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Chemical composition of the bark essential oil of *Cercis canadensis* L. (Fabaceae)

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Abstract
The volatile components from the bark of *Cercis canadensis* L. (Fabaceae) were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry as well as enantioselective gas chromatography. The bark volatiles were dominated by C_6 fatty-acid-derived compounds 1-hexanol (23.3%), hexanoic acid (18.2%), and (2*E*)-hexenoic acid (3.4%). The concentration of monoterpenoids in *C. canadensis* bark was low (4.1%), but did allow determination of the enantiomeric distribution of α-pinene (racemic), limonene (exclusively *d*-enantiomer), linalool and α-terpineol (predominantly *l*-stereoisomers).

Keywords: Essential oil composition, *Cercis canadensis*, enantiomeric distribution, Native American ethnopharmacology

1. Introduction
Cercis canadensis L. (Fabaceae), commonly known as "eastern redbud", ranges throughout the southeastern United States. The tree was used by Native Americans for food as well as medicine. The bark of *C. canadensis* was used to make a tea as a remedy for whooping cough (pertussis), congestion, fever, and vomiting [1]. As part of our continuing interest in Cherokee traditional medicines [2-5], we have investigated the essential oil composition, including enantiomeric distribution of monoterpenoids, of the bark of *C. canadensis*. To our knowledge, this is the first examination of the essential oil of *C. canadensis* and the first report of enantiomeric distribution of monoterpenoids in the Fabaceae.

2. Materials and Methods
2.1 Plant Material
Branches of *C. canadensis* were collected from Huntsville, Alabama (34° 38′ 46″ N, 86° 33′ 27″ W, 191 m elevation) on November 5, 2016. The bark was stripped from the limbs and finely chopped. The chopped bark (87.78 g) was hydrodistilled using a Likens-Nickerson apparatus, with continuous extraction with dichloromethane, for 4 h. *C. canadensis* bark essential oil (1.6709 g, 1.904% yield) was obtained as a colorless liquid, which was stored at –20 °C until further analysis.

2.2 Gas Chromatography – Mass Spectrometry
GC-MS analysis was carried out using a Shimadzu GCMS-QP2010 Ultra. This instrument was operated in the electron impact (EI) mode set at electron energy 70eV with a scan range of 40-400 amu, a scan rate of 3.0 scans per second, and with GC-MS solution software. A ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 μm was used as the GC column. Helium was used as the carrier gas and the pressure was set at 80 psi with a flow rate of 1.37 mL/min on the column head. The temperature of the injector was set at 250 °C and the temperature of the ion source was set at 200 °C. The temperature of the GC oven was programmed to be 50 °C initially and was programmed to increase at a rate of 2 °C/ min to a final temperature of 260 °C. The sample was prepared with CH_2Cl_2 in a 5% w/v solution. Then, 0.1 μL of the solution was injected into the instrument with the splitting mode with a split ratio of 30:1. The retention indices were determined by reference to a homologous series of *n*-alkanes. The components of each essential oil sample were identified based on their retention indices and mass spectral fragmentation patterns compared to reference literature [6] and our in-house library.

2.3 Chiral Gas Chromatography – Mass Spectrometry

The essential oil from *C. canadensis* was also analyzed enantioselectively with a Shimadzu GCMS-QP2010S. The instrument was operated in the EI mode with electron energy of 70 eV, a scan range of 40–400 amu, and a scan rate of 3.0 scans/s. The capillary column used was a Restek B-Dex 325 with film dimensions of 30 m × 0.25 mm ID × 0.25 µm. The temperature of the oven was programmed to start at 50 °C and to rise at a rate of 1.5 °C/min to a final temperature of 120 °C. Then, the oven was raised to 200 °C at a faster rate of 2 °C/min and maintained for 5 min. The carrier gas, helium, was set at a constant flow rate of 1.8 mL/min. A solution (0.1 µL) of 3% w/v of the essential oil in CH_2Cl_2 was injected into the instrument in split mode with the split ratio of 1:45.

3. Results and Discussion

The composition of *C. canadensis* bark essential oil is compiled in Table 1. A total of 57 compounds were identified in *C. canadensis* bark oil accounting for 97.9% of the composition. The essential oil was dominated by fatty acid-derived compounds (76.0%), including 1-hexanol (23.3%), hexanoic acid (18.2%), (2*E*)-hexenoic acid (3.4%), oleamide (3.2%), and 1-docosanol (3.0%). *n*-Alkanes (10.2%), and aromatics (5.5%), were also present. Fatty acids and fatty acid-derived alcohols and aldehydes have sometimes been shown to be a feature of essential oils of the Fabaceae. For example, the bark essential oil of *Cassia bakeriana* from Brazil revealed 51.3% fatty acids, 23.2% aldehydes, and 11.1% alcohols [7]; although it did not contain any C_6 compounds, the bark essential oil of *Inga laurina* from Brazil was composed of 46.8% fatty acids [8]; and the leaf essential oil of *Robinia pseudoacacia* growing in Poland was composed of 65.1% aliphatic alcohols [9].

Nakamura and Hatanaka have shown that C_6 alcohols and aldehydes are bacteriostatic to several different strains of bacteria [10], but, in general, longer chain alcohols are more active [11, 12]. Huang and co-workers have demonstrated that medium-chain fatty acids as well as long-chain fatty acids exhibit antimicrobial activity; hexanoic acid was particularly active against *Candida albicans*, *Fusobacterium nucleatum*, and *Streptococcus mutans* [13].

Although the concentration of monoterpenoids was somewhat low, only 4.1%, it was possible to determine their enantiomeric distribution using chiral gas chromatography – mass spectrometry. α-Pinene was present as a racemic mixture, but limonene was present as the pure (+)-enantiomer. The (–)-enantiomers were the major stereoisomers for linalool (65%) and α-terpineol (70%). This, we believe, represents the first examination of the enantiomeric distribution of monoterpenoids in the Fabaceae.

4. Conclusions

The bark essential oil of *Cercis canadensis* was found to be rich in medium-chain and long-chain alcohols, aldehydes, and carboxylic acids, in particular C_6 compounds. The presence of these compounds may account for the traditional use of *C. canadensis* bark by the Cherokee and other Native Americans. Although monoterpenoid concentrations were low, the chiral gas chromatographic analysis was able to discern the relative enantiomeric concentrations of α-pinene, limonene, linalool, and α-terpineol.

Table 1: Volatile components of *Cercis canadensis* bark.

RI[a]	Components	%		RI[a]	Components	%
799	Hexanal	0.9		1349	Eugenol	0.7
832	2-Methylbutanoic acid	1.0		1397	Methyleugenol	0.6
844	(3*Z*)-Hexenol	0.3		1418	β-Caryophyllene	0.7
849	(3*E*)-Hexenol	2.2		1446	Geranyl acetone	0.5
859	(2*Z*)-Hexenol	0.7		1508	Dicyclohexyl ketone	0.5
862	1-Hexanol	23.3		1580	Caryophyllene oxide	1.1
885	(4*Z*)-Hepten-2-ol	0.8		1600	*n*-Hexadecane	0.6
900	2-Heptanol	1.7		1607	1,10-di-*epi*-Cubenol	0.7
931	α-Pinene	0.2[b]		1654	α-Cadinol	0.9
967	1-Heptanol	0.6		1700	*n*-Heptadecane	0.8
975	Hexanoic acid	18.2		1793	1-Octadecene	0.5
977	1-Octen-3-ol	1.5		1800	*n*-Octadecane	0.6
1003	Octanal	0.4		1894	1-Nonadecene	0.7
1008	(2*E*)-Hexenoic acid	3.4		1900	*n*-Nonadecane	0.9
1028	Limonene	2.0[c]		1956	Palmitic acid	2.5
1032	Benzyl alcohol	1.3		1986	1-Eicosene	0.7
1042	Benzene acetaldehyde	1.0		2000	*n*-Eicosane	0.8
1069	1-Octanol	1.2		2100	*n*-Heneicosane	2.7
1083	*o*-Guaiacol	0.3		2110	Methyl linoleate	1.2
1092	Unidentified[d]	2.1		2200	*n*-Docosane	0.7
1099	Linalool	0.8[e]		2300	*n*-Tricosane	0.9
1104	Nonanal	1.8		2371	Oleamide	3.2
1111	2-Phenylethanol	0.6		2454	Docosanal	0.7
1159	(2*E*)-Nonenal	0.6		2517	1-Docosanol	3.0
1164	Octanoic acid	1.1		2600	*n*-Hexacosane	0.4
1194	α-Terpineol	0.7[f]		2700	*n*-Heptacosane	0.6
1205	Decanal	1.2		2806	(*E*,*E*,*E*)-Squalene	0.5
1230	2-Coumaranone	0.5		2900	*n*-Nonacosane	1.2
1248	Chavicol + Geraniol	0.8			Total Identified	97.8

[a] RI = "Retention Index", determined with respect to a series of *n*-alkanes on a ZB-5 column. [b] 50% (+)-α-pinene / 50% (–)-α-pinene. [c] 100% (+)-limonene. [d] Unidentified: MS, m/e 196(2%), 128(2%), 101(23%), 99(44%), 83(52%), 71(48%), 55(100%), 45(38%), 43(74%), 41(34%). [e] 35% (+)-linalool / 65% (–)-linalool. [f] 30% (+)-α-terpineol / 70% (–)-α-terpineol.

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