

SUPPLEMENTARY MATERIAL

Chemical composition and antibacterial activity of *Tussilago farfara* (L.) essential oil from Quebec, Canada

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Abstract

The chemical composition of *Tussilago farfara* L. essential oil from the Saguenay-Lac-St-Jean region of Quebec, Canada was analysed by gas chromatography – flame ionisation detector (GC-FID) and gas chromatography – mass spectrometry (GC-MS), and the antibacterial activity of the oil was tested against *Escherichia coli* and *Staphylococcus aureus*. Forty-five (45) compounds were identified from the GC profile. The main components were 1-nonene (40.1%), α -phellandrene (26.0%) and *p*-cymene (6.6%). The essential oil demonstrated antibacterial activity against *E. coli* (MIC₅₀ = 468 $\mu\text{g}\cdot\text{mL}^{-1}$; MIC₉₀ = 6869 $\mu\text{g}\cdot\text{mL}^{-1}$) and *S. aureus* (MIC₅₀ = 368 $\mu\text{g}\cdot\text{mL}^{-1}$; MIC₉₀ = 773 $\mu\text{g}\cdot\text{mL}^{-1}$). Dodecanoic acid was found to be active against both bacteria having a MIC₅₀ and MIC₉₀ of 16.4 $\mu\text{g}\cdot\text{mL}^{-1}$ and 95 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively for *E. coli* and a MIC₅₀ and MIC₉₀ of 9.8 $\mu\text{g}\cdot\text{mL}^{-1}$ and 27.3 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively for *S. aureus*. In addition, 1-decene and (E)-cyclodecene were also found to be active against *E. coli*.

Keywords: *Tussilago farfara*, essential oil, antibacterial activity, volatile compounds, terpenes, aliphatic compounds

3. Experimental

3.1. Chemicals

For gas chromatography (GC) analyses and biological testing, we used standard compounds obtained from Sigma-Aldrich (St. Louis, MO, USA) (1-nonene, ρ -cymene, α -phellandrene, (E)-cyclodecene, tetracosane), Fluka (Fluka Chemie GmbH, Buchs, Switzerland) (α -humulene), Takasago (Takasago International Corporation, Tokyo, Japan) (β -caryophyllene) and Acros Organics (Fisher Scientific Company, Ottawa, ON, Canada) (1-decene, dodecanoic acid, tricosane).

3.2. Plant material and extraction of essential oil

The flowers of *T. farfara* were collected in May 2014 and May 2015 in Saguenay, Quebec, Canada. Stem and flower come from 3 different locations (48°14'42.45" 71°15'16.20"; 48°30'1.55" 70°59'29.13"; 48°31'33.12" 71°5'18.78") in Saguenay-Lac-St-Jean, all plant are pooled for hydrodistillation. Voucher specimen no. QFA0610841 has been filed at the Louis-Marie herbarium of Laval University, Quebec, QC, Canada. Essential oil was obtained from freshly harvested flowers by hydrodistillation over a 3-hour period (yield = 0.08% (w/w); refractive index = 1.4534; density = 0.84594 g·mL⁻¹). The essential oil extraction yield depended on the total amount of the raw material. The extracted essential oil was then stored in the dark at a temperature of 4 °C. Essential oil of *Thymus vulgaris* L. was purchased from Aliksir Inc (thymol chemotype).

3.3. GC-FID and GC-MS analyses

All chromatographic analyses were run on an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with a non-polar DB-5 column and a

polar SolGel-Wax column (30 m × 0.25 mm × 0.25 mm) as well as two flame ionisation (FID) detectors (Agilent Technologies, Santa Clara, CA, USA). The oils were injected in an undiluted (0.1 µL injection volume, split 1:235) and undried state. The temperature program began at 40 °C for 2 min, then rose 2 °C·min⁻¹ up to 210 °C. The temperature was then held at 210 °C for 13 min. Samples were also injected on an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5975C InertXL EI/CI mass spectrometer (MS) equipped with a DB-5MS column using the same temperature program as above and a split of 1:1000. Compounds were identified from their retention indexes as calculated from even-numbered C8 to C36 alkane standards and/or from MS databases (NIST08), HPCH 2205 and custom libraries built from pure compounds (LASEVE, UQAC, Chicoutimi, QC, Canada). Quantification was derived from the FID detector response on the DB-5 column without any correction factor. All standards were co-injected to validate the identification.

3.4. Evaluation of antibacterial activity

The antibacterial activity of *T. farfara* essential oils and compounds was evaluated against gram-negative *Escherichia coli* (ATCC bacteria 25922) and gram-positive *Staphylococcus aureus* (ATCC bacteria 25923) using an antibacterial hydrophobic assay as described by Côté et al. (2016). First, 100 µL of nutrient agar was distributed in 96-well plates and were kept at room temperature for 1 h. Subsequently, 20 µL of methanol containing growing concentrations of *T. farfara* essential oil or compounds was distributed on the 96-well plates. After evaporation of methanol, 20 µL of nutrient broth containing bacteria (2.5×10^5 colony forming units per mL) was added to each well. The 96-well plates were then incubated at 37 °C for 5 h. After, 100 µL of a 50 µg·mL⁻¹ resazurin sodium salt solution (Sigma R-2127, St-Louis, MO, USA) was

added to each well. Fluorescence was read on an automated Fluoroskan Ascent FL™ plate reader (Labsystems, Milford, MA, USA) after 2 h of incubation for *S. aureus* and 3 h of incubation for *E. coli*. The MIC₅₀ and MIC₉₀ were determined as the lowest concentration resulting in a 50% and 90% inhibition, respectively, of bacterial growth.

References

Coté H., Boucher M.-A., Pichette A., Roger B., Legault J. 2016. New antibacterial hydrophobic assay reveals *Abies balsamea* oleoresin activity against *Staphylococcus aureus* and MRSA. *J. Ethnopharmacol.* 194: 684–689.

Table S1. Chemical composition of *T. farfara* essential oil.

Name	DB-5 column		SolGel-Wax column	
	I. R.	%	%	I. R.
1-octene	787	0.2	0.2	821
1-nonene	877	40.1	40.5	910
(E)-1,3-nonadiene	912	0.2	0.2	910
α -thujene	915	<0.1	<0.1	1010
α -pinene	917	<0.1	<0.1	1006
myrcene	987	1.0	0.8	1138
1-decene	987	1.1	0.9	1026
α -phellandrene	1001	26.0	25.4	1133
ρ -cymene	1019	6.6	6.6	1243
limonene	1023	0.1	0.1	1172
β -phellandrene	1023	0.1	0.1	1181
(E)- β -ocimene	1045	<0.1	<0.1	1233
γ -terpinene	1053	<0.1	<0.1	1220
1,10-undecadiene	1094	4.1	4.2	1158
unknown ¹	1098	3.1	3.1	1220
1-undecene	1089	0.4	0.4	1121
(E)-cyclodecene	1247	1.0	-	-
unknown ²	1278	0.8	-	-
pregeijerene B	1282	0.6	-	-
unknown ³	1321	1.3	-	-
unknown ⁴	1331	0.4	-	-
7-epi-silphiperfol-5-ene	1337	0.3	-	-
silphiperfol-6-ene	1374	<0.1	-	-
α -copaene	1373	<0.1	<0.1	1463
β -cubebene	1384	<0.1	<0.1	1509
unknown ⁵	1388	0.5	-	-
β -caryophyllene	1411	0.7	0.5	1556
α -humulene	1446	0.2	0.2	1625
<i>trans</i> - β -farnesene	1456	<0.1	<0.1	1655
germacrene D	1475	1.1	1.1	1667
β -selinene	1486	0.2	0.2	1673
(<i>Z,E</i>)- α -farnesene	1494	<0.1	<0.1	1688
α -muurolene	1496	<0.1	<0.1	1692
β -bisabolene	1506	0.1	0.1	1698
γ -cadinene	1509	<0.1	<0.1	1728
δ -cadinene	1525	0.1	0.1	1718
spathulenol	1572	0.3	0.3	2075
dodecanoic acid	1572	2.4	2.7	2454

Name	DB-5 column		SolGel-Wax column	
	I. R.	%	%	I. R.
lauric acid. ethyl ester	1594	0.3	0.3	1826
humulene epoxyde II	1599	0.2	0.2	2012
τ -muurolol	1635	<0.1	<0.1	2138
τ -cadinol	1635	<0.1	<0.1	2122
α -cadinol	1647	<0.1	<0.1	2183
tetradecanoic acid	1767	0.4	0.4	2697
ethyl myristate	1793	<0.1	<0.1	2031
palmitic acid	1962	0.3	0.3	2882
ethyl palmitate	1993	<0.1	<0.1	2236
tricosane	2291	0.3	0.3	2178
tetracosane	2316	0.3	-	-
Total		88.4	86.7	

¹Non identified compound: MS fragmentation peaks 67, 79 (44), 108 (29), 55 (25), 81 (20), 93 (15), 77 (15) 135 (2) m/z

²Non identified compound: MS fragmentation peaks 67, 81 (80), 55 (71), 54 (62), 82 (57), 79 (54), 95 (46) 180 (1) m/z

³Non identified compound: MS fragmentation peaks 111, 126 (97), 71 (58), 109 (34), 95 (28), 93 (24), 55 (23) 175 (1) m/z

⁴Non identified compound: 97, 107 (44), 55 (29), 83 (22), 81 (22), 109 (20), 69 (18) 139 (12) m/z

⁵Non identified compound: 100, 71 (100), 111 (80), 109 (43), 69 (41), 55 (28), 95 (23) 155 (4) m/z

* Result is representative of two different hydrodistillations.

Table S2. Antibacterial activity of *T. farfara* essential oil (EO) and some selected compounds against *E. coli* and *S. aureus*.

<i>Tested product</i>	<i>E. coli</i>		<i>S. aureus</i>	
	MIC ₅₀ ($\mu\text{g}\cdot\text{mL}^{-1}$)	MIC ₉₀ ($\mu\text{g}\cdot\text{mL}^{-1}$)	MIC ₅₀ ($\mu\text{g}\cdot\text{mL}^{-1}$)	MIC ₉₀ ($\mu\text{g}\cdot\text{mL}^{-1}$)
<i>Tussilago farfara</i> EO	468 ± 23	6869 ± 390	368 ± 27	773 ± 50
1-nonene	> 200	> 200	> 200	> 200
1-decene	4.4 ± 0.4	48 ± 4	36 ± 4	> 200
α -humulene	> 200	> 200	> 200	> 200
α -phellandrene	63 ± 9	> 200	> 200	> 200
β -caryophyllene	106 ± 19	> 200	> 200	> 200
p-cymene	> 200	> 200	> 200	> 200
(E)-cyclodecene	6.8 ± 0.5	117 ± 4	100 ± 9	> 200
dodecanoic acid	16.4 ± 0.9	95 ± 4	9.8 ± 0.3	27.3 ± 0.9
tetracosane	12 ± 1	> 200	> 200	> 200
tricosane	14 ± 1	> 200	> 200	> 200
<i>Thymus vulgaris</i> EO	121 ± 12	342 ± 33	26 ± 2	397 ± 15

* Result is representative of two different hydrodistillations.