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Research Article

Chemical composition of the aerial parts essential oil of *Chrysothamnus viscidiflorus* from southwestern Idaho

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Abstract

Yellow rabbitbrush (*Chrysothamnus viscidiflorus*) is native to the Great Basin of North America and the plant was part of the traditional medicine of Native Americans in the region. There has been very little previous work on essential oils of *Chrysothamnus*, and no reports on *C. viscidiflorus* essential oil. Therefore, the purpose of this work was to evaluate the chemical composition of *C. viscidiflorus* essential oil. The aerial parts of *C. viscidiflorus* were collected from southwestern Idaho, the essential oil obtained by hydrodistillation and analyzed by gas chromatographic methods. The essential oil was obtained in 1.121% yield and was dominated by monoterpene hydrocarbons (82.6%), including (–)-β-pinene (41.3%), (+)-limonene (17.4%), (+)-sabinene (9.1%), myrcene (4.2%), and (*E*)-β-ocimene (4.2%). This is the first report on the essential oil characterization of *C. viscidiflorus*, and adds to our understanding of the volatile phytochemistry of *Chrysothamnus*. Biological activities of the major components in the essential oil are consistent with the traditional Native American use of the plant.

1. Introduction

Chrysothamnus viscidiflorus Nutt. (Asteraceae) is fairly widespread throughout the Great Basin of North America, ranging from British Columbia and Montana south to New Mexico, Arizona, and eastern California [1]. Shoshoni Native Americans applied a poultice of crushed stems and leaves of *C. viscidiflorus* to treat rheumatism; the Paiute people took an infusion of crushed leaves to treat colds [2]. There are probably three subspecies in Idaho: *C. viscidiflorus* subsp. *lanceolatus* H.M. Hall & Clem., *C. viscidiflorus* subsp. *puberulus* H.M. Hall & Clem., and *C. viscidiflorus* subsp. *viscidiflorus* (Hook.) Nutt. [3]. *Chrysothamnus viscidiflorus* subsp. *viscidiflorus* grows up to 1 m tall. The leaves are glabrous and viscid, 1 to 6 cm long and generally more than 1.5 cm wide (Fig. 1) [1,4]. The *viscidiflorus* subspecies ranges throughout the Great Basin (Fig. 2) [4].

Previous phytochemical investigations of *C. viscidiflorus* have shown the plant to be a source of flavonoids [5], labdane diterpenoids [6], benzofuranoids, chromanones [7], guaiane, germacrane, and eudesmane sesquiterpenoids, coumarins, and *p*-coumaric acid derivatives [8]. To our knowledge, there have been no previous reports on the essential oil composition of *C. viscidiflorus*. Because *C. viscidiflorus* was important in Native American ethnopharmacology and the essential oil had not been previously investigated, the purpose of

this investigation was to obtain and analyze the essential oil of *C. viscidiflorus*.

2. Materials and methods

2.1 Plant Material

Aerial parts of *Chrysothamnus viscidiflorus* were collected from several plants near Pine, Idaho (43°24'20"N, 115°17'33"W, 1426 m elevation) on June 28, 2022. The plant was identified by W.N. Setzer. Based on botanical descriptions [1,4] and comparison with herbarium samples from the New York Botanical Garden [9], the plant was identified as *C. viscidiflorus* subsp. *viscidiflorus* (Fig. 1).

Figure 1. *Chrysothamnus viscidiflorus* subsp. *viscidiflorus* from southwestern Idaho. Photograph by K. Swor.

A voucher specimen (WNS-Cvv-5686) has been deposited in the University of Alabama in Huntsville herbarium. The fresh plant material from several plants was combined and 108.9 g was hydrodistilled using a Likens-Nickerson apparatus to give 1.221 g of a yellow essential oil.

Figure 2. Range of *Chrysothamnus viscidiflorus* subsp. *viscidiflorus*. Adapted from Anderson [4].

2.2 Gas Chromatographic Analysis

The essential oil of *C. viscidiflorus* subsp. *viscidiflorus* aerial parts was analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD, USA) operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software. The GC column was a ZB-5ms fused silica capillary column (60 m length × 0.25 mm inner diameter) with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm (Phenomenex, Torrance, CA, USA). The carrier gas was helium with a column head pressure of 208.3 kPa and flow rate of 2.0 mL/min. Injector temperature was 260 °C and the ion source temperature was 260 °C. The GC oven temperature program was programmed for 50 °C initial temperature, temperature increased at a rate of 2 °C/min to 260 °C, then held at 260 °C for 5 min. A 5% *w/v* solution of the sample in CH_2Cl_2 was prepared and 0.1 µL was injected with a splitting mode (24.5:1). Retention index (RI) values were calculated using a

homologous series of *n*-alkanes [10]. The essential oil components were identified by comparing their RI values and their MS fragmentation patterns with those reported in the Adams [11], FFNSC3 [12], NIST20 [13], and Satyal [14] databases.

Gas chromatography – flame ionization detection (GC-FID) was carried out using a Shimadzu GC 2010 with FID detector (Shimadzu Scientific Instruments, Columbia, MD, USA) and a ZB-5 GC column (60 m × 0.25 mm × 0.25 μm film thickness) (Phenomenex, Torrance, CA, USA), using the same operating conditions as above for GC-MS. The percent compositions were determined from raw peak areas without standardization.

The *C. viscidiflorus* subsp. *viscidiflorus* essential oil was analyzed by chiral GC-MS using a Shimadzu GCMS-QP2010S (Shimadzu Scientific Instruments, Columbia, MD, USA) instrument fitted with a Restek B-Dex 325 column (30 m × 0.25 mm diameter × 0.25 μm film thickness) (Restek Corp., Bellefonte, PA, USA). The injector and detector temperatures were 240 °C. Helium was the carrier gas with a column head pressure of 53.6 kPa and a flow rate of 1.00 mL/min. The GC oven was programmed with an initial temperature of 50 °C, held for 5 min, then increased to 100 °C at a rate of 1.0 °C/min, then increased to 220 °C at a rate of 2 °C/min. For each essential oil sample, 0.3 μL of a 5% (w/v) solution in dichloromethane was injected using a splitting mode of 24.0:1. The enantiomeric distributions were determined by comparison of retention times with authentic samples obtained from Sigma-Aldrich (Milwaukee, WI, USA). The enantiomer percentages were determined from raw peak areas.

2.3 Antimicrobial Screening

The essential oil components were screened for antibacterial activity against *Staphylococcus aureus* (ATCC No. 29213), *Streptococcus pneumoniae* (ATCC No. 49136), and *Streptococcus pyogenes* (ATCC No. 19615); and antifungal activity against *Cryptococcus neoformans* (ATCC No. 32045) using the microbroth dilution technique [15], as previously reported [16]. The individual essential oil components, (–)-β-pinene, (+)-limonene, and myrcene, 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.

3. Results and discussion

A yellow essential oil in 1.121% yield (*w*/*w*, based on fresh plant material) was obtained by hydrodistillation of the aerial parts of *C. viscidiflorus*. Gas chromatographic analysis (GC-MS and GC-FID) revealed a total of 66 compounds accounting for 93.6% of the total composition (Table 1). The essential oil was dominated by monoterpene hydrocarbons (82.6%), including the major components β-pinene (41.4%), limonene (18.8%), sabinene (9.1%), myrcene (4.2%), and (*E*)-β-ocimene (4.2%).

Very few essential oils of *Chrysothamnus* species have been reported. The essential oil of *Chrysothamnus pulchellus* Green (syn. *Lorandersonia pulchella* (A. Gray) Urbatsch, R.P. Robers & Neubig) from Mexico had sesquicineole (22.7%), β-phellandrene (14.9%), (*Z*)-β-ocimene (9–4%), β-pinene (8.8%), (*E*)-β-ocimene (6.4%), (*E*)-β-caryophyllene (3.3%), and δ-cadinene (2.8%) as major components [17]. There are three reports on the essential oil composition of *Chrysothamnus nauseosus* (Pall. ex Pursh) Britton (syn. *Ericameria nauseosa* (Pursh) G.L. Nesom & G.I. Baird) [18–20]. The major components were β-phellandrene (1.8-56.5%), β-pinene (0.3-23.3%), limonene (0.7-22.3%), (*Z*)-β-ocimene (0.0-29.3%), and myrcene (0.0-12.9%).

The enantiomeric distributions of chiral terpenoids in *C. viscidiflorus* subsp. *viscidiflorus* were determined by chiral GC-MS (Table 2). In the essential oil of *C. viscidiflorus* subsp. *viscidiflorus*, (+)-α-thujene (75.5%) was the dominant enantiomer, (–)-α-pinene (94.5%) predominated over (+)-α-pinene (5.5%), (+)-sabinene was the exclusive enantiomer, and (–)-β-pinene (99.8%) was the major enantiomer. Interestingly, (+)-limonene (92.3%) dominated while (–)-β-phellandrene (95.6%) was the dominant enantiomer. (–)-α-Thujone was the exclusive enantiomer observed. Both (+)-*cis*-sabinene hydrate (86.1%) and (+)-*trans*-sabinene hydrate (90.5%) were the major enantiomers, (+)-terpinen-4-ol (71.9%) was the major enantiomer while (–)-α-terpineol (87.4%) was dominant. (–)-Germacrene D was the major enantiomer. These enantiomeric distributions are very different from

Table 1. Chemical composition (percentages based on peak areas) of the essential oil from the aerial parts of *Chrysothamnus viscidiflorus* subsp. *viscidiflorus*.

RI_{calc}	RI_{db}	Compound	%	RI_{calc}	RI_{db}	Compound	%
886	880	1-Methyl-2-pentylcyclopropane	2.7	1323	1322	Myrtenyl acetate	tr
925	925	α-Thujene	0.1	1335	1335	δ-Elemene	0.2
933	933	α-Pinene	2.6	1359	1361	Neryl acetate	tr
973	971	Sabinene	9.1	1380	1382	(3*Z*)-Hexenyl hexanoate	tr
979	978	β-Pinene	41.4	1383	1382	β-Bourbonene	tr
989	989	Myrcene	4.2	1384	1383	*cis*-β-Elemene	tr
998	1003	Ethyl hexanoate	tr	1389	1390	*trans*-β-Elemene	tr
1017	1017	α-Terpinene	0.2	1392	1392	(*Z*)-Jasmone	tr
1025	1024	*p*-Cymene	0.3	1418	1417	(*E*)-β-Caryophyllene	0.1
1030	1030	Limonene	18.8	1429	1427	γ-Elemene	tr
1032	1031	β-Phellandrene	1.1	1454	1454	α-Humulene	0.1
1035	1034	(*Z*)-β-Ocimene	0.1	1474	1475	γ-Muurolene	tr
1046	1045	(*E*)-β-Ocimene	4.2	1477	1478	γ-Curcumene	tr
1057	1057	γ-Terpinene	0.4	1480	1480	Germacrene D	1.0
1069	1069	*cis*-Sabinene hydrate	0.3	1497	---	Unidentified[a]	1.2
1084	1086	Terpinolene	0.2	1512	1512	γ-Cadinene	0.1
1099	1101	Linalool	tr	1517	1518	δ-Cadinene	0.1
1100	1101	*trans*-Sabinene hydrate	0.2	1547	1546	α-Elemol	0.9
1106	1105	α-Thujone	0.2	1560	1561	(*E*)-Nerolidol	0.1
1113	1113	(*E*)-4,8-Dimethylnona-1,3,7-triene	tr	1570	1571	(3*Z*)-Hexenyl benzoate	0.2
1124	1124	*cis*-*p*-Menth-2-en-1-ol	0.1	1575	1574	Germacra-1(10),5-dien-4β-ol	0.1
1138	1137	Nopinone	tr	1592	---	Unidentified[b]	4.1
1139	1139	(*E*)-Myroxide	tr	1626	1624	Selin-6-en-4β-ol	tr
1141	1141	*trans*-Pinocarveol	0.1	1628	1629	*iso*-Spathulenol	0.4
1142	1142	*trans*-*p*-Menth-2-en-1-ol	0.1	1632	1632	γ-Eudesmol	0.1
1153	1153	*p*-Vinylanisole	tr	1643	1643	τ-Cadinol	0.9
1157	1157	Sabina ketone	tr	1645	1645	τ-Muurolol	0.1
1162	1164	Pinocarvone	tr	1655	1656	β-Eudesmol	0.3
1181	1180	Terpinen-4-ol	1.7	1656	1655	α-Cadinol	0.4
1185	1187	(3*Z*)-Hexenyl butanoate	0.1	1672	1677	Cadalene	0.1
1187	1187	Cryptone	tr			Monoterpene hydrocarbons	82.6
1191	1190	Methyl salicylate	tr			Oxygenated monoterpenoids	3.1
1195	1195	α-Terpineol	0.4			Sesquiterpene hydrocarbons	1.5
1196	1198	*cis*-Piperitol	tr			Oxygenated sesquiterpenoids	3.4
1209	1209	*trans*-Piperitol	tr			Benzenoid aromatics	0.2
1284	1285	Bornyl acetate	tr			Others	2.8
1295	1296	*trans*-Pinocarvyl acetate	tr			Total identified	93.6
1320	1315	*p*-Mentha-1,4-dien-7-ol	tr				

RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Reference retention index obtained from the databases [11–14]. tr = trace (< 0.05%).

[a] Unidentified sesquiterpenoid, MS(EI): 220(3%), 177(4%), 159(18%), 134(100%), 132(44%), 121(27%), 119(48%), 105(20%), 93(27%), 91(19%), 79(16%), 77(11%), 71(10%), 55(9%), 43(55%), 41(17%).

[b] Unidentified sesquiterpenoid, MS(EI): 218(1%), 203(1%), 189(3%), 175(23%), 147(15%), 132(16%), 120(51%), 105(100%), 91(19%), 79(9%), 77(11%), 55(8%), 43(14%), 41(18%).

those observed for *Chrysothamnus nauseosus* (syn *Ericameria nauseosa*) where (–)-α-thujene, (–)-sabinene, (–)-limonene, (–)-*cis*-sabinene hydrate, (–)-*trans*-sabinene hydrate, and (–)-terpinen-4-ol were the dominant enantiomers [20].

The major components found in *C. viscidiflorus* essential oil have demonstrated biological activities consistent with the traditional Native American use of the plant to treat rheumatism. β-Pinene has demonstrated both antinociceptive [21] and anti-inflammatory activities [22]. Limonene has shown both antinociceptive and anti-inflammatory activities [23, 24]. Importantly, (+)-limonene has shown antinociceptive activity [25] and anti-inflammatory activity [26,27]. Sabinene has shown anti-inflammatory effects [28,29]. Myrcene demonstrated antinociceptive activity [30, 31] as well as anti-inflammatory activity [28].

Numerous essential oil components have been identified that exhibit inhibitory activity against respiratory tract infections and pathogens [32, 33]. The commercially-available essential oil components, (–)-

Table 2. Chemical compositions (% enantiomer) of the essential oil from the aerial parts of *Chrysothamnus viscidiflorus* subsp. *viscidiflorus*.

Compound	RT_{std}	RT_{EO}	Composition (%)
(+)-α-Thujene	13.92	13.90	75.5
(−)-α-Thujene	13.99	13.97	24.5
(−)-α-Pinene	15.92	15.84	94.5
(+)-α-Pinene	16.40	16.42	5.5
(+)-Sabinene	19.68	19.16	100.0
(−)-Sabinene	20.60	nd	0.0
(+)-β-Pinene	20.08	19.60	0.2
(−)-β-Pinene	20.28	19.96	99.8
(−)-Limonene	25.06	25.02	7.7
(+)-Limonene	25.99	25.44	92.3
(−)-β-Phellandrene	26.15	26.34	95.6
(+)-β-Phellandrene	26.88	26.87	4.4
(+)-*cis*-Sabinene hydrate	40.70	40.70	86.1
(−)-*cis*-Sabinene hydrate	41.25	41.26	13.9
(+)-α-Thujone	43.56	nd	0.0
(−)-α-Thujone	44.88	45.06	100.0
(+)-*trans*-Sabinene hydrate	46.15	46.11	90.5
(−)-*trans*-Sabinene hydrate	46.84	46.84	9.5
(+)-Terpinen-4-ol	54.64	54.56	71.9
(−)-Terpinen-4-ol	54.93	55.00	28.1
(−)-α-Terpineol	59.73	59.74	87.4
(+)-α-Terpineol	60.58	60.54	12.6
(+)-Germacrene D	73.48	73.44	33.4
(−)-Germacrene D	73.73	73.70	66.6

RT_{std} = Retention time (min) of the standard chemicals. RT_{EO} = Retention time (min) of the essential oil components. nd = not detected.

β-pinene, (+)-limonene, and myrcene, have been screened for antimicrobial activity against the respiratory pathogens *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Cryptococcus neoformans* (Table 3).

Table 3. Antimicrobial activity (MIC, μg/mL) of *Chrysothamnus viscidiflorus* subsp. *viscidiflorus* major essential oil components.[a]

Compound	*S. aureus*	*S. pneumoniae*	*S. pyogenes*	*C. neoformans*
(−)-β-Pinene	156.3	39.1	625	625
(+)-Limonene	312.5	78.1	312.5	312.5
Myrcene	312.5	625	625	312.5
Positive control[b]	0.61	< 19.5	< 19.5	0.39
DMSO[c]	1250	1250	1250	1250

[a]Based on three replicates. [b] Gentamicin for bacteria, Amphotericin B for *C. neoformans*. [c] Dimethylsulfoxide negative control. *S. aureus: Staphylococcus aureus; S. pneumoniae: Streptococcus pneumoniae; S. pyogenes: Streptococcus pyogenes; C. neoformans: Cryptococcus neoformans*

Both *S. aureus* and *S. pneumoniae* were particularly susceptible to the essential oil components. Furthermore, β-pinene and (+)-limonene have shown good anti-*Klebsiella pneumoniae* (MIC 8-64 μg/mL) [34]; β-pinene, (+)-limonene, myrcene, and sabinene have shown good activity against *Mycobacterium tuberculosis* (MIC 10.4, 25.0, 25.0, and 33.3 μg/mL, respectively) [35]. The antimicrobial activities of the major components of *C. viscidiflorus* essential oil indicate potential effectiveness against respiratory infections and may account for the Native American use of *C. viscidiflorus* to treat colds.

4. Conclusions

As far as we are aware, this is the first report on the essential oil characterization of *C. viscidiflorus*, and adds to our understanding of the volatile phytochemistry of *Chrysothamnus*. The biological activities of the major components are consistent with the traditional Native American use of *C. viscidiflorus* to treat rheumatism and colds. Clearly, additional research is needed on other species of *Chrysothamnus* as well as infraspecific taxa of *Chrysothamnus* species in order to more fully define the volatile phytochemistry of the genus in terms of common components or marker compounds or to provide chemical profiles to differentiate species and subspecies.

Authors' contributions

Conceptualization, W.N.S.; Methodology, P.S., N.S.D., and W.N.S.; Software, P.S.; Validation, W.N.S., Formal Analysis, P.S., N.S.D., A.P., and W.N.S.; Investigation, P.S., N.S.D., A.P., K.S., and W.N.S.; Resources, P.S. and W.N.S.; Data Curation, W.N.S.; Writing – Original Draft Preparation, W.N.S.; Writing, Review & Editing, P.S., N.S.D., A.P., K.S., and W.N.S.; Project Administration, W.N.S.

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Conflicts of interest

The authors declare no conflict of interest.

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