
1014	α-Terpinene	0.8	0.6	0.0	0.2
1020	p-Cymene	0.1	0.1	0.1	0.2
1022	o-Cymene	2.3	1.3	3.2	1.2
1024	Limonene	3.1	2.1	3.1	4.8
1025	β-phellandrene	**51.1**	**28.9**	**9.5**	**14.9**
1054	γ-terpinene	0.4	0.3	0.0	0.3
1065	Trans-sabinene hydrate	t	t	t	t
1085	p-Menth-2,4(8)-diene	0.1	0.1	t	0.1
1086	Terpinolene	0.9	0.8	0.1	1.4
1095	Linalool	0.1	0.1	t	0.1
1108	1,3,8-p-Menthatriene	t	t	t	t
1118	*cis-p*-Menth-2-en-1-ol	t	t	t	t
1122	α-Campholenal	0.1	0.1	t	t
1136	Trans-p-menth-2-en-1-ol	t	t	t	0.1
1139	Trans-pinocarveol	t	0.1	0.2	0.1
1160	Pinocarvone	t	t	t	t
1174	Terpinen-4-ol	0.1	0.2	0.1	0.1
1183	Cryptone	0.2	0.1	0.4	0.1
1195	Methyl chavicol	0.4	0.5	0.6	0.2
1207	Trans-piperitol	t	t	t	t
1232	Thymol-methyl-ether	0.1	t	0.1	t
1238	Cumin aldehyde	t	t	0.1	t
1273	p-Menth-1-En-7al	0.1	0.1	0.1	t
1284	Bornyl acetate	t	t	t	t
1356	Eugenol	t	t	t	t
1403	Methyl eugenol	t	t	t	t
1411	*α-cis*-Bergamotene	t	t	t	t
1513	γ-Cadinene	t	t	t	t
1522	δ-Cadinene	t	t	t	t
1968	Sandaracopimara-8(14),15-diene	t	t	0.1	t
2016	Phyllocladene	t	t	0.1	0.1
2184	Sandaracopimarinal	0.1	t	0.2	t
	Total Identified	***98.9***	***99.1***	***97.2***	***98.9***

Samples A-C are individual trees, while sample D represents an aggregate from 10+ trees. Reported values below represent averages from samples analyzed in triplicate, which was done to ensure repeatability (standard deviation ≤ 0.6 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.

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Table 3. Prominent (> 0.5 in at least one sample) essential oil constituents of samples A-C.

Compound Name	*Pinus contorta* var. *latifolia* area (%)			STDev
	A	B	C	
Tricyclene	0.3	0.6	0.9	0.2
α-Thujene	0.5	0.9	0.9	0.2
α-pinene	9.2	15.9	24.3	6.2
3,7,7-Trimethyl-1,3,5-cycloheptatriene	0.4	0.9	0.4	0.2
β-pinene	6.2	17.8	40.4	14.2
Myrcene	1.7	1.1	0.4	0.5
α-Phellandrene	2.2	1.6	0.1	0.9
δ-3-Carene	16.3	22.0	7.8	5.8
α-Terpinene	0.8	0.6	0.0	0.3
o-Cymene	2.3	1.3	3.2	0.8
Limonene	3.1	2.1	3.1	0.5
β-Phellandrene	51.1	28.9	9.5	17.0
Terpinolene	0.9	0.8	0.1	0.4
Total Identified	***95.0***	***94.4***	***91.1***	

Values reported below indicate the average % of each compound in samples A-C, and respective standard deviation between samples. Relative % for constituents were obtained via GC/FID.

compositions of constitutive terpenes such as increased limonene and δ-3-carene, and decreased α-pinene, myrcene, and terpinolene [19]. Future studies should explore how naturally exuded resin essential oil could be used as a tool in forest health and management, particularly regarding bark beetle infestation.

While the current study presents averages for resin essential oil terpene constituents that are specific to the Rocky Mountain lodgepole pine population in Rich County, UT, other studies have observed variation in constituents between different geographic locations [23]. In the study conducted by Clark et al. [23], prominent terpenes identified remained the same, with variation being observed in the total terpenes measured and their relative amounts. In a separate study, Roth et al. [24] found that trees attacked by mountain pine beetles had 247% higher monoterpene levels than healthy trees. These two examples help better explain observed differences in essential oil composition in the current study. Internal resin isolated in phloem tissue shared prominent constituents with resin essential oil with the exception of myrcene and terpinolene, which were observed at a much lower percentage in samples from the current study. In multiple studies on internal tissue resin, myrcene comprised on average 3% and terpinolene 2% of total terpenes [10,18, 23]. Evaluation of the exuded resin essential oil in the current study observed myrcene and terpinolene to have a maximum of 1.7% and 1.4%, respectively. Some of these variations have been explained by simple changes in the geographic location of the trees sampled, however, differences between the internal resin and exuded resin have been understudied in Rocky Mountain lodgepole pine and should be the focus of future studies.

4. Conclusions

Rocky Mountain lodgepole pine stands as a highly recognizable and ecologically significant tree in North America. Important studies have highlighted the terpene composition of internal resin in plant defense against mountain bark beetles and associated fungi. The current study has established an average yield (w/w) of 2.49% and GC profile ((δ-3-carene (avg. 40.3%), β-pinene (avg. 19.3%), β-phellandrene (avg. 14.9%), α-pinene (avg. 9.3%), limonene (avg. 4.8%), o-cymene (avg. 1.2%), camphene (avg. 1.1%)) of essential oil obtained from exuded Rocky Mountain lodgepole pine resin. Commercially, Rocky Mountain lodgepole pine resin essential oil requires more resources to distill with a lower percentage yield (w/w) when compared to other conifers such as Douglas Fir

(*Pseudotsuga menziesii* var. *glauca*). As this is a manual process time, abundance of resin, and yield are important to consider. In addition, the current study has reviewed recent research on Rocky Mountain lodgepole pine and mountain bark beetles as they relate to constituents found in resin essential oil. Looking ahead, future queries hold promise in understanding if and how resin changes following exudation to the outer surface could help further forest health analysis as bark beetles are projected to increase in spread.

Authors' contributions

Conceptualization, R.B.J., T.M.W, C.P.; Methodology, T.M.W.; Software, T.M.W.; Validation, R.E.C.; formal analysis, R.B.J., T.M.W.; Investigation, R.B.J., T.M.W., C.P; Data Curation, R.B.J.; Writing-Original draft preparation, R.B.J.; Writing-Review and Editing, T.M.W., C.P., C.R.B., R.E.C.; Funding acquisition, C.R.B., R.E.C. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

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

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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