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Short Communication

Pinus monophylla (Pinaceae) resin: A novel technique for extracting terpenoids from aromatic plant materials

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Abstract

Pinus monophylla is an aromatic tree in the Pinaceae family, that grows throughout the western United States. This conifer species naturally exudes large quantities of essential oil-rich resins, which, similar to other commercially available tree resins, can be hydrodistilled to extract the essential oil (EO). Following an initial hydrodistillation, a patents pending process was used herein, in which the resin EO was used as a solvent to extract additional compounds from the spent conifer resin, including non-volatile terpenoids. GC/MS and LC/MS analyses of samples (n = 6) were conducted to establish the terpenoid profiles of both EO and secondary extracted samples (DeepSpectra® samples). The volatile profiles of *P. monophylla* resin extractions were somewhat similar between sample types, EO and DeepSpectra® samples, with prominent compounds in α-pinene (avg. 83.2%, 80.0%), δ-3-carene (avg. 2.4%, 2.2%), and α-copaene (avg. 2.7%, 3.5%), respectively. DeepSpectra® samples contained abietic acid (avg. 6.3 mg/mL) as well as other non-volatile compounds, although they were not detected in the EO samples. To the best of our knowledge, this is the first study to establish the complete terpenoid profile of *P. monophylla* resin using a novel sequential extraction technique.

1. Introduction

Pinus monophylla Torr. & Frém (single-needle pinyon pine) is an evergreen tree in the Pinaceae family [1, 2]. *P. monophylla* grows on desert mountain slopes throughout the western United States and is often associated with Utah Juniper (*Juniperus osteosperma*), creating the common pinyon-juniper forests [2]. The low-growing aromatic tree typically grows 5-20 m in height [2, 3].

Various parts of the *P. monophylla* tree contain extractable essential oils (EO), however, to date, only the aromatic leaves (needles) and oleoresin have been studied. The leaf EO is primarily composed of light monoterpenes, such as α-pinene, β-pinene, and β-phellandrene [4-5]. The oleoresin of *P. monophylla* extracted from the woody materials, is also largely composed of α-pinene [6].

Resin from several commercial oils (frankincense, myrrh, etc.) is typically obtained by tapping (mechanical incision) of the trees, which causes the resin to exude [7]. While this could also be practiced with *P. monophylla*, forests cover substantial acreage in North America [2, 3], and the tree is observed to naturally exude relatively large quantities of resin. Given its availability, naturally exuded *P. monophylla* resin can be collected in a sustainable approach and without further wounding of trees [8, 9].

The current study uses a novel, patents pending extraction technique [10] that uses hydrodistilled *P. monophylla* EO (distilled from the resin) as a secondary solvent to then extract non-volatile compounds from the spent resin. This approach eliminates the use of harsh chemical solvents (DCM, methanol, etc.) and,

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since the secondary extraction is conducted on spent resin, establishes an environmentally sustainable approach to obtaining additional biologically active and beneficial compounds that are otherwise not detectable in the hydrodistilled EO. The current study established the different chemical profiles of *P. monophylla* EO (n = 3) and secondary extracted (aka, DeepSpectra® extraction) samples (n = 3) by GC/MS and LC/MS analyses. The current research is the first to establish the complete terpenoid profile of *P. monophylla* resin and to investigate a novel patents pending extraction technique to recover non-volatile compounds from the spent resin. While the aforementioned patents pending technology [10] has a seemingly endless list of applications, the current study demonstrates its application and utility with a single plant material, *P. monophylla* resin.

2. Materials and methods

2.1. Distillation and extraction techniques

Pinus monophylla resin was collected on 23 April 2025 from native populations located on public lands (Bureau of Land Management). The collection site (37.140251, -113.144893) was located on the Gooseberry Mesa (UT, USA). A representative voucher sample was produced from the site and is held in the Young Living Aromatic Herbarium (YLAH): *P. monophylla* Torr. & Frém, Wilson 2025-01. The collected resin comprises an assortment ranging from soft-fresh to hard-old resin. Naturally exuded resin is typically a reaction to a traumatic event (boring insect, broken limb, etc.), may drip to a secondary location (tree branch, low section of the trunk, etc.), and may eventually make its way to a tertiary location, such as the ground. For this research, available naturally exuded resin was collected from all sources (Fig. 1).

Essential oil (EO) samples (n = 3) were produced by laboratory-scale hydrodistillation as follows: 6 L of water was added to the bottom of a 12-L distillation chamber (Albrigi Luigi S.R.L., Grezzana, Italy), approximately 2 kg of resin was accurately weighed and added to the distillation chamber. Hydrodistillation was performed for 3 h, and the volatile oil was separated using a cooled condenser and Florentine flask. The EO samples were filtered and stored in a sealed amber glass bottle at room temperature until use for secondary extraction or analysis. The *P. monophylla* resin that no longer had EO (spent resin) was separated from any remaining water, allowed to dry at room temperature for 72 h, and broken into small (approx. 3 cm x 3 cm) pieces.

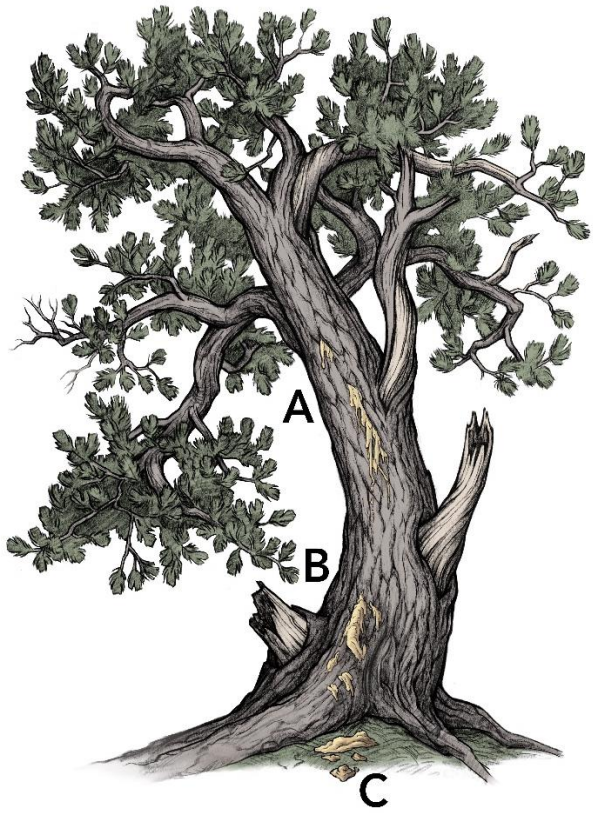

Figure 1. Illustration depicting sources of naturally exuded *Pinus monophylla* resin. (**A**) The initial trauma site, which contains soft-fresh resin, (**B**) the secondary site where resin falls, which contains either soft-fresh or moderately hard resin, (**C**) the tertiary site where resin reaches the ground and it ranges typically from moderately hard to very hard resin. Botanical illustration by Zach Nielsen

Secondary extraction DeepSpectra® samples (n = 3) were produced as follows: Dried pieces of spent resin were ground to #18 particle size (1000 microns) using a mortar and pestle and an ASTM E-11 USA Standard Sieve (Dual Manufacturing Co., Inc., Franklin Park, IL, USA), accurately weighed and added to EO (approx. 1:3), mixed in a beaker at 200 rpm for 2 h, and filtered using a 0.22 µm PVDF Luer lock filter (Restek Corporation, Bellefonte, PA, USA). DeepSpectra® samples (n = 3) were derived from the respective EO samples and spent materials (i.e., DeepSpectra® sample A produced by mixing EO sample A with spent resin from EO sample A hydrodistillation, etc.).

The DeepSpectra® sample extraction details are presented in Table 1.

Table 1. Secondary extraction, or DeepSpectra® (DS) extraction.

Products	DS Sample A	DS Sample B	DS Sample C
Spent Resin Mass (g)	20.09	20.22	20.11
Essential Oil Mass (g)	60.10	60.04	60.03

Details including spent resin mass (g) and essential oil mass (g) used for production of each sample.

2.2. Analysis methods

Relative density (specific gravity) analysis was conducted using a density meter (Anton Paar, Graz, Austria) in accordance with the International Organization for Standardizations (ISO) 279 [11].

To determine volatile compound profiles, EO and DeepSpectra® samples were analyzed, and compounds were identified and quantified by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J & W DB-5, 60 m × 0.25 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for EO in ethanol), 100:1 split ratio, initial oven temp. of 40 °C with an initial hold time of 5 min, and 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 was 35–650 amu, scan rate was 2.4 scans per s, source temp. 230 °C, and quadrupole temp. 150 °C. Compounds were identified using the Adams volatile oil library [12] and a Chemstation library search in conjunction with retention indices.

To determine the non-volatile compound profile, EO and DeepSpectra® samples were analyzed by LC/MS. Samples were prepared for analysis by adding 50 µL of sample to 9.95 mL of HPLC grade ethanol (Sigma-Aldrich, 200 proof, item 459828) with a pipette to a 15 mL light sensitive centrifuge tube. The samples were inverted several times to mix and then sonicated at room temperature for 10 min. Each sample was then filtered (Restek syringe filter, PVDF, 0.22 µm x 30mm) into an amber HPLC vial and analyzed for abietic acid content by LC/MS using a Waters ACQUITY UPLC H-Class PLUS system coupled with a Waters QDa Mass Detector operating in ESI positive ion mode (Waters Corporation, Milford, Massachusetts, USA). Analyte separation was achieved using an AQCUITY Premier HSS T3 column (2.1 x 150 mm, 1.8 µm) under the following operating conditions: 0.5 µL of the sample was injected onto the column and subjected to a 20-min mobile phase and flow rate gradient (Table 2).

Table 2. Mobile phase gradient details (method time, flow rate, concentrations of mobile phases A and B).

Number	Time (min)	Flow (mL/min)	A (%)	B (%)
1	Initial	0.3	25	75
2	2.00	0.3	25	75
3	17.00	0.3	20	80
4	17.10	0.3	25	75
5	20.00	0.3	25	75

Mobile phase A was 10 mM ammonium formate (LiChropur, LC/MS grade, Sigma-Aldrich item 70221) in ultra-pure water (Milli-Q IQ 7000, 0.22 µm Millipak filter), with 0.05% formic acid (LiChropur, LC/MS grade, Sigma-Aldrich item 5.33002). Mobile phase B was Acetonitrile (J.T.Baker, LC/MS grade, Avantor item 9829-03), with 0.05% formic acid (LiChropur, LC-MS grade, Sigma-Aldrich item 5.33002). Column temp was 25 ^{0}C. Positive identification was achieved by both retention time comparison and specific Single Ion Recording (SIR). Quantitation of the analyte was achieved by comparing peak area responses to an established calibration curve (Linear regression, minimum R2 value of 0.995) with a range of 10 to 50 µg/mL (ppm). The SIR for abietic acid was 303.22 m/z. The general QDa conditions were as follows: MS Scan Mass Range 100 Da – 800 Da, Cone Voltage 15 (V), Positive Capillary voltage 0.8 (kV), Sampling Rate 1 points/sec and Probe temperature 600 ^{0}C. Calibration curves and retention times were established using certified reference materials (Sigma-Aldrich, abietic acid, item 00010).

2. Results

Hydrodistillation of *Pinus monophylla* essential oil (EO) resulted in three samples, A-C. The yields (w/w) ranged from 5.9-7.4% (w/w) (Table 3). The color and appearance of all EO samples were colorless and clear liquids.

The secondary extraction and DeepSpectra® extraction details are presented in Table 1.

Table 3. Hydrodistillation and essential oil (EO) production details include *Pinus monophylla* fresh resin mass (g), EO yield (g), and EO % (w/w).

Products	EO Sample A	EO Sample B	EO Sample C
Resin Mass (g)	1979.98	2030.81	1923.84
EO Yield (g)	146.49	118.81	130.73
EO % (w/w)	7.4	5.9	6.8

When measuring the pre- and post-weights of the EO and DeepSpectra® samples, trivial amounts of samples were lost in the filtering process; so, an accurately calculated increase in mass resulting from the DeepSpectra® extraction process was not feasible and is not recorded within the manuscript. The color and appearance of all DeepSpectra® samples were pale-yellow and clear liquids.

As an initial check on sample characteristics and differences, specific gravity was measured for the initial extraction (EO) and DeepSpectra® samples. The results are presented in Table 4.

Table 4. Specific gravity values for *Pinus monophylla* essential oil (EO) and DeepSpectra® (DS) samples.

Samples	*P. monophylla* EO	*P. monophylla* DS
Sample A	0.87	0.90
Sample B	0.87	0.90
Sample C	0.87	0.90

The GC/MS analysis identified 31 volatile compounds in the EO samples and 34 volatile compounds in the DeepSpectra® samples. The GC findings are presented in Table 5.

Abietic acid was present in each DeepSpectra® sample, however, was not detected in any EO sample. A summary of LC findings are provided in Table 6.

3. Discussion

Upon completion of hydrodistillation and secondary extraction (DeepSpectra® extraction), the oil samples changed from colorless to pale-yellow respectively. Additionally, the specific gravity values increased from 0.87 (essential oil samples) to 0.90 (DeepSpectra® samples). These data suggest that the essential oil (EO) was a reliable solvent for extracting additional compounds of higher molecular weight from the spent resins.

GC/MS analysis resulted in similar volatile profiles for both EO samples (n = 3) and DeepSpectra® samples (n = 3); however, subtle differences were observed. Some compounds were only detected in the DeepSpectra® samples such as, carvone (trace), γ-amorphene (avg. 0.1%), and α-calacorene (trace). More telling is the average relative abundance of monoterpenoids and sesquiterpenoids in EO samples compared to DeepSpectra® samples. On average of the three samples, monoterpenoids comprised 91.4% of EO samples and 88.0% of DeepSpectra® samples. Sesquiterpenoids comprised an average of 7.8% of EO samples and 11.1% of DeepSpectra® samples. These data suggest that the DeepSpectra® process increases the sesquiterpenoid recovery efficiency. Similar research has been conducted in the Intermountain region on naturally exuded resins from *Pseudotsuga menziesii* and *Pinus contorta* [8, 9], where the volatile profiles were also primarily composed of monoterpenoids. However, the volatile profile of *P. monophylla* resin EO is more similar to that of *P. menziesii* (α-pinene 57.7%) than to that of *P. contorta* (α-pinene 9.3%), despite the two species sharing the same genus.

LC/MS analysis detected abietic acid (avg. 6.3 mg/mL) in the DeepSpectra® samples. While abietic acid appears to be present in the resin of multiple *Pinus spp*., it is also present in non-coniferous plant species [13, 14]. Previous studies have investigated the potential medicinal properties of abietic acid, which has been used as an antifungal, antiviral, anti-inflammatory, and oncological agent [14-16]. Although, the current study does not focus on the purported health benefits of abietic acid, future studies should investigate the biological activity of abietic acid and other possible compounds, as it interacts with other naturally derived terpenoids from *P. monophylla* resin. Additionally, it is expected that other non-volatile compounds (diterpenoids, etc.) were extracted from the spent resin through DeepSpectra® extraction. However, additional reference standards are needed to determine their identification, which will also be the focus of future research (Fig. 2).

4. Conclusions

The current study investigated the terpenoid profile of *P. monophylla* resin. While typical terpenoid

Table 5. Volatile compounds detected (≥ 0.5 %) in at least one *Pinus monophylla* essential oil (EO) or DeepSpectra® (DS) samples.

Compound name	KI	*P. monophylla* EO (area %)			*P. monophylla* DS (area %)		
		A	B	C	A	B	C
Tricyclene	921	0.3	0.3	0.3	0.3	0.3	0.2
α-Thujene	924	0.4	0.5	0.7	0.3	0.5	0.6
α-Pinene	932	84.4	84.6	80.4	83.1	82.3	74.7
Camphene	946	1.3	1.2	1.1	1.1	1.2	1.0
Thuja-2,4(10)-diene	953	0.4	0.6	0.4	0.3	0.5	0.4
Sabinene	969	0.1	0.1	0.4	0.1	0.1	0.3
β-Pinene	974	0.9	0.9	1.0	0.8	0.8	0.9
Myrcene	988	0.2	0.1	0.1	0.1	0.1	0.1
δ-3-carene	1008	1.4	1.9	3.8	1.3	1.8	3.5
p-Cymene	1020	0.4	0.5	0.6	0.3	0.4	0.5
Limonene	1024	1.0	1.0	1.0	0.9	0.9	1.0
(Z)-β-ocimene	1032	0.1	0.1	0.1	0.1	0.1	0.1
γ-Terpinene	1054	0.1	0.1	0.1	0.1	0.1	0.2
Terpinolene	1086	0.2	0.1	0.3	0.1	0.1	0.3
α-Campholenal	1122	0.1	tr	0.1	0.1	0.1	0.1
(E)-Pinocarveol	1135	0.1	0.1	0.1	0.2	0.2	0.2
(E)-Verbenol	1140	0.2	tr	0.1	0.2	0.1	0.2
Ethyl octanoate	1196	0.1	tr	tr	0.1	0.1	0.1
Carvone	1239	nd	nd	nd	tr	tr	0.1
Bornyl acetate	1283	0.2	0.1	0.1	0.2	0.1	0.2
α-Cubebene	1348	0.2	0.3	0.3	0.3	0.3	0.4
α-Ylangene	1373	0.1	0.1	0.1	0.1	0.1	0.1
α-Copaene	1374	2.6	2.5	3.1	3.1	2.9	4.4
β-Bourbonene	1387	1.4	1.0	1.4	1.6	1.2	2.2
Longifolene	1407	0.8	1.1	1.0	1.0	1.3	1.6
(E)-Caryophyllene	1417	0.2	0.1	0.2	0.2	0.2	0.3
β-Copaene	1430	0.1	0.1	0.1	0.2	0.1	0.2
γ-Muurolene	1478	0.5	0.6	0.7	0.8	0.8	1.3
Germacrene D	1480	0.3	0.1	0.3	0.4	0.1	0.5
γ-Amorphene	1495	nd	nd	nd	0.1	0.1	0.2
α-Muurolene	1500	0.5	0.4	0.5	0.7	0.6	1.0
γ-Cadinene	1513	0.2	0.2	0.2	0.3	0.3	0.5
δ-Cadinene	1522	0.6	0.7	0.8	1.0	1.0	1.6
α-Calacorene	1544	nd	nd	nd	tr	tr	tr
Total		***99.2***	***99.4***	***99.3***	***99.4***	***99.2***	***99.0***

The compound name, KI, and relative area % are reported. KI is the Kovat's Index value, (manually calculated) and was previously calculated by Robert Adams using a linear calculation on a DB-5 column [12].

Table 6. Non-volatile compounds detected in *Pinus monophylla* DeepSpectra® (DS) samples.

Compound name	*P. monophylla* DS (mg/mL)		
	A	B	C
Abietic acid	6.2	6.2	6.6

Compounds were measured in mg/mL. None of the below mentioned non-volatile compounds were detected in the *P. monophylla* essential oil samples (LOD 2 μg/mL).

investigations may include only a volatile profile characterization of the essential oil (analysis by GC/MS), this study differed in that: (1) the hydrodistilled essential oil (EO) was used as a solvent for a secondary extraction (DeepSpectra® extraction) on spent resin, and (2) LC/MS analysis was conducted for a non-volatile compound analysis on both sample types.

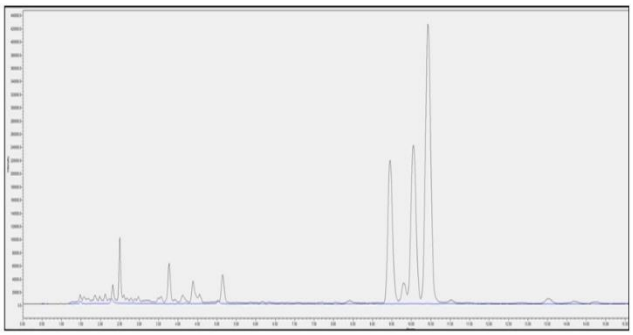

Figure 2. LC/MS chromatographic overlay of *Pinus monophylla* essential oil (blue) and DeepSpectra® sample (black) at SIR 303.22 m/z. Abietic acid peak at 10.430 min.

The volatile profiles were somewhat similar between the sample types, EO and DeepSpectra® samples, with prominent compounds of α-pinene (avg. 83.2%, 80.0%), δ-3-carene (avg. 2.4%, 2.2%), and α-copaene (avg. 2.7%, 3.5%), respectively.

DeepSpectra® samples contained an average of 6.3 mg/mL abietic acid, as well as other non-volatile compounds. Neither abietic acid, nor additional unidentified non-volatile compounds were detected in any of the EO samples. Future studies will focus on the procurement of reference standards and the identification of aforementioned non-volatile compounds for a more thorough terpenoid profiling of *P. monophylla* resin extractions.

Abbreviations

EO: Essential Oil
DCM: Dichloromethane
DS: DeepSpectra®
GC/MS: Gas Chromatography/Mass Spectrometry
LC/MS: Liquid Chromatography/Mass Spectrometry.

Patents

United States Patent Application Publication Number: US 2024/0084217 A1. Publication Date: 14 March 2024. Publication Title: METHODS AND SYSTEMS FOR EXTRACTING ADDITIONAL BENEFICIAL LIPID-SOLUBLE COMPOUNDS FROM PLANT MATERIALS IN ENVIRONEMENTIALLY SUSTAINABLE WAYS.

Authors' contributions

Conceptualization, T.M.W., H.K.L., R.B.J., C.P.; sample procurement and production, T.M.W., R.B.J.; methodology, T.M.W., M.C.J.; software, T.M.W., M.C.J.; validation, C.R.B.; formal analysis, T.M.W., M.C.J.; data curation, T.M.W., M.C.J.; writing—original draft preparation, T.M.W., M.C.J.; writing—review and editing, H.K.L, C.P., C.R.B.; funding acquisition, C.R.B.

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Availability of data and materials

All data are either presented within the current manuscript.

Conflicts of interest

The authors declare no conflict of interest. While the funders (Young Living Essential Oils) hold the patent for DeepSpectra® technology, the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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