Journal of Medicinally Active Plants

Volume 12
Issue 2 *Vol 12 Issue 2*

6-30-2023

Characterization of the Essential Oil of Holodiscus dumosus (S. Watson) A. Heller (Rosaceae)

Characterization of the Essential Oil of *Holodiscus dumosus* (S. Watson) A. Heller (Rosaceae)

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Manuscript received: April 6, 2023

Keywords: Idaho, Gas chromatography, Enantiomers, Chiral, Antibacterial, Antifungal

ABSTRACT

Leaves of *Holodiscus dumosus* were obtained from a wild-growing plant in southern Idaho. The leaf essential oil was obtained by hydrodistillation (0.151% yield) and analyzed by gas chromatography (GC-MS, GC-FID, and chiral GC-MS). The major components in the leaf oil were geraniol (17.4%), germacrene B (8.9%), (*E*)-β-caryophyllene (6.1%), α-cadinol (5.7%), linalool (4.7%), and γ-elemene (4.0%). Linalool was nearly racemic (54.0% (–)-linalool, 46% (+)-linalool) while (–)-(*E*)-β-caryophyllene was the exclusive enantiomer. Linalool, geraniol, and (*E*)-β-caryophyllene were screened for antimicrobial activity and showed strong activity (MIC < 500 μg/mL) against *Staphylococcus aureus*, *S. epidermidis*, *Candida albicans*, *Microsporum canis*, *M. gypseum*, and *Trichophyton rubrum*, which may account for the Native American traditional use of the plant as an antiseptic wash.

Keywords: Idaho, gas chromatography, enantiomers, chiral, antibacterial, antifungal

INTRODUCTION

Holodiscus (K. Koch) Maxim. (Rosaceae) is a taxonomically complex genus with two currently recognized species in North America, *Holodiscus discolor* (Pursh) Maxim. (creambush, ocean-spray) and *Holodiscus dumosus* (S. Watson) A. Heller. (Ley, 1943). *Holodiscus discolor* is most abundant along coastal North America from British Columbia to southern California, from sea level to 2150 m in elevation (Shaw et al., 2008). *Holodiscus dumosus*, on the other hand, ranges east of the Cascade and Sierra Nevada Mountains from north central Oregon and southern Idaho, through Nevada, Utah, Colorado, Arizona, and New Mexico, south to Chihuahua, Mexico, ranging in elevation from 1400 m to 3350 m above sea level. (Shaw et al., 2008; Kartesz 2015).

In the Great Basin, *H. dumosus* grows on hillsides, talus slopes, rock outcrops, slickrock plateaus, dry rocky areas, and river bottoms with soils that are well-drained, sandy or gravelly, and dry to moderately dry. The plant occurs in various plant communities including sagebrush, juniper, and pine (Shaw et al., 2004). Flowering occurs in June and into August and fruiting occurs in August. Although the palatability is low, *H. dumosus* is browsed by mule deer (*Odocoilus hemionus*) and elk (*Cervus elaphus*) in the autumn and winter, and by bighorn sheep (*Ovis canadensis*) in the summer (Shaw et al., 2008). *Holodiscus dumosus* (S. Watson) A. Heller, Rosaceae (bush rock spirea, glandular oceanspray) is a compact shrub 1-4 m tall. The leaves are elliptic-ovate or ovate, longer than broad, deeply toothed, densely villous and tomentose beneath (Figure 1) (Ley, 1943). The Paiute Native Americans took a

decoction of the stems to treat colds, while the Shoshoni people used a decoction of the aerial parts as an antiseptic wash (Moerman, 1998). There have been no previous reports on phytochemical constituents of this plant; this is the first report of the essential oil for this species. The purpose of this work, therefore, was to obtain and characterize the essential oil of this plant.

Figure 1. *Holodiscus dumosus* (S. Watson) A. Heller collected near Prairie, Idaho (43°32′16″ N, 115°42′19″ W, 1109 m elevation). Photograph by K. Swor.

MATERIALS AND METHODS

Plant Material. Leaves were collected on May 16, 2022, near Prairie, Idaho (43°32′16″ N, 115°42′19″ W, 1109 m elevation). The plant was identified by W.N. Setzer based on botanical descriptions (Ley, 1943) and by comparison with herbarium samples in the New York Botanical Garden (New York Botanical Garden). A voucher specimen (WNS-Hd-5328) has been deposited in the University of Alabama in Huntsville herbarium. The fresh leaves were stored frozen (–20 °C) until distilled. The leaves (18.00 g) were hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane for 3 h to give 27.1 mg pale-yellow essential oil (0.151% yield).

Gas Chromatographic Analysis. The essential oils were analyzed by gas chromatography with flame ionization detection (GC-FID), gas chromatography – mass spectrometry (GC-MS) and chiral GC-MS as previously described (Satyal et al., 2023; Swor et al., 2022): Shimadzu GCMS-QP2010 Ultra (GC-MS), ZB-5ms column, oven temperature program was 50 °C increased 2 °C/min to 260 °C, held at 260 °C for 5 min; Shimadzu GC 2010 (GC-FID), ZB-5 column, same conditions as GC-MS; Shimadzu GCMS-QP2010S (chiral GC-MS), Restek B-Dex 325 column, oven temperature program was 50 °C, held for 5 min, increased to 100 °C at a rate of 1.0 °C/min, then increased to 220 °C at a rate of 2 °C/min. Retention index values were determined using a homologous series of *n*-alkanes on a ZB-5ms column using the linear formula of van den Dool and Kratz (van den Dool and Kratz, 1963). The essential oil components were identified by comparison of the mass spectral fragmentation patterns and by comparison of retention index (RI) values available in the Adams (Adams, 2007), FFNSC 3 (Mondello, 2016), NIST20 (NIST20, 2020), and our own in-house database (Satyal, 2015). The identification of enantiomers was determined by comparison of retention times with authentic samples obtained from Sigma-Aldrich (St. Louis, MO, USA).

Antimicrobial Screening. The essential oil components were screened for antibacterial activity against Gram-positive bacteria (*Cutibacterium acnes* (ATCC No. 11827), *Staphylococcus aureus* (ATCC No. 29213), and *Staphylococcus epidermidis* (ATCC No. 12228); for antifungal activity against dermatophyte molds (*Microsporum canis* (ATCC No. 11621), *Microsporum gypseum* (ATCC No. 24102), *Trichophyton mentagrophytes* (ATCC No. 18748), and *Trichophyton rubrum* (ATCC No. 28188); and the pathogenic yeast *Candida albicans* (ATCC No. 18804) using the microbroth dilution technique (EUCAST, 2003) using two-fold dilutions (2500, 1250, 625, 312.5, 156.3, and 78.1 µg/mL), as

previously reported (Poudel et al., 2022). Individual essential oil components, (±)-linalool, geraniol, and (*E*)-β-caryophyllene, were obtained from Sigma-Aldrich (St. Louis, MO) and were used as received, without additional purification. Antibacterial and antifungal positive controls were gentamicin and amphotericin B (Sigma-Aldrich, St. Louis, MO), respectively; dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO) was the negative control.

RESULTS AND DISCUSSION

Hydrodistillation of the leaves of *H. dumosus* gave a pale-yellow essential oil in a relatively low yield of 0.151%. Gas chromatographic analysis (GC-MS and GC-FID) were carried out to characterize the volatile phytochemicals in the essential oil (Table 1).

A total of 83 compounds were identified in the leaf essential oil accounting for 95.5% of the total composition. The components with the highest concentrations in the essential oil were geraniol (17.4%), germacrene B (8.9%), (*E*)-β-caryophyllene (6.1%), α-cadinol (5.7%), linalool (4.7%), and γ-elemene (4.0%). Interestingly, there were 37 sesquiterpene hydrocarbons accounting for 50.3% of the composition. Indeed, terpenoids made up 94.2% of the composition (including an unidentified oxygenated sesquiterpenoid, RI 1622), which is not characteristic of essential oils of the Rosaceae. With the exception of floral essential oils, the Rosaceae is not considered an essential oil-bearing plant family and long-chain alkanes and other fatty acid derivatives generally dominate the compositions of Rosaceae foliar essential oils (Swor et al., 2023).

Because *H. dumosus* was used by Native Americans as an antiseptic wash, the commercially available major components of the essential oil were screened for antimicrobial activity against bacterial (*Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and fungal dermatophytes (*Candida albicans*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*) (Table 2). Unfortunately, the low essential oil yield precluded screening the essential oil itself. Neither linalool, geraniol, nor (*E*)-β-caryophyllene showed exceptional antimicrobial activity. However, *S. aureus*, *S. epidermidis*, *C. albicans*, *M. canis*, *M. gypseum*, and *T. rubrum* were notably susceptible to the essential oil components with MIC values < 500 µg/mL (Duarte et al., 2007; Van Vuuren and Holl, 2017). In addition, linalool was broadly active against all microorganisms tested (MIC < 500 µg/mL). Because the essential oil yield was relatively low and the major components were not in high concentrations, it is unlikely that the components themselves played a significant role in the any antiseptic activity of *H. dumosus*. They may, however, act synergistically with other essential oil components or with non-volatile components in the plant to account for the ethnobotanical use of *H. dumosus*.

The enantiomeric distribution of chiral terpenoid components was investigated using chiral GC-MS (Table 3). Both linalool and α-terpineol were nearly racemic in distribution, while (*E*)-β-caryophyllene and germacrene D were exclusively the (–)-enantiomers. δ-Cadinene, on the other hand, was 87.6% (+)-δ-cadinene. The enantiomeric distributions found in the essential oil of *Purshia tridentata* (Rosaceae), also from southern Idaho, were comparable with (+)-linalool (54.3-62.3%), (+)-α-terpineol (31.2-38.4%), 100% (–)-(*E*)-β-caryophyllene and (–)-germacrene D and 100% (+)-δ-cadinene (Swor et al., 2023).

This report presents, for the first time, the essential oil characterization of *Holodiscus dumosus*, and the *Holodiscus* genus. The essential oil composition may account for the ethnobotanical antiseptic use of the plant. However, even though the leaf essential oil of *H. dumosus* was rich in terpenoids, the low yield likely precludes consideration of this plant as a commercial source of essential oil.

Table 1. Leaf essential oil composition of *Holodiscus dumosus* from southwestern Idaho.

RI_{calc}[1]	RI_{db}[2]	Compound	%	RI_{calc}	RI_{db}	Compound	%
1008	1007	α-Phellandrene	0.1	1503	1505	α-Bulnesene	0.5
1100	1101	Linalool	4.7	1505	1506	δ-Amorphene	2.7
1104	1104	Hotrienol	0.1	1508	1511	Germacrene A	0.1
1106	1107	Nonanal	0.4	1514	1514	γ-Cadinene	0.6
1187	1188	*p*-Cymen-8-ol	0.4	1517	1515	Cubebol	0.1
1195	1195	α-Terpineol	0.7	1520	1520	δ-Cadinene	2.3
1206	1206	Decanal	0.1	1524	1526	Zonarene	0.1
1208	1207	(3*E*)-Octenyl acetate	0.1	1538	1540	Selina-4(15),7(11)-diene	0.4
1216	1217	Coumaran	0.2	1542	1541	α-Calacorene	0.2
1224	1226	Nerol	0.1	1543	1542	Selina-3,7(11)-diene	0.4
1238	1239	Neral	0.2	1550	1549	α-Elemol	0.9
1251	1249	Geraniol	17.4	1560	1560	Germacrene B	8.9
1262	1263	(2*E*)-Decenal	0.1	1563	1564	β-Calacorene	0.2
1267	1268	Geranial	0.4	1583	1587	Caryophyllene oxide	0.4
1289	1289	Thymol	0.1	1594	1600	Khusimone	0.5
1297	1296	Carvacrol	0.2	1616	1609	Salvial-4(14)-en-1-one	0.4
1308	1309	4-Vinylguaicol	0.5	1622	---	Unidentified sesquiterpenoid[3]	2.2
1331	1330	Bicycloelemene	0.2	1624	1624	Selina-6-en-4β-ol	0.2
1334	1336	δ-Elemene	1.4	1629	1629	*iso*-Spathulenol	1.2
1346	1348	α-Cubebene	0.3	1633	1633	γ-Eudesmol	0.9
1350	1356	Eugenol	0.1	1635	1634	*cis*-Cadin-4-en-7-ol	1.4
1368	1371	α-Ylangene	0.2	1643	1643	τ-Cadinol	0.7
1374	1375	α-Copaene	0.2	1645	1645	τ-Muurolol	0.5
1381	1383	*cis*-β-Elemene	0.2	1648	1645	δ-Cadinol	0.3
1382	1382	β-Bourbonene	1.0	1648	1645	Selina-3,11-dien-6α-ol	0.3
1385	1385	α-Bourbonene	0.1	1656	1655	α-Cadinol	5.7
1388	1390	*trans*-β-Elemene	3.0	1659	1658	*neo*-Intermedeol	2.4
1400	1400	Tetradecane	0.1	1665	1662	9-Methoxycalamenene	0.2
1409	1410	Dodecanal	0.1	1670	1668	Intermedeol	0.2
1418	1417	(*E*)-β-Caryophyllene	6.1	1694	1688	Shyobunol	0.3
1430	1430	γ-Elemene	4.0	1698	1698	Juniper camphor	0.5
1438	1438	α-Guaiene	0.9	1717	1715	Pentadecanal	0.4
1443	1442	Guaia-6,9-diene	0.2	1727	1730	(*Z*)-Ligustilide	0.7
1446	1447	*iso*-Germacrene D	0.1	1794	1793	α-Phellandrene dimer A	0.5
1451	1451	*trans*-Muurola-3,5-diene	0.1	2101	2100	Heneicosane	0.2
1457	1454	α-Humulene	2.4	2300	2300	Tricosane	0.2
1475	1476	Selina-4,11-diene	1.0	2499	2500	Pentacosane	0.1
1477	1478	γ-Muurolene	0.8	2699	2700	Heptacosane	0.2
1479	1478	γ-Gurjunene	0.2			Monoterpene hydrocarbons	0.1
1481	1482	α-Amorphene	3.2			Oxygenated monoterpenoids	24.3
1483	1483	Germacrene D	2.0			Sesquiterpene hydrocarbons	50.3
1489	1488	δ-Selinene	0.5			Oxygenated sesquiterpenoids	16.9
1491	1489	β-Selinene	1.9			Diterpenoids	0.5
1494	1490	γ-Amorphene	0.7			Benzenoid aromatics	1.5
1498	1497	α-Selinene	2.2			Others	2.0
1500	1500	α-Muurolene	0.6			Total identified	95.5

[1] RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column.
[2] RI_{db} = Reference retention index obtained from the databases (Adams 2007; Satyal 2015; Mondello 2016; NIST20 2020).
[3] MS(EI): 222(1%), 207(19%), 204(10%), 189(3%), 179(3%), 161(38%), 123(18%), 121(20%), 119(22%), 109(23%), 105(31%), 95(28%), 93(27%), 81(100%), 71(23%), 69(21%), 67(22%), 55(23%), 43(72%), 41(32%).

Table 2. Antimicrobial activity (MIC, µg/mL) of *Holodiscus dumosus* major essential oil components.

Compound	Gram-positive bacteria			Yeast
	Cutibacterium acnes	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
(±)-Linalool	313	156	313	156
Geraniol	625	313	313	156
(*E*)-β-Caryophyllene	625	313	313	156
Positive control[1]	< 19.5	0.61	< 19.5	0.61
DMSO[2]	1250	1250	1250	1250
	Fungal dermatophytes			
	Microsporum canis	*Microsporum gypseum*	*Trichophyton mentagrophytes*	*Trichophyton rubrum*
(±)-Linalool	313	313	156	313
Geraniol	313	156	625	313
(*E*)-β-Caryophyllene	313	313	625	313
Positive control[1]	< 19.5	< 19.5	< 19.5	< 19.5
DMSO[2]	1250	1250	1250	1250

[1] Gentamicin for bacteria, Amphotericin B for fungi.
[2] Dimethylsulfoxide negative control.

Table 3. Enantiomer percentages for chiral terpenoids found in *Holodiscus dumosus* leaf essential oil.

Compound	RT_{std}[1]	RT_{EO}[2]	Enantiomer %
(–)-Linalool	45.69	45.67	54.0
(+)-Linalool	46.24	46.20	46.0
(–)-α-Terpineol	59.73	59.73	46.3
(+)-α-Terpineol	60.58	60.55	53.7
(–)-(*E*)-β-Caryophyllene	69.33	69.33	100.0
(+)-(*E*)-β-Caryophyllene	na[3]	nd[4]	0.0
(+)-Germacrene D	73.48	nd	0.0
(–)-Germacrene D	73.73	73.71	100.0
(–)-δ-Cadinene	76.50	76.50	12.4
(+)-δ-Cadinene	77.33	77.33	87.6

[1] RT_{std} = Retention time of the standard compound.
[2] RT_{EO} = Retention time of the component in the essential oil.
[3] na = Standard compound not available.
[4] nd = Enantiomer not detected in the essential oil.

ACKNOWLEDGMENTS

This work was carried out as part of the activities of the Aromatic Plant Research Center (APRC, https://aromaticplant.org/).

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