Volatile compound profile of *Osmorhiza occidentalis* Torr. (Apiaceae) root essential oil

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ABSTRACT

Osmorhiza occidentalis Torr. is an essential-oil-bearing plant in the Apiaceae family. Previous research was conducted by the same group of researchers on the essential oil produced by and extracted from the above-ground plant parts (herb). The current study was conducted on the steam distilled essential oil from the roots of the same plant, *O. occidentalis*. The root essential oil was analyzed by gas chromatography (GC/FID, GC/MS) and was found to be comprised of 39 compounds. Prominent volatile compounds include sabinene (24.2%), o-cymene (2.3%), (Z)-β-ocimene (2.0%), γ-terpinene (19.6%), methyl chavicol (22.4%), 2-allyl-1,4-dimethoxybenzene (2.4%), and 1-allyl-2,4-dimethoxybenzene (18.4%). The current study establishes for the first time, to the best knowledge of the authors, the essential oil yield and composition of *O. occidentalis* root. These results provide fundamental data for substantiation of ethnobotanical applications and for future investigations on the secondary metabolites from this plant species. *Published online* ***www.phytologia.org*** *Phytologia 106(2): 33-38 (June 20, 2024). ISSN 030319430.*

KEY WORDS: Apiaceae, essential oil, gas chromatography, *Osmorhiza occidentalis*, root

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Osmorhiza occidentalis, commonly known as western sweet-cicely or mountain sweet-cicely, is a perennial herbaceous plant belonging to the Apiaceae family (WFO: accessed 12 April 2024). It is native to the western regions of North America, particularly the Intermountain Region extending into the Pacific Northwest of the United States and northward into Canada.

This aromatic plant is characterized by its finely divided, pinnate leaves and small yellow to greenish-white flowers arranged in umbels (Cronquist et al. 1997) (Fig. 1). The plant typically grows in moist, shaded areas such as forests, woodlands, and along stream banks. Pleasantly, one of the distinguishing features of *O. occidentalis* is its licorice-like aroma, which emanates from both its roots and foliage (Cronquist et al. 1997). This aroma is attributed to the presence of certain volatile compounds within the plant (Wilson et al. 2022).

Figure 1. Botanical illustration of *Osmorhiza occidentalis* whole plant (left) and close perspective of the root (right). The main stem (left) is truncated to focus on and detail the herbaceous and reproductive plant parts. Illustrated by Zach Nielsen.

Throughout history, indigenous peoples have utilized various parts of *O. occidentalis* for medicinal, culinary, and cultural purposes. The roots were often brewed into teas to treat respiratory issues such as colds and pneumonia (Murphy 1959), while the seeds and stems were used for flavoring foods and dyeing garments (Moerman 1998), respectively. Specifically, the Paiute and Shoshone peoples referred to the plant as “Bossowey” and valued its medicinal properties in treating various ailments (Murphy 1959; Uphof 1968).

While *O. occidentalis* has a rich ethnobotanical history, modern scientific research on its medicinal properties is relatively limited. However, some studies have identified compounds such as falcarindiol (Kern and Cardellina 1982) in the plant, which is known for its antifungal properties. The objective of this ongoing study was to extend the scope of prior research conducted by the same research team (Wilson et al. 2022). This study compares the root essential oil profile and yield with previous findings of the herb essential oil profile and yield.

MATERIALS AND METHODS

Osmorhiza occidentalis plant material was collected on July 17, 2023, from native populations located on public lands (Bureau of Land Management) on the Oquirrh Mountain Range in Tooele County, Utah, USA (40°28′15″ N 112°10′18″ W; 2737 m elevation). Both above-ground portions (during the flowering stage) and roots were collected to determine the weight, yield, and the composition of the volatile oil extracted from the herb and root. A representative voucher sample is held in the Young Living Aromatic Herbarium (YLAH): *O. occidentalis* Torr., Wilson 2023-01 (YLAH).

Organic chervil, *Anthriscus cerefolium* Hoffm. (Apiaceae), seeds were purchased (Johnny’s Selected Seeds, Winslow, ME, USA), grown at the Young Living Research Greenhouse for a 3-month period, and the herb was harvested as inflorescence developed. The distilled essential oil was used as a reference standard for the identification of two compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene.

Plant material was prepared for laboratory-scale distillation as follows: the below-ground portion (root) was cleaned and dried. The above-ground portions (herb) and root were grouped separately, bagged, and stored at −20 ± 2 °C until steam distilled.

Laboratory-scale distillation was as follows: 1.5 L of water was added to 2-L steam generator that fed into a 2-L distillation chamber, plant material was accurately weighed and added to the distillation chamber, distillation for 1.5 h from pass-over by indirect steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were each 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 × 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in methylene chloride), 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 (Adams 1997) using Chemstation library search in conjunction with retention indices. Note that limonene/β-phellandrene eluted as a single peak. Their amounts were determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene). 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 × 60 m, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in methylene chloride, 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 of relative area % values. Compounds were identified using retention indices coupled with retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MS, USA).

RESULTS AND DISCUSSION

Previously published research on the essential oil profile of *Osmorhiza occidentalis* herb by Wilson and associates (2022) was conducted on plant material from the same mountain range (Oquirrh Mountains). However, for both studies (Wilson et al. 2022; current), plant material was collected from two different populations of *O. occidentalis*, which lie approximately 250 meters apart and are separated by a dense Douglas fir (*Pseudotsuga menziesii*) forest. In the Oquirrh Mountain range, *O. occidentalis* appears to grow best at high altitudes (> 2500 m) and with either full sun exposure or partial shade, but it does not grow well under thick forest canopy.

Since plant material was collected from a different population of *O. occidentalis*, above-ground plant parts (herb) were once again collected and distilled for comparative analysis. Additionally, roots were collected and distilled to establish essential oil yield (Table 1) and essential oil composition (Table 2).

Table 1. Yield data, including mass of the plant material distilled (g), essential oil yield (g), and calculated yield (%) from *Osmorhiza occidentalis* herb and root samples.

Sample	Plant Mass Distilled (g)	EO Yield (g)	EO Yield (%)
Herb	410.08	0.77	0.19
Root	423.90	9.08	2.14

The calculated essential oil yield (*w/w*) of the herb in the current study (0.19%) was similar to the average calculated yield of herb from the previous study (0.12%) (Wilson et al. 2022). The calculated essential oil yield (*w/w*) of the root was 2.14%, or just over 11x the yield of the herb.

Table 2. Essential oil profile of *Osmorhiza occidentalis* herb and root samples. Reported values represent averages from samples analyzed in triplicate, which was done to ensure repeatability of values (standard deviation ≤ 0.7 for all compounds). Values less than 0.1% are denoted as trace (tr) and those not detected as nd. KI is the Kovat's Index value and was previously calculated by Robert Adams using a linear calculation on a DB-5 column (Adams 1997). Relative area percent was determined by GC/FID.

Compound Name	KI	Herb EO (area %)	Root EO (area%)
α-thujene	924	0.1	0.3
α-pinene	932	0.2	1.1
camphene	946	tr	tr
sabinene	969	9.8	24.2
β-pinene	974	0.1	0.6
myrcene	988	0.4	0.7
α-phellandrene	1002	0.1	tr
o-cresol methyl ether	1005	nd	tr
δ-3-carene	1008	nd	tr
α-terpinene	1014	tr	0.3
o-cymene	1022	2.1	2.3
limonene	1024	0.1	tr
β-phellandrene	1025	0.1	0.3
(*Z*)-β-ocimene	1032	6.1	2.0
(*E*)-β-ocimene	1044	0.2	0.1
γ-terpinene	1054	1.8	19.6

Compound Name	KI	Herb EO (area %)	Root EO (area%)
(*Z*)-sabinene hydrate	1065	tr	0.1
terpinolene	1086	0.3	0.1
3-isopropyl-2-methoxypyrazine	1090	nd	tr
linalool	1095	0.1	nd
(*E*)-sabinene hydrate	1098	nd	0.1
(*Z*)-4-methoxy thujane	1118*	nd	0.3
allo-ocimene	1128	0.6	0.2
citronellal	1148	tr	nd
terpinen-4-ol	1174	0.3	0.3
α-terpineol	1186	tr	0.1
methyl chavicol	1195	74.7	22.4
citronellol	1223	0.2	nd
thymol methyl ether	1232	nd	tr
pulegone	1233	tr	nd
neral	1235	0.1	nd
(*E*)-anethol	1282	1.3	0.2
bornyl acetate	1284	nd	tr
α-copaene	1374	nd	tr
2-allyl-1,4-dimethoxybenzene	1393*	nd	2.4
methyleugenol	1403	tr	1.6
1-allyl-2,4-dimethoxybenzene	1407*	tr	18.4
isoamyl benzoate	1433	nd	tr
bicyclogermacrene	1500	tr	0.6
kessane	1529	nd	tr
(*E*)-nerolidol	1561	tr	nd
(*E*)-p-methoxycinnamaldehyde	1562	tr	nd
spathulenol	1577	nd	tr
γ-eudesmol	1630	nd	tr
shyobunol	1688	nd	tr
benzyl benzoate	1759	tr	0.3
	sum	**98.6**	**98.5**

*KI not included in the Adam's library (1997). KI was manually calculated using alkane standards on a DB-5 column.

As with previous findings (Wilson et al. 2022), the composition of the herb essential oil is largely composed of five volatile compounds, including sabinene (9.8%), o-cymene (2.1%), (*Z*)-β-ocimene (6.1%), γ-terpinene (1.8%), and methyl chavicol (74.7%), which, together, comprise approximately 95% of the identified compounds. The same five volatile compounds comprise approximately 70% of the root sample (sabinene 24.2%, o-cymene 2.3%, (*Z*)-β-ocimene 2.0%, γ-terpinene 19.6%, methyl chavicol 22.4%). Additionally, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene are other prominent compounds of the root, comprising 2.4% and 18.4% of the essential oil profile, respectively.

These same two compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene, are not in the Adams' library (1997). Their preliminary identification was made by referencing the 2020 NIST mass spectral library and referencing previously published research on chervil herb (*Anthriscus cerefolium*), which contains both compounds (Baser et al. 1998; Chizzola 2011; Lemberkovics et al. 1994). Their identification was confirmed by distilling and analyzing in-house cultivated chervil herb, which contained both compounds with matching mass spectra and compound elution times (Table 3). Additionally, chervil herb and *O. occidentalis* root samples were analyzed using GC columns with different stationary phases (Rtx-1, DB-5) to confirm that elution times of the two compounds still matched between samples. Prominent ions for 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene include 103, 135, 178 and 121, 147, 178, respectively.

Table 3. Essential oil profile of *Anthriscus cerefolium* (chervil) herb and *Osmorhiza occidentalis* root samples. Reported values represent averages from samples analyzed in triplicate, which was done to ensure repeatability of values (standard deviation ≤ 1.0 for all compounds). KI is the Kovat's Index value and was previously calculated by Robert Adams using a linear calculation on a DB-5 column (Adams 1997). Relative area percent was determined by GC/FID.

Compound Name	KI	Chervil Herb EO (area %)	Root EO (area%)
β-pinene	974	2.2	0.6
methyl chavicol	1195	20.4	22.4
2-allyl-1,4-dimethoxybenzene	1393*	3.2	2.4
1-allyl-2,4-dimethoxybenzene	1407*	68.3	18.4

*KI not included in the Adam's library (1997). KI was manually calculated using alkane standards on a DB-5 column.

CONCLUSIONS

Findings from the current study confirm those from previous research on the essential oil from the above-ground portions (herb) of *Osmorhiza occidentalis*. Namely, that the herb essential oil profile is largely composed of five volatile compounds (sabinene, o-cymene, (*Z*)-β-ocimene, γ-terpinene, methyl chavicol) and that volatile compound profile variability exists from sample-to-sample (Wilson et al. 2022).

Unfortunately, the limited population size of *O. occidentalis* on the Oquirrh Mountain range, particularly on public lands, did not allow for collection and distillation of additional root essential oil samples. An investigation of additional samples in the future would provide an understanding if, similar to herb samples, yield and volatile compound profile variability exist from sample-to-sample and/or population-to-population in root samples.

In the current study, it was found that the root essential oil of *O. occidentalis* had a much higher yield (> 11x) compared to the herb of the same plant. Also, while the volatile compound profile of the root essential oil does somewhat overlap with that of the herb, the root essential oil contains two prominent compounds, 2-allyl-1,4-dimethoxybenzene and 1-allyl-2,4-dimethoxybenzene, that were not previously detected in the herb. Future research could investigate reasons for the differences in essential oil yield and volatile compound profiles from the root and herb of *O. occidentalis*.

The current study focuses on the identification and quantification of volatile compounds from *O. occidentalis*. Future research could use other analytical techniques (LC/MS) to investigate concentrations of falcarindiol and other non-volatile compounds. Additionally, future studies could investigate the application of both volatile and non-volatile secondary metabolites from *O. occidentalis*.

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