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Research Article

Essential oil composition and enantioselective profile of *Juniperus communis* var. *depressa* (Cupressaceae) from Utah

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Abstract

Juniperus communis var *depressa* (common juniper) is an essential oil-bearing evergreen shrub native to North America, including the state of Utah. Cones (berries), leaves, and limb material were collected, and steam distilled together. The resulting essential oil samples ($n = 3$) were analyzed, and both the achiral and chiral profiles established by GC/FID, GC/MS, enantioselective GC/FID, and molecular rotational resonance (MRR). Prominent compounds of the achiral profile include (averages) α-pinene (63.9%), β-pinene (6.2%), myrcene (6.9%), δ-3-carene (6.8%), and limonene (3.3%). Four prominent chiral pairs (α-pinene, β-pinene, δ-3-carene, limonene) were analyzed to determine the enantioselective profile. Where enantiopure standards were not commercially available, (-)-δ-3-carene, MRR was used as a quick and reliable analytical technique for chiral analysis. This study verifies the achiral profile for North American common juniper and, for the first time to the authors' knowledge, establishes the achiral and chiral profiles for *J. communis* var. *depressa*, specifically from Utah. This study confirms the utility and practicality of using MRR for determining chiral profiles in essential oils. Additionally, results provide a foundation for future research in the flavor and fragrance industries for common juniper of North American origin.

1. Introduction

Common juniper (*Juniperus communis* L.) is a small coniferous evergreen shrub or tree in the Cupressaceae family, and the most widespread juniper species in the world [1, 2]. This circumboreal species is native to both the Eastern and Western Hemispheres and is subject to much geographic variation [1,3]. Nine different varieties of *J. communis* have been identified worldwide [3]. Species from the eastern and western hemispheres can easily be distinguished through genetic investigation [4, 5]. In the western hemisphere, distinguishing the native varieties of *J. communis* is more difficult, but currently there are five accepted varieties of the species based on DNA and morphology: var. *charlottensis*, var. *depressa*, var. *jackii*, var. *megostocarpa*, and var. *saxatilis* [4, 6]. *J. communis* var. *depressa* is common throughout North America [2, 7, 8].

The only documented variety of common juniper in Utah is *Juniperus communis* var. *depressa* Pursh [1,9-12]. Typically, it is found in alpine regions among aspen

and spruce-fir communities, is less than 1 m in height, has awl shaped leaves in whorls of three that are dark green with a white band on the upper portion, and is generally dioecious, although it can be monecious [1,10-12]. The berry-like cones ripen and mature from green to dark blue-black over 2 years [1,10-13].

One of the most common uses of juniper cones is in making beverages, liqueurs (Borovička and Steinhäger), and in flavoring gin [1, 2, 11]. During the second World War, North America was cut off from European sources of commercial juniper cones (typically var. *erecta*), and several attempts were made to replace the demand with domestic sources (var. *depressa*). While domestic supplies were mostly considered inferior to European supplies, due to a turpentine-like off-note, some sources contained a similar aroma and flavor to var. *erecta* [2].

Since each volatile compound, and enantiomer, displays a unique aroma and flavor, the achiral and chiral essential oil composition are integral to understanding the use of common juniper essential oil in the flavor and fragrance industry. Many different varieties exist with established achiral essential oil [14-21] and chiral (enantioselective) profiles [16-23]. To the author's best knowledge, the essential oil composition and enantioselective profile of *J.* communis var. *depressa* from Utah have never been characterized. In this study limb, leaf, and cone material of *J. communis* var. *depressa* were steam distilled together, and the resulting essential oil analyzed by GC/FID, GC/MS, enantioselective GC, and molecular rotational resonance (MRR). The achiral, essential oil composition was determined by gas chromatography and enantioselective profile established utilizing gas chromatography and MRR, which characterizes volatile compounds in the gas phase through their characteristic pure rotational momentum transitions [24]. As MRR is an extremely high-resolution spectroscopic technique and is highly sensitive to slight changes in a molecule's three-dimensional mass distribution, distinct compounds (including isomers), using the chiral tagging technique, can be identified and quantified in a mixture without the need for enantiopure reference standards or chromatographic separation [24-27]. Results provide a foundation for future research in the flavor and fragrance industries for common juniper of North American origin.

2. Materials and methods

Juniperus communis var *depressa* plant material (cones, leaves, limb) was collected during the third week of August 2020 from private land in Tabiona, UT, USA. Plant material was collected from the following location: 40°20′43″ N 110°45′10″W (elevation 2350 m) and stored at -20 ± 2 °C until distillation. A representative voucher sample is held in the Young Living Aromatic Herbarium (YLAH): *Juniperus communis* var. *depressa* Pursh, Wilson 2021-01 (YLAH).

Prior to distillation, the frozen limbs material was cut into 5-10 cm segments and all plant parts (cones, leaves, limbs) were distilled together/simultaneously. Laboratory-scale distillation was as follows: 3 L of water was added to the bottom of a 12 L distillation chamber (Albrigi Luigi S.R.L., Italy), plant material accurately weighed and added to the distillation chamber, distillation for 2 hours from pass-over by direct steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples ($n = 3$) were filtered and stored in a sealed amber glass bottle until analysis. For simplicity and consistency, each sample will be referred to by a letter, A-C.

Essential oils 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, 60 m x 0.25 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in ethanol), 150:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 5 min, oven ramp rate of 4.5 °C per minute to 310 °C with a hold time of 5 min, helium carrier gas. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library (version 4) [28] using Chemstation library search in conjunction with retention indices. Note that limonene/β-phellandrene and epi-α-cadinol/epi-α-muurolol elute as unresolved peaks. Their ratios were determined by the ratio of masses 41, 68, 93 (limonene), 65, 77, 93 (β-phellandrene) and 81, 105, 161 (epi-α-cadinol), 43, 95, 121 (epi-α-muurolol), respectively. Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B and Agilent J&W DB-5, 60 m x 0.25 mm, 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 injection, initial oven temperature at 40 °C with an initial hold time of 2 min, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 min, helium carrier gas. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were assigned using retention indices coupled with the retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, USA).

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.

Enantioselective analysis was performed on chiral compounds that had an average area > 2% (achiral profile). Essential oils were analyzed, and chiral pairs identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Restek Rt-β, 30 m x 0.32 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.2 µL of sample (0.5% soln. for essential oils in ethanol), 25:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 20 min, oven ramp rate of 2.0 °C per minute to 140 °C with a hold time of 35 min, second oven ramp rate of 30.0 °C per minute to 230 °C with a hold time of 2 min, helium carrier gas. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library (version 4) [28] using Chemstation library search. Chiral pairs were quantified and are reported as enantiomeric excess (ee %) by GC/FID using an Agilent 7890B and Restek Rt-β, 30 m x 0.32 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.2 µL of sample (0.5% or 2% soln. for essential oils in ethanol, 0.1% for enantiopure reference compounds in ethanol), 10:1 split injection, initial oven temperature at 40 °C with an initial hold time of 20 min, oven ramp rate of 2.0 °C per minute to 140 °C with a hold time of 35 min, second oven ramp rate of 30.0 °C per minute to 230 °C with a hold time of 2 min, helium carrier gas. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 0.5 when calculating ee % for each chiral pair). Enantiopure reference standards were used for (-)-α-pinene, (+)-α-pinene, (+)-β-pinene, (-)-β-pinene, (+)-δ-3-carene, (S)-(-)-limonene, (R)-(+)-limonene (MilliporeSigma, Sigma-Aldrich, St. Louis, USA). Black pepper (*Piper nigrum* L.) essential oil was used for identification of the (-)-δ-3-carene enantiomer (Young Living Essential Oils, Lehi, USA).

$$ee\ (\%) = \frac{area\ \%\ of\ predominant\ enantiomer - area\ \%\ of\ minor\ enantiomer}{area\ \%\ of\ predominant\ enantiomer + area\ \%\ of\ minor\ enantiomer} \times 100$$

The enantiomeric composition of δ-3-carene and α-pinene in the common juniper essential oil samples was also measured with MRR, using the chiral tagging technique. In these measurements, the analytes of interest are mixed with a small chiral molecule (tag) of known enantiomeric composition to prepare noncovalently bound diastereomeric complexes in the gas phase. These complexes, which have different mass distributions, can be resolved using MRR [26]. First, a broadband MRR spectrometer was used to characterize the MRR spectrum of each compound and to select the appropriate chiral tag. Racemic δ-3-carene (MilliporeSigma, Sigma-Aldrich, St. Louis, USA) and a commercial sample of (-)-a-pinene (TCI America, Portland, USA) were used for this screening. Neon carrier gas, pre-mixed with the chiral tag of interest at approx. 0.1% concentration, was seeded with the vapor from the analyte sample. The samples had enough vapor pressure at room temperature to achieve sufficient analyte concentration in the gas phase. The resulting gas mixture was then injected into a high vacuum chamber through a pulsed supersonic expansion nozzle to create a rotationally cold sample for analysis. The structures of the resulting non-covalent complexes formed between the analyte and chiral tag are determined by comparison of the experimentally derived rotational constants to those calculated from quantum chemical calculations using dispersion-corrected density functional theory (B3LYP-GD3BJ/def2TZVP) [29]. For δ-3-carene, 2,2,2-trifluoroisopropanol (TFIP) was used as the chiral tag, while propylene oxide (PO) was used for the α-pinene enantiomer measurement. A racemic tag sample was used in these measurements to generate the two

diastereomeric complexes of analyte and tag at equal concentration.

After this initial characterization, the targeted IsoMRR spectrometer (BrightSpec, Inc., Charlottesville, USA) was used to measure chiral purity of these two analytes in the common juniper oil samples [26]. The IsoMRR instrument can run measurements more quickly, and with much lower sample consumption, due to the use of a cavity to enhance the measurement sensitivity. In these measurements, 5 μL of the neat oil was injected into the sample inlet, which was held at 30°C. Strong lines of the two diastereomeric complexes of the analyte and tag were measured to determine the enantiomeric composition of each analyte in the sample. The instrument response to the two complexes was calibrated by additionally measuring the analyte signal levels using a racemic tag sample. The total measurement time per sample was approximately 18 minutes for each analyte. Additional details of the analyses can be found in Supplementary Table S1 –S8 and Figure S1- S2.

3. Results and discussion

The aromatic profile of *Juniperus communis* var. *depressa* is detailed in Table 1. Essential oil samples were analyzed in triplicate to ensure reproducibility (standard deviation < 1 for all compounds). Prominent compounds (defined as averages > 2%) detected included α-pinene (63.9%), β-pinene (6.2%), myrcene (6.9%), δ-3-carene (6.8%), and limonene (3.3%), averaged over all samples. The achiral profile established herein shows similarities to previously established results for *J. communis* var. *depressa* from New Mexico, with α-pinene (53.9%), β-pinene (5.5%), myrcene (4.1%), δ-3-carene (9.3%), and limonene (2.6%) also comprising most of the essential oil profile [14]. However, from the same study, *J. communis* (common juniper) samples of North American origin from different taxonomic varieties demonstrated widely varying profiles. Similar findings were observed when comparing the profiles from the current study to those of European and North African origin, which sources are of economic relevance.

Previously published literature revealed that most sources of common juniper analyzed (Algeria, Austria, Estonia, Italy, Lithuania, Poland) share some achiral profile similarities, with prominent compounds and values largely being α-pinene (10.3-90%), myrcene

Table 1. Aromatic profile of *Juniperus communis* var *depressa* essential oil (n = 3).

KI	Compound	A	B	C
849	Ethyl isovalerate	t	t	t
869	Isopentyl acetate	t	t	t
921	Tricyclene	0.1	0.1	0.1
924	α-Thujene	t	t	t
932	α-Pinene	66.8	63.9	61.1
945	α-Fenchene	0.4	0.4	0.4
946	Camphene	0.6	0.6	0.6
953	Thuja-2,4(10)-diene	0.1	0.1	0.1
969	Sabinene	0.3	0.3	0.3
974	β-Pinene	6.3	6.1	6.2
988	Myrcene	6.1	7.4	7.1
997	Ethyl hexanoate	t	t	t
1001	δ-2-Carene	0.3	0.2	0.2
1002	α-Phellandrene	0.1	0.1	0.1
1005	o-Cresol methyl ether	0.1	0.1	0.1
1008	δ-3-Carene	6.0	6.3	8.0
1014	α-Terpinene	0.1	0.1	0.1
1020	p-Cymene	0.1	0.1	0.1
1024	Limonene	2.9	3.6	3.4
1025	β-Phellandrene	1.2	1.1	1.2
1032	(Z)-β-Ocimene	t	t	t
1044	(E)-β-Ocimene	t	t	t
1054	γ-Terpinene	0.1	0.1	0.1
1085	p-Mentha-2,4(8)-diene	0.1	0.1	0.1
1086	Terpinolene	0.8	0.9	1.0
1095	Linalool	0.2	0.3	0.2
1102	Isopentyl isovalerate	t	0.1	0.1
1112	3-Methyl-3-butenyl-3-methyl-butanoate	0.1	0.1	0.1
1122	α-Campholenal	0.1	0.1	0.2
1135	trans-Pinocarveol	0.1	0.1	0.1
1137	cis-Verbenol	t	t	t
1140	trans-Verbenol	0.1	0.1	0.1
1148	Citronellal	0.1	0.1	0.1
1165	Borneol	t	t	t
1166	p-Mentha-1,5-dien-8-ol	t	t	0.1
1172	cis-Pinocamphone	t	t	t
1174	Terpinen-4-ol	0.1	0.1	0.1
1179	p-Cymen-8-ol	t	t	t
1186	α-Terpineol	0.1	0.1	0.1
1194	Myrtenol	t	t	t
1195	Myrtenal	t	t	t
1223	Citronellol	0.2	0.2	0.3
1232	Thymol methyl ether	t	t	t
1249	Geraniol	t	t	t
1257	Methyl citronellate	0.2	0.2	0.2

Table 1. *(Continued)*

KI	Compound	A	B	C
1284	Bornyl acetate	0.4	0.5	0.5
1324	Myrtenyl acetate	0.7	0.7	0.9
1346	α-Terpinyl acetate	0.6	0.8	0.9
1350	Citronellyl acetate	0.1	0.1	0.1
1356	Eugenol	0.1	0.1	0.1
1359	Neryl acetate	0.1	0.1	0.1
1379	Geranyl acetate	0.2	0.2	0.2
1385	trans-Myrtanol acetate	0.1	0.1	0.1
1389	β-Elemene	0.3	0.3	0.3
1417	(E)-Caryophyllene	0.1	0.1	t
1434	γ-Elemene	0.1	0.1	0.1
1452	α-Humulene	0.1	0.1	t
1478	γ-Muurolene	t	t	0.1
1480	Germacrene D	0.2	0.3	0.3
1489	β-Selinene	t	t	t
1500	α-Muurolene	0.4	t	t
1505	β-Bisabolene	0.1	0.1	0.2
1513	γ-Cadinene	0.1	0.1	0.2
1522	δ-Cadinene	0.3	0.3	0.4
1537	α-Cadinene	t	t	t
1559	Germacrene B	t	t	t
1561	(E)-Nerolidol	0.1	0.1	0.1
1574	Germacrene D-4-ol	0.1	0.1	0.1
1577	Spathulenol	t	t	0.1
1608	Humulene epoxide II	t	t	t
1638	epi-α-Cadinol	t	t	t
1640	epi-α-Muurolol	t	t	t
1644	α-Muurolol	t	t	t
1652	α-Cadinol	0.1	0.1	0.1
1685	α-Bisabolol	0.2	0.2	0.3

Each sample is referred to by a letter, A-C. The Kovat's Index (KI), volatile compound name, and compound average area % for each sample are provided. Each essential oil sample was analyzed in triplicate to ensure reproducibility (Standard Deviation < 1 for all values). Values less than 0.1% are denoted as trace (t). The KI values were previously calculated and obtained using a linear calculation on DB-5 column [28].

(1.8-52.4%), and limonene (0.2-15.8%) [16-21]. However, many common juniper samples also contained additional or different prominent volatile compounds such as sabinene (12.4-42.5%) [16, 19, 20], β-phellandrene (19.1%) [19], γ-terpinene (11.8%) [19], terpinene-4-ol (14.1%) [16], (E)-caryophyllene (10.3-11.4%) [18,20], and/or caryophyllene oxide (17.9%) [18]. The variability in these profiles was credited to sample origin, chemotype, morphotype, and/or plant part from which the essential oil was extracted [16-21].

Enantiopure reference standards were commercially available for 3 of 4 prominent (defined as averages > 2%) chiral pairs found in these essential oils but was not available for δ-3-carene. Chiral tagging molecular rotational resonance (MRR) was therefore used to perform the chiral analysis of δ-3-carene in common juniper essential oil. Fig. 1 illustrates the analysis of δ-3-carene by chiral tagging MRR. The geometries of the non-covalent complexes of δ-3-carene and TFIP used in the analysis are shown. These complexes are named either as homochiral (where the optical rotations of the analyte and tag are the same, e.g. (+)-δ-3-carene / (+)-(*R*)-TFIP) or heterochiral (where the optical rotations are different[1]). When a racemic chiral tag ((*RS*)-TFIP) is used, the signals of the two complexes are approximately the same; but in the measurement with enantiopure (*R*)-TFIP, only the homochiral complex is observed, allowing us to conclude that (+)-δ-3-carene is the major enantiomer in common juniper oil. Due to the low fractional composition of δ-3-carene in the common juniper oil and the resulting reduced signal intensity in the chiral tag complexes, we were not able to detect the weaker enantiomer of δ-3-carene and are only able to determine a lower limit of ee% >85%.

To illustrate the similarity between values from conventional techniques (GC/MS and GC/FID) and the novel application of chiral tagging MRR for determining chiral profiles, ee% was determined for α-pinene using both techniques (Table 2). The results show the same trend in enantiomeric excess between the three fractions. Values (ee%) compared between each technique agree within 6% or less. We noted a systematic offset between the GC/FID and MRR results of approximately 5% but were not able to determine the source of this difference. Previous studies have validated the quantitative accuracy of MRR in comparison to chiral GC [26, 30]. Determining the systematic offset between values is beyond the scope of the current study and will be explored in future studies.

Using ee% data (MRR) and elution order of compounds (GC/MS) from the current study, ee% was determined for δ-3-carene by GC/FID. In addition, and using enantiopure reference standards, chiral pairs were analyzed in the current study for α-pinene, β-pinene, and limonene. In these samples (-)-α-

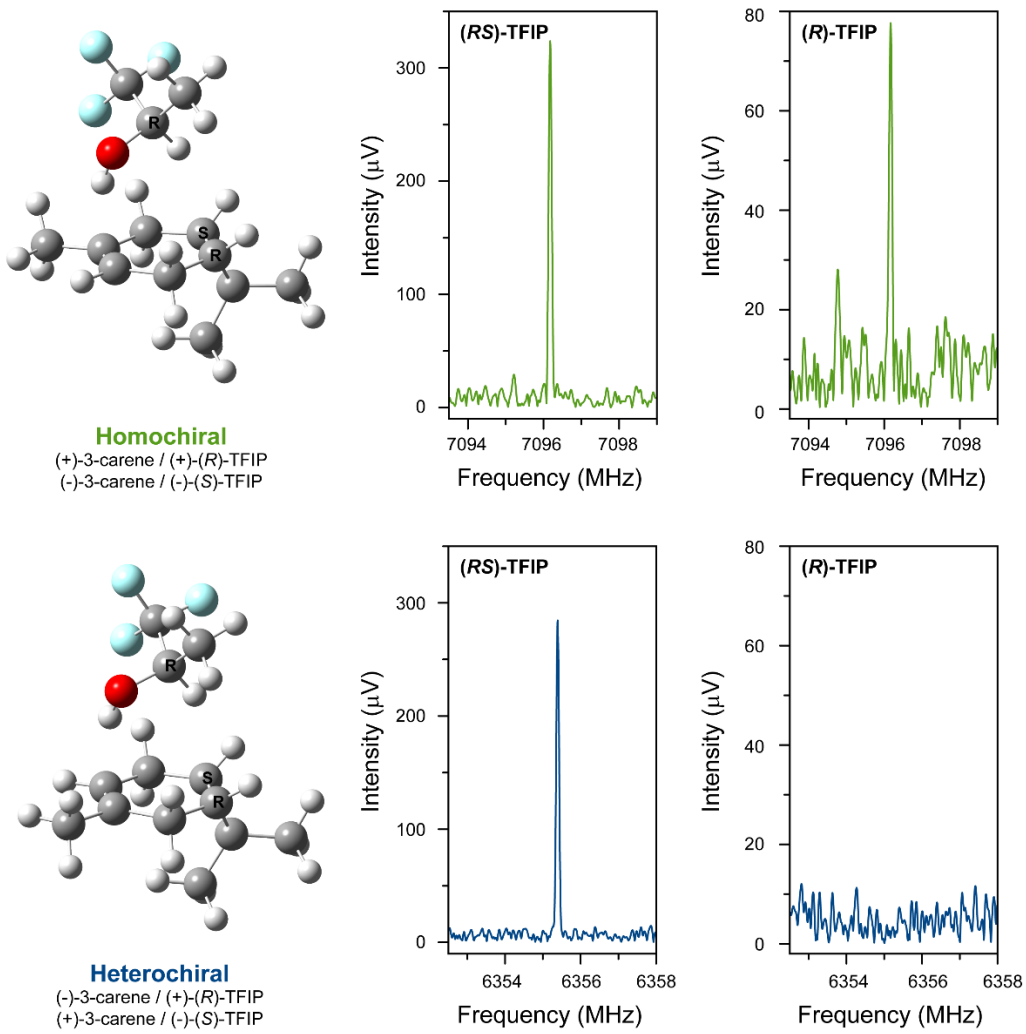

Figure 1. Chiral analysis of δ-3-carene (3-carene) by chiral tagging MRR. The complex geometries of the assigned spectra that are used for the analysis are shown in the left panel. The middle panel shows the measurement of a standard of δ-3-carene with racemic TFIP, which is used to normalize the instrument response. The right panel shows the measurement of the common juniper EO sample with (*R*)-TFIP (ee=94.6%).

Table 2. Enantiomeric excess (ee%) of α-pinene in *Juniperus communis* var. *depressa* essential oil determined by GC/FID and molecular rotational resonance (MRR).

Method	Enantiomer	A	B	C
GC/FID	(-)-α-Pinene	67.7	62.1	71.1
	(+)-α-Pinene	-	-	-
MRR	(-)-α-Pinene	62.8	56.7	67.9
	(+)-α-Pinene	-	-	-

Each sample (n = 3) is referred to by a letter, A-C. Each essential oil sample was analyzed in triplicate to ensure repeatability (standard deviation for analysis by GC/FID ≤ 0.3 for repeat injections; standard deviation for analysis by MRR < 3% for repeat injections). Values (ee%) between techniques agree within 6% or less.

pinene, (-)-β-pinene, (+)-δ-3-carene, and (R)-(+)-limonene were the prominent enantiomers (Table 3). For β-pinene, comparable results were found in samples of European and North African origin, where (-)-β-pinene was the prominent enantiomer [16, 17, 19]. However, enantiomeric prominence appears to switch between (-)/(+)-α-pinene and (S)-(-)/(R)-(+)-limonene [16-19, 21, 22] depending on sample origin, chemotype, morphotype, and/or plant part from which the essential oil was extracted. To the authors' best knowledge, this is the first time that chiral analysis of δ-3-carene has been performed in common juniper samples of any region.

In addition to investigating the chiral profile of common juniper samples, the current study verifies chiral chromatography from previous studies on other plant species. While (+)-δ-3-carene enantiopure reference standards are commercially available, researchers have historically relied on natural standards of black pepper (*Piper nigrum*) essential oil to reference the (-)-δ-3-carene enantiomer [31-34]. The

Table 3. Enantiomeric excess of chiral compounds that had an average area % (achiral profile) > 2% for *Juniperus communis* var. *depressa*.

Enantiomer	A	B	C
(-)-α-Pinene	67.7	62.1	71.1
(+)-α-Pinene	-	-	-
(+)-β-Pinene	-	-	-
(-)-β-Pinene	95.4	94.8	94.8
(+)-δ-3-Carene	>99.0	>99.0	>99.0
(-)-δ-3-Carene	-	-	-
(S)-(-)-Limonene	-	-	-
(R)-(+)-Limonene	39.5	48.4	42.3

Chiral ratios (calculated as ee%) determined by GC/FID. Each sample (n = 3) is referred to by a letter, A-C. Each essential oil sample was analyzed in triplicate to ensure repeatability (standard deviation for analysis by GC/FID ≤ 0.3 for all compounds). For δ-3-carene, the (-)-δ-3-carene enantiomer was not detected in any sample. The value (>99.0) was determined based on the GC/FID limit of detection.

use of MRR in this study verifies that authentic and natural sources of black pepper essential oil can be used as a reference for (-)-δ-3-carene.

Essential oil yield is detailed in Table 4. The average essential oil yield for *J. communis* var. *depressa* is 0.14%(w/w). Yields from the current study are lower than those of European and North African origin (0.2-1.6%) [16, 20, 22, 23].

To the authors' knowledge, this is the first time that the achiral and chiral profiles of *Juniperus communis* var. *depressa* of Utahn origin has been fully detailed.

4. Conclusions

The achiral profile established herein is similar to that of *J. communis* var. *depressa* from New Mexico, and both the achiral and chiral profiles from the current study show similarities to profiles of other varieties of *J. communis* from European and North African origin. However, previous studies have shown that there is substantial variation in both achiral and chiral profiles of *J. communis* essential oil from commercially important sources (Europe and North Africa). The profiles established herein provide fundamental data for understanding the potential use of *J. communis* of North American origin in the flavor and fragrance industry. Given that juniper cones (berries) are highly sought after in the flavor and fragrance industry, essential oil extracted from only cones (berries) of *J. communis* var. *depressa* should be investigated in future studies.

The current study also demonstrates the utility and practicality of using molecular rotational resonance (MRR) for determining chiral profiles in essential oils. Since a complete understanding of the achiral and chiral profiles are inherent to the utility of essential oils, MRR is a novel technique that could have important applications in the flavor and fragrance industry.

Table 4. Yield data, including weight of plant material distilled (g), essential oil yield (g), and calculated essential oil yield (%) for *Juniperus communis* var. *depressa* samples (n=3).

Plant Name	Plant Sample	Plant Material Weight (g)	Essential Oil Yield (g)	Essential Oil Yield (%)
J. communis var. *depressa*	A	1058.83	1.53	0.14
	B	1044.73	1.46	0.14
	C	1042.88	1.27	0.12
	Avg.	1048.81	1.42	0.14
	Avg. RSD (n = 3)			8.85

Each sample is referred to by a letter, A-C. The average calculated yield for samples is 0.14%. The relative standard deviation (RSD) is provided for essential oil yield.

Footnotes

[1] In other chiral tagging MRR studies it is more common to use the Cahn-Ingold-Prelog nomenclature to name complexes as homochiral or heterochiral; however, as δ-3-carene has two chiral centers with opposite nomenclature, we use the optical rotations instead.

Supplementary Data

Supplementary Table S1-S8

Supplementary Figure S1-S2

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Authors' contributions

Conceptualization, A.P., T.M.W., and R.E.C.; methodology, A.P., T.M.W., R.E.S., and A.D.; software,

A.P., T.M.W., R.E.S., and A.D.; validation, J.L.N and R.E.C..; formal analysis, A.P., T.M.W., R.E.S., and A.D.; investigation, A.P. and T.M.W.; data curation, T.M.W. and R.E.S.; writing—original draft preparation, A.P., T.M.W., R.E.S, and J.L.N; writing—review and editing, A.P., A.D., R.E.S., J.L.N., and R.E.C.; funding acquisition, 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

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

Conflicts of interest

Authors R.E.S. and J.L.N. have equity in BrightSpec, Inc.

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