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Volatile composition of *Vicia caroliniana* growing in Huntsville, Alabama

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Abstract
The volatile oils from the aerial parts of three populations of *Vicia caroliniana*, a Cherokee traditional medicine, were obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. The oils were devoid of monoterpenoids and sesquiterpenoids, but rich in phytol (16-36%), phytone (2-22%), and fatty acid derivatives (44-74%). The major components of *V. caroliniana* volatiles may be responsible for the traditional uses of this plant to treat cramps and pains.

Keywords: Carolina vetch, phytol, phytone, Cherokee traditional medicine

1. Introduction
Vicia caroliniana Walter (Carolina vetch) is a sprawling, sometimes climbing herbaceous member of the Fabaceae [1]. The plant was used by Cherokee Native Americans to treat several maladies [2]. An infusion of the plant was rubbed into scratches made over the location of muscular cramps or back pains; a decoction of the plant was taken internally to treat indigestion, rheumatism, or as an emetic [3]. A related species, *Vicia americana* Muhl. ex Willd. (American vetch), was used by the Navajo and Ramah Native Americans as an eyewash, while the Squaxin people used an infusion of the plant as a bath for soreness [3]. As part of our investigations on volatile compositions of Cherokee aromatic medicinal plants [4-8], we have collected and analyzed the volatiles from *V. caroliniana*. To our knowledge, there have been no previous reports on the phytochemistry of this plant.

2. Materials and methods
2.1 Plant Material
The aerial parts of *V. caroliniana* were collected from three different populations growing on the campus of the University of Alabama in Huntsville (34°43′ N; 86°38′ W; 197 m elevation) in March, 2016. The plant was identified by W.N. Setzer [1], The fresh plant material was chopped and hydrodistilled using a Likens-Nickerson apparatus with continuous extraction with dichloromethane to give pale yellow volatile oils in low yield (Vc-1, 0.0045%; Vc-2, 0.0039%; Vc-3, 0.0040%).

2.2 Gas Chromatography – Mass Spectrometry
The *V. caroliniana* volatile oils were analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 40-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 k Pa and a flow rate of 1.0 mL/min. Inlet temperature was 200 °C and interface temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2°/min to 220 °C. A 0.1% w/v solution of each sample in dichloromethane was prepared and 1 µL was injected using a splitless injection technique.
Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [9] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version

C.00.01.080]. The percentages of each component are reported as raw percentages based on total ion current without standardization.

2.3 Molecular Docking

Protein-ligand docking studies were carried out based on the structures of ovine cyclooxygenase-1 (COX-1, PDB 2AYL) [10] and human cyclooxygenase-2 (COX-2, PDB 5KIR) [11]. Prior to docking, all solvent molecules and the co-crystallized ligands were removed from the structures. The heme cofactors were retained in each protein model. Molecular docking calculations for the major volatile components with each of the proteins were undertaken using Molegro Virtual Docker (Version 6.0.1, Molegro ApS, Aarhus, Denmark) [12], with a sphere (15 Å radius) large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. The site of the co-crystallized inhibitor was chosen as the binding site. Standard protonation states of the proteins based on neutral pH were used in the docking studies. Each protein was used as a rigid model structure; no relaxation of the protein was performed. Assignments of the charges on each protein were based on standard templates as part of the Molegro Virtual Docker program; no other charges were necessary to set. Each ligand structure was built using Spartan '16 for Windows (Version 2.0.3, Wavefunction Inc., Irvine, CA, USA). Flexible ligand models were used in the docking and subsequent optimization scheme. Variable orientations of each of the ligands were searched and ranked based on their re-rank score. For each docking simulation, the maximum number of iterations for the docking algorithm was set to 1500, with a maximum population size of 50 and 30 runs per ligand. The RMSD threshold for multiple poses was set to 1.00 Å. The generated poses from each ligand were sorted by the calculated re-rank score.

3. Results and Discussion

Hydrodistillation of the aerial parts of *V. caroliniana* gave very low yields of essential oils (Vc-1, 0.0045%; Vc-2, 0.0039%; Vc-3, 0.0040%). The compositions of the volatile oils are summarized in Table 1. The compositions were qualitatively similar between the three samples, but did show quantitative differences. The volatiles were dominated by (*E*)-phytol, phytone, and fatty acids, principally palmitic acid. *Lathryrus ochrus* (Cyprus vetch) essential oil was rich in both phytol and palmitic acid [13]. A purge-and-trap collection of volatiles from *Vicia faba* leaves showed only the very volatile fatty-acid-derived green leaf volatiles hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 1-hexanol, and (*Z*)-3-hexen-1-ol [14]. Likewise, the headspace volatiles of *Vicia sativa* were dominated by green leaf volatiles [15]. The leaf essential oil of *Vicia dadianorum*, on the other hand, was rich in sesquiterpene hydrocarbons as well as fatty-acid-derived compounds and alkanes [16].

The major components of *V. caroliniana* volatiles may be responsible, in part, for the traditional uses of this plant. Phytol has shown antinociceptive [17] and anti-inflammatory [18, 19] activities. Additionally, fatty acids and phytol have been shown to inhibit cyclooxygenase [20].

In order to test whether the major components are cyclooxygenase inhibitors comparable to known COX inhibitors, a molecular docking analysis was carried out using the major components from *V. caroliniana* (phytone, methyl roughanate, palmitic acid, and (*E*)-phytol) with the crystal structures of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). The MolDock docking energies for the lowest-energy poses of the ligands are summarized in Table 2. Each of the major components from *V. caroliniana* docked with COX-1 with docking energies comparable to that of the known COX-1 inhibitor and co-crystallized ligand flurbiprofen. However, for COX-2, the known inhibitor rofecoxib (Vioxx®) showed stronger docking than the phytochemical ligands. Likewise, the phytochemical ligands did not show selectivity for either of the cyclooxygenase isozymes; this is not surprising considering the very flexible nature of these compounds.

Table 1: Essential oil composition of *Vicia caroliniana* essential oils

RI	Compound	Vc-1	Vc-2	Vc-3
981	1-Octen-3-ol	1.2	---	0.4
1488	(*E*)-β-Ionone	1.2	0.6	0.9
1508	Unidentified	tr	1.4	---
1515	7-Ethyl-2-methyl-2-nonen-4-one	---	---	0.6
1653	Unidentified	1.1	---	---
1711	Pentadecanal	tr	5.7	1.5
1761	Myristic acid	2.2	---	2.5
1840	6,10,14-Trimethylpentadecan-2 one (= Phytone)	5.3	21.5	2.2
1850	Unidentified	---	2.6	---
1860	Pentadecanoic acid	tr	---	0.8
1879	*n*-Hexadecanol	tr	---	1.1
1881	(*Z*,*Z*)-9,12-Hexadecadienoic acid methyl ester	tr	3.6	1.2
1887	(*Z*,*Z*,*Z*)-7,10,13 Hexadecatrienoic acid methyl ester (= Methyl roughanate)	1.9	29.5	4.3
1916	Unidentified	1.4	---	tr
1960	Palmitic acid	28.1	9.9	26.7
2022	Isopropyl palmitate	1.1	---	---
2050	(*Z*)-18-Octadec-9-enolide	---	---	15.1
2074	*n*-Octadecanol	---	---	8.2
2091	Methyl linoleate	---	2.5	1.2
2108	(*E*)-Phytol	36.1	15.8	19.2
2130	Linoleic acid	2.5	---	10.2
2138	Linolenic acid	7.2	---	tr
2151	(*E*)-Phytol acetate	1.2	---	---
2184	Unidentified	3.8	2.4	1.9
2300	Tricosane	1.0	1.8	---
2436	Unidentified	tr	0.9	tr
2500	Pentacosane	2.1	1.8	tr
2700	Heptacosane	2.4	tr	1.2
2800	Octacosane	tr	---	0.7
	Fatty acid derivatives	44.2	51.1	73.9
	Terpenoids	43.8	37.9	22.2
	Alkanes	5.6	3.6	1.9
	Total Identified	93.6	92.6	98.1

Table 2: MolDock (rerank) docking scores (kJ/mol) for the major *Vicia caroliniana* volatiles with cyclooxygenase.

Compound	COX-1 (PDB 2AYL)	COX-2 (PDB 5KIR)
6,10,14-Trimethylpentadecan-2-one (= Phytone)	-91.7	-95.4
(*Z*,*Z*,*Z*)-7,10,13-Hexadecatrienoic acid methyl ester (= Methyl roughanate)	-96.9	-100.5
Palmitic acid	-87.5	-97.5
(*E*)-Phytol	-96.9	-92.3
Co-crystallized ligand	-96.9[a]	-124.6[b]

[a] Flurbiprofen. [b] Rofecoxib.

4. Conclusions

The volatile oils of *V. caroliniana* were dominated by phytol, phytone, and fatty acid derivatives, these compounds showed excellent docking properties to cyclooxygenase, and it is therefore likely that these constituents are responsible for any analgesic and antinociceptive properties of the plant and are consistent with the traditional uses of the plant to treat aches, pains, and cramps.

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