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Leaf essential oil composition of *Tsuga canadensis* growing wild in North Alabama and Northwest Georgia

Jonathan D Craft and William N Setzer

Abstract
Tsuga canadensis was an important traditional medicine for Native Americans for the treatment of rheumatism. Leaves of *T. canadensis* were collected from two disjunctive populations: one from north Alabama and the other from northwest Georgia. The leaf essential oils were obtained by hydrodistillation and analyzed by GC-FID and GC-MS. The major components in the leaf oils were isobornyl acetate, α-pinene, camphene, limonene, piperitone, and manool. The presence of these constituents, which had previously shown analgesic and anti-inflammatory activities, support the traditional uses of this plant to treat rheumatism.

Keywords: Native American traditional medicine, isobornyl acetate, α-pinene, camphene, limonene, piperitone, manool

1. Introduction
The genus *Tsuga* (Pinaceae) is comprised of nine species, four of which occur in North America [1]. *Tsuga canadensis* (L.) Carrière, "eastern hemlock", was an important traditional medicine for Native Americans. The Abenaki and the Iriquois people consumed a decoction of the leaves to treat rheumatism; a decoction of the twigs was used by the Delaware Native Americans as an herbal steam to treat rheumatism [2]. In 1951, Shaw obtained the steam-distilled essential oil of *T. canadensis*, and was able to isolate and identify seven monoterpene hydrocarbons: Tricyclene, α-pinene, camphene, β-pinene, myrcene, α-phellandrene, and limonene [3]. More recently, there have been several headspace solid-phase microextractions[4–7] and solvent extractions [8] along with mass spectral analyses of the leaves of *T. canadensis*. In addition to the seven major monoterpene hydrocarbons, the oxygenated monoterpenoids piperitone and isobornyl acetate, and the sesquiterpene hydrocarbons β-caryophyllene and α-humulene, were also found to be major volatile components. In this work, we report the chemical compositions of the leaf essential oils of two different specimens of *T. canadensis* collected in northern Alabama and northwestern Georgia.

2. Materials and Methods
2.1 Plant Material
Branches of *T. canadensis* were collected from north Alabama (34.308° N, 87.502° E, 230 m elevation) on November 1, 2017, and from northwestern Georgia (34.819° N, 85.495° E, 546 m elevation) on November 6, 2017.The fresh leaves were removed from the branches, stored at -20 °C overnight, and hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane for four hours.

2.2Gas Chromatography and Gas Chromatography-Mass Spectrometry
The leaf essential oils of *T. canadensis* were analyzed by gas chromatography – flame ionization detection (GC-FID, Agilent 6890 GC with Agilent FID) and gas chromatography – mass spectrometry (GC-MS, Agilent 6890 GC, Agilent 5973 MSD, HP-5ms capillary column) as described previously [9].

3. Results and Discussion
The masses of the frozen leaves prior to extraction were 36.1 g and 33.0 g for the Alabama and Georgia samples, respectively. Hydrodistillation yielded 149 mg (Alabama) and 136 mg

(Georgia) of essential oils. The leaf essential oil compositions of *T. canadensis* from Alabama and Georgia are compiled in Table 1. The oils were dominated by monoterpene hydrocarbons (44.6 and 27.4%, respectively) and oxygenated monoterpenoids (48.4 and 51.9%, respectively). The major components of *T. canadensis* leaf essential oils were tricyclene (4.5% in the oil from Alabama), α-pinene (13.9 and 5.4% in the oils from Alabama and Georgia, respectively), camphene (13.3 and 3.4%, respectively), limonene (6.0 and 7.0%, respectively), piperitone (4.3 and 7.7%, respectively), bornyl acetate (38.6 and 37.0%, respectively), β-caryophyllene (3.4% in the oil from Georgia), α-humulene (4.4% in the oil from Georgia), and the diterpenoid manool (6.8% in the oil from Georgia).

Table 1: Chemical compositions of the leaf essential oils of *Tsuga canadensis* collected from Bankhead National Forest, Alabama, and Rising Fawn, Georgia.

RI	Compound	Percent Composition	
		Alabama	Georgia
851	(2*E*)-Hexenal	0.6	0.2
881	Santene	tr	---
924	Tricyclene	4.5	0.6
929	α-Thujene	0.2	0.1
936	α-Pinene	13.9	5.4
948	Camphene	13.3	3.4
952	Thuja-2,4(10)-diene	tr	tr
970	Sabinene	0.1	0.1
973	β-Pinene	2.0	2.2
989	Myrcene	1.6	1.7
1001	α-Phellandrene	0.7	3.7
1006	δ-3-Carene	0.1	0.1
1013	α-Terpinene	0.3	0.3
1021	*p*-Cymene	0.2	0.7
1027	Limonene	6.0	7.0
1028	1,8-Cineole	0.4	---
1038	(*Z*)-β-Ocimene	tr	tr
1049	(*E*)-β-Ocimene	tr	tr
1058	γ-Terpinene	0.5	0.6
1066	*cis*-Sabinene hydrate	tr	0.1
1089	Terpinolene	1.3	1.5
1098	*trans*-Sabinene hydrate	tr	0.1
1103	Linalool	tr	0.1
1114	*endo*-Fenchol	tr	tr
1123	*cis*-*p*-Menth-2-en-1-ol	0.2	0.7
1128	α-Campholenal	tr	tr
1139	*trans*-Pinocarveol	tr	tr
1142	*trans*-*p*-Menth-2-en-1-ol	0.1	0.5
1145	Camphor	0.6	0.6
1147	*trans*-Verbenol	tr	0.1
1149	Camphene hydrate	0.3	0.1
1151	α-Phellandrene-8-ol	tr	tr
1158	Isoborneol	tr	---
1164	Pinocarvone	tr	tr
1167	Borneol	0.7	0.9
1170	*p*-Mentha-1,5-dien-8-ol	0.1	0.1
1175	*cis*-Pinocamphone	tr	tr
1180	Terpinen-4-ol	0.8	1.5
1194	α-Terpineol	1.2	0.8
1198	*cis*-Piperitol	0.1	0.2
1210	Verbenone	0.1	0.2
1211	*trans*-Piperitol	0.1	0.2
1234	Citronellol	tr	0.1
1238	Thymol methyl ether	0.1	---
1253	Piperitone	4.3	7.7
1286	Isobornyl acetate	38.6	37.0
1290	Thymol	0.5	0.4
1295	*trans*-Pinocarvyl acetate	tr	0.1
1327	Myrtenyl acetate	0.1	0.2
1350	α-Cubebene	tr	0.1
1358	Citronellyl acetate	0.1	tr
1369	Neryl acetate	tr	---
1370	α-Ylangene	tr	tr
1375	α-Copaene	tr	0.1
1383	β-Bourbonene	tr	tr
1384	*trans*-Myrtanyl acetate	tr	0.1

1392	7-*epi*-Sesquithujene	---	tr
1392	β-Cubebene	---	tr
1418	β-Caryophyllene	1.0	3.4
1427	β-Copaene	tr	tr
1437	α-*trans*-Bergamotene	tr	0.1
1452	α-Humulene	1.6	4.4
1460	Sesquisabinene	tr	tr
1462	*cis*-Cadina-1(6),4-diene	tr	tr
1473	*trans*-Cadina-1(6),4-diene	tr	tr
1476	γ-Muurolene	0.2	0.4
1479	Germacrene D	0.1	0.4
1484	β-Selinene	0.1	0.3
1490	*trans*-Muurola-4(14),5-diene	tr	tr
1493	α-Selinene	tr	0.1
1494	γ-Amorphene	tr	0.1
1501	α-Muurolene	0.1	0.1
1507	Epizonarene	tr	---
1511	β-Bisabolene	tr	0.2
1513	γ-Cadinene	0.1	0.5
1517	(*Z*)-γ-Bisabolene	tr	0.3
1524	δ-Cadinene	0.3	0.8
1534	(*E*)-γ-Bisabolene	tr	0.3
1542	α-Calacorene	tr	tr
1567	(*E*)-Nerolidol	0.5	0.8
1581	Caryophyllene oxide	0.2	0.2
1607	Humulene epoxide II	0.2	0.2
1642	τ-Cadinol	0.2	0.3
1647	α-Muurolol	0.1	0.1
1654	α-Cadinol	0.2	0.2
1688	α-Bisabolol	tr	0.1
1726	(2*Z*,6*E*)-Farnesol	tr	0.1
2053	Manool	1.6	6.8
	Monoterpene hydrocarbons	44.6	27.4
	Oxygenated monoterpenoids	48.4	51.9
	Sesquiterpene hydrocarbons	3.4	11.5
	Oxygenated sesquiterpenoids	1.3	2.2
	Diterpenoids	1.6	6.8
	Others	0.6	0.2
	Total Identified	99.9	99.6

The compositions of *T. canadensis* essential oils are consistent with the headspace solid-phase microextractions reported earlier [4–7]: Tricyclene (3.1-7.8%), α-pinene (10.4-23.7%), camphene (7.8-15.9%), limonene (0.7-6.0%), piperitone (up to 5.9%), isobornyl acetate (26.8-50.7%), β-caryophyllene (1.3-6.0%), and α-humulene (up to 9.2%). However, these headspace techniques did not reveal less volatile constituents such as oxygenated sesquiterpenoids and the diterpenoid manool, which were observed in the essential oils in this study.

Several monoterpenes have shown analgesic [10, 11] and anti-inflammatory [12–14] effects, including α-pinene, myrcene, and limonene. In addition, bornyl acetate [15–17] has shown anti-inflammatory activity, β-caryophyllene has demonstrated analgesic [18–21] and anti-inflammatory [14, 22] activities, α-humulene has shown anti-inflammatory effects [23], and manool was also shown to be anti-inflammatory [24]. Thus, the major components of *T. canadensis* essential oil show analgesic and anti-inflammatory activities, which are consistent with the traditional uses of this plant to treat rheumatism.

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Conflicts of Interest

The authors declare no conflicts of interest. The funding sponsor, dōTERRA International, played no role in the design of the study; in the collection, analysis, or interpretation of the data; conclusions of the study; or in the decision to publish the results.

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