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Research Article

An investigation of cones (berries) of *Juniperus spp.* of Utahn origin as a flavoring source for gin

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Abstract

Juniperus spp., cones (berries), particularly *J. communis* of European origin, are routinely used to flavor gin and other beverages. *Juniperus communis* (common juniper) is a circumboreal species with multiple varieties native to both Eastern and Western Hemispheres. While native to North America, typically only berries of European origin are used in the flavor and fragrance industry. In the current study, ethanol extracts of common juniper berries of European origin (n = 3) were compared to common juniper berries of Utahn origin (var. *depressa*) (n = 3) and two other species native to Utah (*J. osteosperma*, *J. scopulorum*) (n = 6) to evaluate their suitability for flavoring purposes. Volatile oil profiles were established by GC/MS. Prominent compounds of *J. communis* (EU), *J. communis* var. *depressa*, *J. osteosperma*, and *J. scopulorum* include α-pinene (41.0%, 17.2%, 51.3%, 5.7%), myrcene (15.0%, 53.3%, 2.5%, 9.2%), and limonene (6.6%, 4.0%, 10.4%, 5.6%), respectively. However, clear differences exist between the volatile profiles of each species as well. While none of the studied species appear to be a suitable direct replacement for common juniper berries of European origin, each is a possible alternative that provides a unique volatile oil profile. This study establishes for the first time, to the authors' knowledge, the volatile profiles of berries from *J. communis* var. *depressa*, *J. osteosperma*, and *J. scopulorum* in full detail. Results from this study provide insights into the use of juniper berries from new sources as flavoring agents.

1. Introduction

Juniperus communis (common juniper) is a circumboreal species that is native to both Eastern and Western Hemispheres, with multiple varieties distinguished throughout the world [1]. One of the most well-known uses of juniper cones (berries), particularly from *J. communis*, is in making beverages/liqueurs (Borovička and Steinhäger) and in flavoring gin [2-4]. During the second World War, North America was cut off from European sources of commercial juniper berries (typically *J. communis* var. *erecta*), and attempts were made to replace the demand with domestic sources (*J. communis* 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 *J. communis* var. *erecta* [3]. Unfortunately, important factors like regional variation did not appear to have been considered when evaluating the flavor profile of juniper berries. While *J. communis* var. *depressa* is native to the state of Utah (USA), two other native species of the same genus are abundant throughout the state as well, *J. osteosperma* and *J. scopulorum* [5, 6]. To the authors' knowledge, none of these species are currently used as flavoring agents and the volatile profiles from the berries have never been fully reported.

The volatile compound profile is integral to understanding the aroma and flavor of juniper berries. In the current study, ethanol extractions of juniper berries of Utahn origin were analyzed and compared to those of common juniper from Europe to evaluate their utility for flavoring gin and other beverages.

2. Materials and methods

Juniper cones (berries) from 3 species native to Utah (*Juniperus communis* var. *depress*, *J. osteosperma*, *J. scopulorum*) were collected on September 3, 2021, from private land in Tabiona, UT, USA. Berries were collected from the following locations: *J. communis*, 40°20′43″ N 110°45′6″W (elevation 2350 m); *J. osteosperma*, 40°20′35″N 110°44′25″W (elevation 2329 m); *J. scopulorum*, 40°20′43″N 110°45′4″W (elevation 2380 m). A representative voucher sample of each species is held in the Young Living Aromatic Herbarium (YLAH): *Juniperus communis* var. *depressa* Pursh, Wilson 2021-02 (YLAH); *Juniperus osteosperma* (Torr.) Little, Wilson 2021-01 (YLAH); *Juniperus scopulorum* Sarg., Wilson 2021-01 (YLAH). Additionally, *J. communis* berries of European origin (Bulgaria) were purchased for comparative analysis (Starwest Botanicals, Sacramento, CA, USA) (Fig. 1).

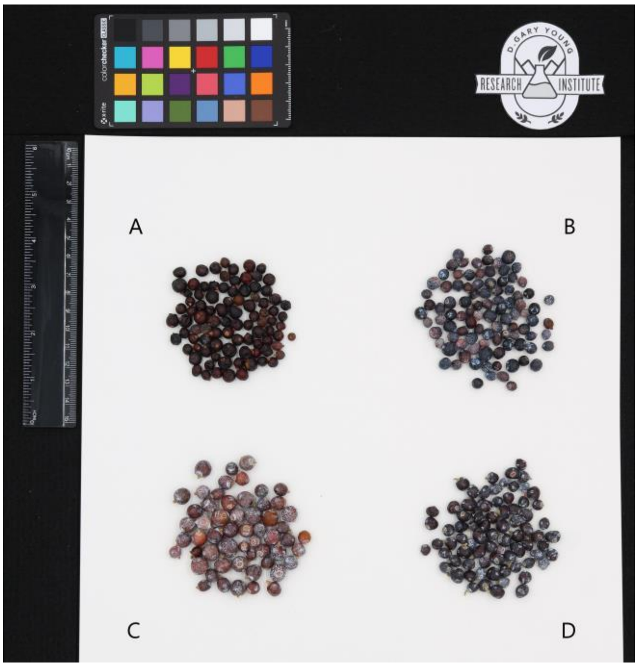

Figure 1. Visual characteristics of *Juniperus spp.* berries: (A) *J. communis* (Bulgarian origin), (B) *J. communis* (Utahn origin), (C) *J. osteosperma* (Utahn origin), and (D) *J. scopulorum* (Utahn origin).

Laboratory-scale extractions were performed in two steps as follows: First, an initial ethanol (Everclear, Luxco, St. Louis, MO, USA) extraction was performed (200 mL ethanol, 3 h) on the intact berries using a 200-mL (55/50) Soxhlet device (Wilmad Labglass, Vineland, NJ, USA), resulting in a light green-brown colored crude extract. Second, the crude extract was distilled (10 minutes) using a custom, 2-L stainless steel device (Fig. 2A). Referring to Fig. 2A, the crude extracts were placed and heated in modular section #1, separated from the non-volatile fraction as a gas phase in modular sections #2/3, and recondensed by the circulating water condenser in section #4. Samples (n = 12) resulted in a clear and colorless aromatic alcohol solution. This process is hereafter described as the '2-step' process.

Given the routine practice of using a gin-basket technique when producing the distilled alcohol (gin), a second distillation unit (Fig. 2B) was constructed to replicate this technique at a laboratory-scale. Referring to Fig. 2B, ethanol (Everclear, Luxco, St. Louis, MO, USA) was placed in section #5, *J. communis* (Bulgarian origin) berries suspended in section #6 by a tri-clamp mesh gasket, and gas-phase volatiles and ethanol (sections #7/8) are recondensed by the circulating water condenser in section #9. The resulting sample (n = 1) resulted in a clear and colorless aromatic alcohol. This process is hereafter described as the 'gin-basket' process.

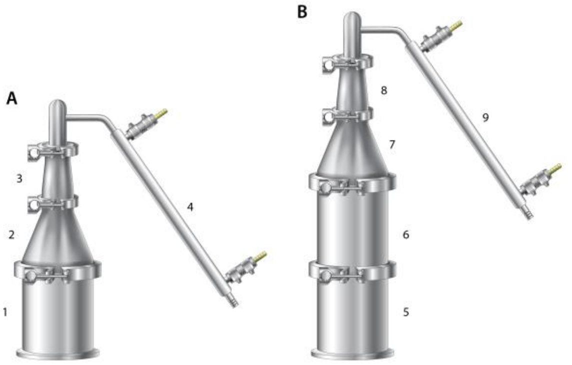

Figure 2. Illustration of custom stainless steel distillation devices. Modular sections referred to in the text are labeled by numbers. Illustrated by Rick Simonson, Science Lab Studios, Inc. (Kearney, NE, USA).

Samples (n = 13) were analyzed, and volatile 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 x 0.25 mm, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of the sample (splitless), 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 [7] using Chemstation library search in conjunction with retention indices. Note that limonene/1,8-cineole and (E)-caryophyllene/β-ylangene elute as unresolved peaks. Their ratios were determined by the ratio of masses 41, 68, 93 (limonene), 43, 81, 108 (1,8-cineole) and 41, 93, 133 ((E)-caryophyllene), 91, 120, 161 (β-ylangene), respectively.

3. Results and discussion

The aromatic profiles of *Juniperus communis* (common juniper) berries of European (Bulgaria) origin are provided in Table 1, for both the 'gin-basket' and '2-step' process. Prominent compounds (defined as > 2%) from the 'gin-basket' and '2-step' process include α-pinene (44.6%, 41.0%), sabinene (11.2%, 13.5%), β-pinene (2.7%, 2.8%), myrcene (16.5%, 15.0%), and limonene (7.0%, 6.6%), respectively. Values for each compound, when comparing the two extraction methods, are similar, with most standard deviations less than 1%. Exceptions include two prominent compounds, α-pinene (1.8%) and sabinene (1.2%). These findings suggest that the '2-step' process, while not the conventional process for producing flavoring for beverages, is suitable for lab-scale evaluation and assessment. The aromatic profiles established herein from European sourced common juniper berries are similar to those observed in previous studies from berries of European and North African origin, where α-pinene (10.3-90%), myrcene (1.8-52.4%), and limonene (0.2-15.8%) were reported as prominent compounds [8-14]. However, many common juniper samples, from berries and/or leaves, from these same geographic sources contained additional or different prominent volatile compounds such as sabinene (0.0-42.5%) [8,12,13], β-phellandrene (19.1%) [12], γ-terpinene (11.8%) [12], terpinene-4-ol (14.1%) [8], (*E*)-caryophyllene (0.8-11.4%) [11,13], and/or caryophyllene oxide (17.9%) [11]. The variability in these profiles was credited to sample origin, chemotype, morphotype, and/or plant part from which the volatile oil was extracted [8-14].

The aromatic profiles ('2-step' process) from berries of *J. communis* (Bulgarian) and those of Utahn origin (*J. communis* var. *depressa*, *J. osteosperma*, *J. scopulorum*) are provided in Table 2. Prominent compounds (defined as > 2%) present in *J. communis* (Bulgarian origin) include α-pinene (41.0%), sabinene (13.5%), β-pinene (2.8%), myrcene (15.0%), and limonene (6.6%). Prominent compounds present in *J. communis* var. *depressa* include α-pinene (17.2%), myrcene (53.3%), limonene (4.0%), β-ylangene (2.4%), β-copaene (2.2%), (*Z*)-β-farnesene (3.1%), and germacrene D (2.7%). Prominent compounds present in *J. osteosperma* include α-pinene (51.3%), sabinene (16.1%), myrcene (2.5%), limonene (10.4%), and bornyl acetate (5.4%). Prominent compounds present in *J. scopulorum* include α-thujene (4.3%), α-pinene (5.7%), sabinene (54.1%), myrcene (9.2%), limonene (5.6%), γ-terpinene (2.9%), terpinolene (2.4%), and pregeijerene B (2.2%). While, based on the aromatic profiles in this study, no *Juniperus spp.* of Utahn origin in this study would be feasibly considered a direct replacement for common juniper berries of European origin for flavoring gin or other beverages, both *J. communis* var. *depressa* and *J. osteosperma* are reasonable substitutes that provide similar flavoring characteristics to common juniper berries from Europe. In European (Bulgarian) common juniper berries, the 2 most prominent compounds, α-pinene and myrcene, are also the most prominent in *J. communis* var. *depressa*, however, the general ratios of those compounds are switched, with α-pinene being the prominent compound in European common juniper berries and myrcene being the prominent compound in Utahn common juniper berries (Fig. 3). When comparing profiles of European common juniper and *J. osteosperma* (Fig. 3), values of α-pinene, sabinene, and limonene are similar, however, *J. osteosperma* also contains more bornyl acetate (0.1% vs. 5.4%, respectively). Additionally, *J. osteosperma* contains the lowest concentration of myrcene of any species in this study. When comparing sesquiterpenoid profiles (Fig. 4), those of *J. communis* (from European and Utahn origin) are the most similar, with β-ylangene, β- copaene, (*Z*)-β-farnesene, and germacrene D being more prevalent than in the other two species evaluated. From this study, *J. scopulorum* would likely

Table 1. Aromatic profiles (GC/MS) of *Juniperus communis* berry (Bulgarian origin) by two extractions techniques, namely, the 'gin basket' and '2-step' process (see Materials and Methods for details).

KI	**Compound**	***Juniperus communis* (EU) 'gin-basket' process (area%)**	***Juniperus communis* (EU) '2-step' process (area%)**	**sd**
921	tricyclene	0.1	nd	-
924	α-thujene	2.0	nd	-
932	α-pinene	44.6	41.0	1.8
946	camphene	0.5	0.4	0.1
969	sabinene	11.2	13.5	1.2
974	β-pinene	2.7	2.8	0.0
988	myrcene	16.5	15.0	0.8
1002	α-phellandrene	0.2	0.1	0.1
1008	δ-3-carene	0.2	0.2	0.0
1014	α-terpinene	0.7	0.1	0.3
1020	p-cymene	1.5	1.2	0.1
1024	limonene	7.0	6.6	0.2
1026	1,8-cineole	t	t	0.0
1032	(*Z*)-β-ocimene	0.3	0.3	0.0
1044	(*E*)-β-ocimene	t	t	0.0
1054	γ-terpinene	1.4	1.2	0.1
1065	cis-sabinene hydrate	0.1	0.1	0.0
1086	terpinolene	1.0	0.4	0.3
1098	*trans*-sabinene hydrate	0.1	0.1	0.0
1100	n-nonanal	0.1	nd	-
1128	allo-ocimene	0.8	0.9	0.1
1174	terpinen-4-ol	0.5	0.4	0.1
1180[1]	verbenyl ethyl ether	0.1	0.1	0.0
1186	α-terpineol	0.1	0.1	0.0
1200	n-dodecane	0.3	0.7	0.2
1204	verbenone	t	t	0.0
1282	(*E*)-anethol	t	t	0.0
1284	bornyl acetate	nd	0.1	-
1289	thymol	0.2	nd	-
1298	carvacrol	1.0	nd	-
1348	α-cubebene	0.3	0.5	0.1
1356	eugenol	1.0	nd	-
1373	α-ylangene	t	0.1	0.1
1374	α-copaene	0.4	0.7	0.2
1400	n-tetradecane	0.6	1.1	0.3
1409	α-gurjunene	0.2	0.3	0.1
1417	(*E*)-caryophyllene	0.8	1.4	0.3
1419	β-ylangene	0.3	0.7	0.2
1430	β-copaene	0.5	0.8	0.2
1440	(*Z*)-β-farnesene	0.1	0.1	0.0
1452	α-humulene	0.5	0.9	0.2
1478	γ-muurolene	0.1	0.2	0.1
1480	germacrene D	0.3	0.7	0.2
1489	β-selinene	0.1	nd	-
1500	α-muurolene	0.2	0.4	0.1
1513	γ-cadinene	0.1	0.2	0.1
1522	δ-cadinene	0.4	0.7	0.2
1564	β-calacorene	nd	0.1	-

Table 1. (*Continued*)

KI	Compound	*Juniperus communis* (EU) 'gin-basket' process (area%)	*Juniperus communis* (EU) '2-step' process (area%)	sd
1577	spathulenol	nd	0.1	-
	Total	**98.8**	**96.1**	**N/A**

The Kovat's Index (KI), volatile compound name, compound average area % for each sample, and the standard deviation between compounds from each method are provided. Values less than 0.1% are denoted as trace (t). Compounds not detected in a sample are denoted as not detected (nd). The KI values were previously calculated and obtained using a linear calculation on DB-5 column [7]. [1]Indicates KI not previously calculated [7] and manual calculation was performed using alkane standards.

Table 2. Aromatic profiles (GC/MS) from berries of *Juniperus communis* (Bulgarian origin) and three *Juniperus spp.* native to the state of Utah (USA): *J. communis* var. depressa, *J. osteosperma*, *J. scopulorum* by the '2-step' extraction process (see Materials and Methods for details).

KI	Compound	*J. communis* (EU) (area%)	*J. communis* var. *depressa* (area%)	*J. osteosperma* (area%)	*J. scopulorum* (area%)
921	tricyclene	nd	nd	0.8	t
924	α-thujene	2.0	nd	0.5	4.3
932	α-pinene	41.0	17.2	51.3	5.7
946	camphene	0.4	0.2	0.9	0.2
969	sabinene	13.5	0.2	16.1	54.1
974	β-pinene	2.8	1.4	0.7	0.4
979	3-octanone	nd	nd	nd	0.1
988	myrcene	15.0	53.3	2.5	9.2
1002	α-phellandrene	0.1	nd	0.1	0.3
1008	δ-3-carene	0.2	1.2	0.1	0.1
1014	α-terpinene	0.1	nd	0.2	1.8
1020	p-cymene	1.2	0.1	0.6	0.7
1024	limonene	6.6	4.0	10.4	5.6
1026	1,8-cineole	t	t	t	t
1032	(*Z*)-β-ocimene	0.3	0.1	0.3	t
1036	benzene acetaldehyde	nd	nd	nd	0.1
1044	(*E*)-β-ocimene	t	nd	0.3	0.1
1054	γ-terpinene	1.2	0.1	0.4	2.9
1065	cis-sabinene hydrate	0.1	0.1	0.2	1.1
1086	terpinolene	0.4	0.3	0.4	2.4
1095	linalool	nd	0.3	nd	nd
1098	*trans*-sabinene hydrate	0.1	nd	0.1	1.3
1112	trans-thujone	nd	nd	nd	0.1
1122	α-campholenal	nd	nd	0.6	nd
1128	allo-ocimene	0.9	0.3	1.1	0.1
1135	*trans*-pinocarveol	nd	nd	0.2	nd
1141	camphor	nd	nd	0.8	nd
1148	citronellal	nd	0.1	nd	0.1
1160	pinocarvone	nd	nd	0.1	nd
1174	terpinen-4-ol	0.4	0.1	0.1	0.8
1180[1]	verbenyl ethyl ether	0.1	nd	0.5	nd
1186	α-terpineol	0.1	0.1	nd	t
1200	n-dodecane	0.7	0.5	0.7	0.2
1204	verbenone	t	nd	0.1	nd
1257	methyl citronellate	nd	0.2	nd	0.2
1274	pregeijerene B	nd	nd	nd	2.2
1282	(*E*)-anethol	t	t	t	nd
1284	bornyl acetate	0.1	0.1	5.4	0.1

Table 2. (*Continued*)

KI	Compound	*J. communis* (EU) (area%)	*J. communis* var. *depressa* (area%)	*J. osteosperma* (area%)	*J. scopulorum* (area%)
1285	pregeijerene	nd	nd	nd	0.7
1289	trans-sabinyl acetate	nd	nd	nd	0.1
1348	α-cubebene	0.5	0.3	nd	nd
1350	α-longipinene	nd	0.1	nd	nd
1373	α-ylangene	0.1	0.2	nd	nd
1374	α-copaene	0.7	0.2	0.1	t
1389	β-elemene	nd	1.1	nd	nd
1400	n-tetradecane	1.1	nd	0.4	0.1
1400	β-longipinene	nd	0.1	nd	nd
1407	longifolene	nd	0.3	nd	nd
1409	α-gurjunene	0.3	nd	nd	nd
1417	(*E*)-caryophyllene	1.4	1.1	0.5	0.4
1419	β-ylangene	0.7	2.4	t	0.2
1430	β-copaene	0.8	2.2	0.1	0.3
1432	α-*trans*-bergamotene	nd	nd	0.1	nd
1440	(*Z*)-β-farnesene	0.1	3.1	nd	nd
1452	α-humulene	0.9	1.5	0.2	nd
1478	γ-muurolene	0.2	0.1	nd	nd
1480	germacrene D	0.7	2.7	nd	nd
1500	α-muurolene	0.4	0.2	nd	nd
1505	β-bisabolene	nd	0.3	nd	nd
1513	γ-cadinene	0.2	0.2	nd	nd
1522	δ-cadinene	0.7	0.4	0.2	0.1
1548	elemol	nd	nd	nd	0.3
1564	β-calacorene	0.1	0.1	0.1	nd
1577	spathulenol	0.1	t	nd	nd
1582	caryophyllene oxide	nd	nd	0.2	nd
	Total	**96.1**	**96.5**	**97.4**	**96.4**

The Kovat's Index (KI), volatile compound name, compound average area % for each sample, and the standard deviation between methods are provided. Values less than 0.1% are denoted as trace (t). Compounds not detected in a sample are denoted as not detected (nd). The KI values were previously calculated and obtained using a linear calculation on DB-5 column [7]. Values are averages (n = 3) per species. ^{1}Indicates KI not previously calculated [7] and manual calculation was performed using alkane standards.

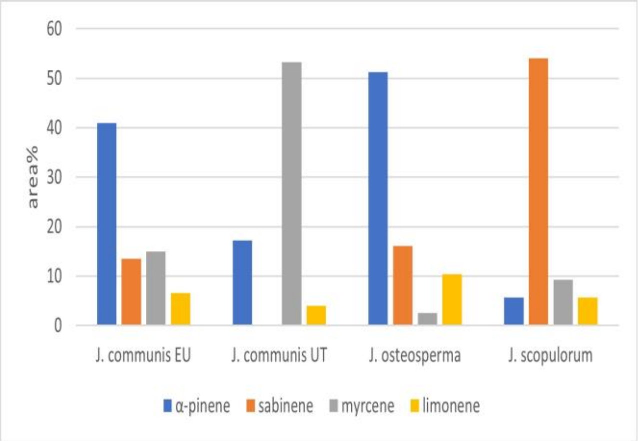

Figure 3. Relative abundance (area%) of prominent volatile compounds (terpenoids) in *Juniperus communis* (EU/Bulgarian origin), *J. communis* var. *depressa* (UT/Utahn origin), *J. osteosperma*, and *J. scopulorum*.

be the poorest direct replacement for European common juniper, given the prevalence of sabinene (Fig. 3) and the presence of the unique compounds pregeijerene B (2.2%) and pregeijerene (0.7%).

The aromatic profiles established in the current study from juniper berries are similar to previous findings from other plant parts of the same species. Common juniper (leaf, cone, stem) volatile oil from Utah (var. *depressa*) was also prominent in α-pinene (63.9%), myrcene (6.9%), and limonene (3.3%), but also in β-pinene (6.2%) and δ-3-carene (6.8%) [15]. The volatile oil profile of *J. osteosperma* whole tree from Utah was prominent in α-pinene (40.5%), sabinene (4.7%), limonene (4.2%), and bornyl acetate (6.7%), but also in camphor (6.7%), cis-thujopsene (5.6%), and cedrol

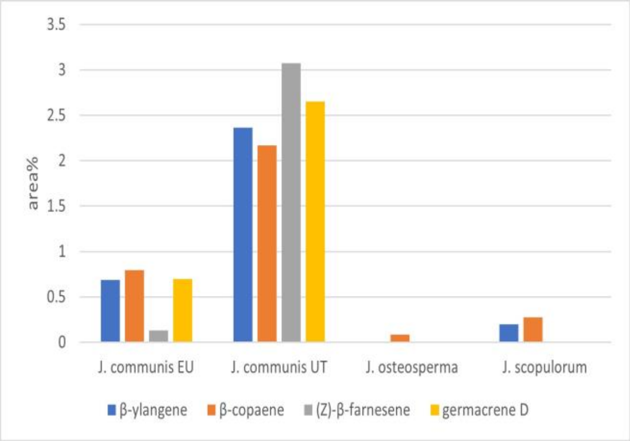

Figure 4. Relative abundance (area%) of prominent volatile compounds (sesquiterpenoids) in *Juniperus communis* (EU/Bulgarian origin), *J. communis* var. *depressa* (UT/Utahn origin), *J. osteosperma*, and *J. scopulorum*.

(2.9%) [16]. The leaf volatile oil profile from *J. scopulorum* was also prominent in α-pinene (4.3%), sabinene (66.9%), limonene (3.4%), γ-terpinene (2.2%), and pregeijerene B (2.8%), but also in terpinen-4-ol (3.8%) [17].

4. Conclusions

To the authors' knowledge, this is the first time that the volatile profiles of berries from *Juniperus communis* var. *depressa*, *J. osteosperma*, and *J. scopulorum* have been fully detailed. The profiles established in the current study, particularly those of both *J. communis* var. *depressa* and *J. osteosperma*, could be considered for flavoring gin and other beverages. Prominent compounds of *J. communis* var. *depressa*, *J. osteosperma*, and *J. scopulorum* include α-pinene (17.2%, 51.3%, 5.7%), myrcene (53.3%, 2.5%, 9.2%), and limonene (4.0%, 10.4%, 5.6%), respectively. Due to the low concentration of the volatile compounds in an ethanol solution, the analytical testing was performed using GC/MS. While GC/MS is the standard technique for identifying volatile compounds, GC/FID is the preferred technique for quantifying volatile compounds. Future studies could concentrate the sample by removing the extraction solvent and perform the quantification using GC/FID, rather than relying on an estimated GC/MS quantification.

Authors' contributions

Conceptualization, T.M.W., C.P., and R.E.C.; methodology, T.M.W.; software, T.M.W.; validation, R.E.C..; formal analysis, T.M.W.; investigation, T.M.W., E.A.Z, A.P. and C.P.; data curation, T.M.W.; writing—original draft preparation, T.M.W.; writing—review and editing, E.A.Z., A.P., C.P., 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

The authors have declared that no competing interests exist.

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