



AkiNik

# American Journal of Essential Oils and Natural Products

Available online at [www.essencejournal.com](http://www.essencejournal.com)

ISSN: 2321-9114  
 AJEONP 2017; 5(1): 15-17  
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 Received: 04-11-2016  
 Accepted: 05-12-2016

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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<sub>6</sub> 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<sub>2</sub>Cl<sub>2</sub> 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.

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### 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<sub>2</sub>Cl<sub>2</sub> 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<sub>6</sub> 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<sub>6</sub> 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.  $\alpha$ -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  $\alpha$ -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<sub>6</sub> 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  $\alpha$ -pinene, limonene, linalool, and  $\alpha$ -terpineol.

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

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

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

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