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Potential interest of *Tussilago farfara* (L.) whole plant of Lithuanian and French origin for essential oil extraction

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Abstract
Tussilago farfara (L.), an Asteraceae of interest for traditional medicine, is known as a source of valuable essential oil (EO). The chemical composition of hydrodistilled EO of *T. farfara* flowers and stems was determined by GC and GC/MS techniques. Comparatively, quantitative and qualitative differences were observed in EO composition from both selected origins. The EO yields were 0.07 ± 0.01% and 0.09 ± 0.00% from Lithuanian and French origin. In total, 37 components were identified in Lithuanian and French oils. The major constituent in Lithuanian oil was n-tricosane (21.7 ± 0.1%) while in French oil it was 1-nonene (34.1 ± 0.00%).

Keywords: Essential oil, *Tussilago farfara* (L.), flower, stem, geographic

1. Introduction
Tussilago farfara (L.), commonly called coltsfoot, is a perennial herbaceous Asteraceae that has traditional medicinal uses. Coltsfoot, which is native to Europe and Asia, is also a common plant in North and South America to which it was introduced, most likely by settlers as a medicinal item [1]. The plant is often found in was and disturbed places and along roadsides and paths. In some areas such as New England, coltsfoot is an invasive weed that threatens native plant habitats. Coltsfoot is spread by seeds and rhizomes and is often found in colonies of dozens of plants. The flowers appear in early spring and are pollinated by a range of flies and bees. Coltsfoot has been used medicinally as a cough suppressant [1]. The plant has been used since at least historical times to treat lung ailments such as asthma as well as various coughs by way of smoking [1, 2]. *T. farfara* leaves and flowers have expectorant activity and are used for chronic dry cough and various pulmonary diseases [2]. Crushed flowers supposedly cure skin conditions. Coltsfoot is not commonly used as a food but it is listed by the Council of Europe as a source of natural food flavoring.
Few studies have been performed on the extraction and chemical composition of *T. farfara* essential oil [3, 4]. Studies have mostly used plant buds for essential oil extraction and there are very few reports on the possibility of using the whole plant for essential oil extraction as well as on the effect of geographical variations on the composition of *T. farfara* essential oil.
The aim of this study was to examine the chemical composition profile of *T. farfara* essential oils of Lithuanian and French origin and to prove the possibility of using both flowers and stems as sources for essential oil extraction.

2. Materials and Methods
2.1 Plant material
The flowers and stems of *T. farfara* plants were collected from the Midi-Pyrenées area (south-west of France 43° 36′ 16.2″ N/ 1° 26′ 38.4″ E) and from the Kaunas Botanical Garden of Vytautas Magnus University, Lithuania (54° 52′ 14″ N/ 23° 54′ 40″ E) during April 2011. The collection period was during the full flowering vegetative phase, which is considered a preferable time in terms of phytochemical composition and functional properties of herbs [4]. Climatic conditions were in both locations favorable for plant development. Nevertheless, mean temperature and rainfall during the month of April were higher in Midi-Pyrenées (10.5 °C and 97.1 mm) than in Kaunas (6.9 °C and 34.0 mm). The *T. farfara* species has been authenticated by the herbalist Benoit Bock and cataloged in the nomenclatural database of the flora of France under the taxonomic number BDNFFnt1284.

2.2 Essential oil extraction
Sixty grams of air-dried aerial plant parts (flowers and stems) were mixed with 800 mL of distilled water and the essential oil was isolated by hydrodistillation in a Clevenger-type apparatus for 3 h. The obtained oil was separated from the water and dried over anhydrous sodium sulfate. Isolation of essential oil was performed in duplicate and the sample was stored in a freezer prior to further analysis.

2.3 GC/FID and GC/MS analysis of oil constituents
The essential oil was analyzed by GC using Hewlett-Packard 6890 apparatus (Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and electronic pressure control (EPC) injector using two fused-silica Agilent DB5 capillary columns (30 m length, 0.25 mm id., 0.25 µm film thickness) and Agilent Carbowax 20M (30 m length, 0.25 mm id., 0.25 µm film thickness). The flow of the carrier gas (N_2) was 1.0 mL/min at a column pressure of 42 kPa. The analysis was performed using the following temperature program for DB5: oven temperature isothermal at 50 °C for 5 min, increase at a rate of 5 °C/min and isothermal at 300 °C for 5 min, and for Carbowax 20M: oven temperature isothermal at 50 °C for 5 min, increase at a rate of 5 °C/min and isothermal at 250 °C for 5 min. Injector and detector temperatures were held at 280 and 300 °C, respectively.
GC/MS analysis was performed on an HP 5890 (II) gas chromatograph interfaced with an HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron ionization (EI) of 70 eV. A DB5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used. The column temperature was programmed from 30 °C (5 min hold) to 280 °C (10 min hold) at a rate of 3 °C/min. The carrier gas was He with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 *m/z*, respectively. The injected volume was 0.1 µL. Identification was based on comparison of peak retention indices relative to (C8–C28) n-alkanes with literature data. Further identification was confirmed by matching recorded spectra with the Wiley/NBS mass spectral library and other published mass spectra [5]. Quantitative data were obtained from the electronic integration of the FID peak areas.

2.4 Microscopy analysis and plant cutting procedure
To confirm the presence of essential oil in both flowers and stems, anatomical study and free hand sections were used. This method allowed examination of the samples in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs [6]. Fresh stem and flower tissues were harvested, free finest hand section was performed and tissues were placed for 1 min in Gazet du Châtelier reagent [7]. Then the sections were mounted on a clean glass slide with the help of glycerin water and covered by a glass cover slip. Then slides were observed directly on a light microscope (Leica DM-LB, magnification 400; Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) and photographed using a digital camera (Canon Power Shot S40; Canon, New York, USA).

2.5 Statistical analysis
All data were subjected to variance analysis using the GLM procedure of SAS [8]. Mean pairwise comparisons were based on the Duncan test. For GC/MS, raw data were imported into the statistical software package MSStat (ANALYT MTC, Muehlheim, Germany, MS Statistical Software version 3.02u); the software was looking for differences in the mass fragments (*m/z*), retention time and abundance of oil constituents.

3. Results and Discussion
3.1 Essential oil yield and whole plant potential
The yield of *T. farfara* essential oil was 0.07 ± 0.01% (Lithuania) and 0.09 ± 0.00% (France) (w/w). A similar essential oil yield of 0.10% (w/w) from *T. farfara* buds grown in China was obtained by Liu *et al*. [3]. However, in this study aerial biomass (flowers and stems) was used. This result highlighted the possibility and interest to use whole plants for essential oil extraction instead of buds. Moreover, the European Food Safety Authority (EFSA) expressed an opinion on the scientific substantiation of health claims in relation to *T. farfara* and normal function of the upper respiratory tract and "immune health" in 2009 [9]. The EFSA report provided information on the conditions of use of flowers and leaves of *T. farfara* plants as infusion, powder, tincture and fluid extract. However, no information was provided on the conditions of use of stem parts. In order to prove the potential interest of the stem as a provider of essential oil, we performed an anatomical study. The result emphasized clearly the presence of glandular trichomes in *T. farfara* stems similar to those shown in flowers (Figure 1(b), (c1) and (c2)) which could be useful organs for essential oil supply. This result reinforces the proposal of using the aerial part (flowers and stems) of *T. farfara* plants for essential oil production. It should be noted that to date, no sufficient scientific study on *T. farfara* species has been conducted in the literature as noted in the EFSA report. Moreover, stems represented an interesting percentage of the aerial part (50 ± 3.5%) of plant dry matter, while those of the bud and flower were two times lower (28.6 ± 2.4% and 21.4 ± 3.7%, respectively). Accordingly, the use of the stem could be suggested as a potential and additional part of *T. farfara* species for essential oil supply.

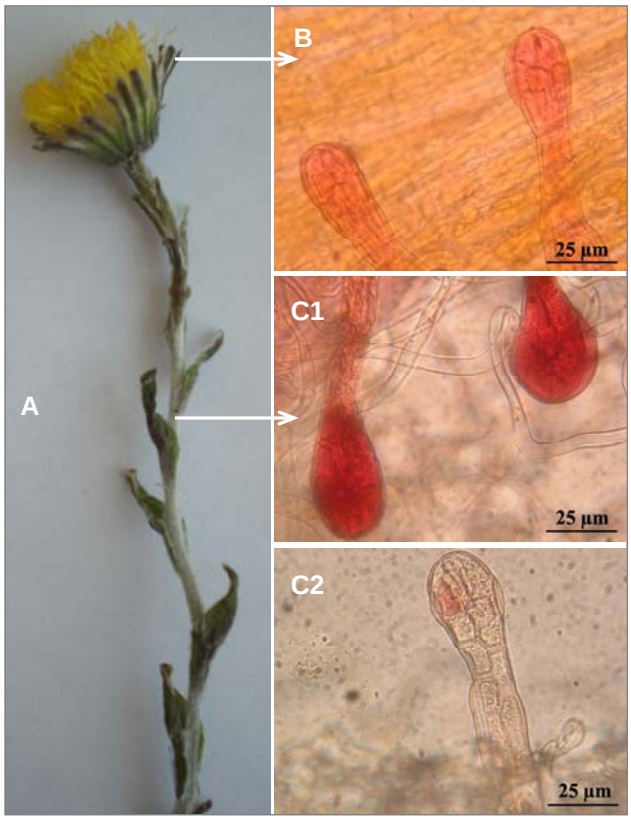

Fig 1: Microscopic photographs of glandular trichomes from two parts of (A) *Tussilago farfara* (L.) plant; (B) flower; (C1 and C2) stem. Magnification 400×.

3.2 Essential oil composition and effect of geographical origin

GC/MS analysis exhibited 37 components detected in Lithuanian and French oil, accounting for 98.7 ± 0.1% and 91.5 ± 0.5% of the total essential oil. The chemical composition of *T. farfara* essential oil previously studied from different origins and reported in several works is presented in Table 1. Up to now, no information has been available on the chemical constituents of *T. farfara* essential oils from plants grown in France or other Southern European countries. There is only one study on the volatile oil of coltsfoot of Eastern European origin [4]. Qualitative and quantitative differences in oil composition of the two origins (Lithuania and France) are presented in Table 1. 1-Nonene (34.1 ± 0.0) was the major compound in French oil; however, n-tricosane (21.7 ± 0.1) was the major compound in Lithuanian oil. Moreover, seven volatile compounds, namely tetracosane, n-tricosane, n-pentacosane, n-hexacosane, n-pentadecane, 1-tetradecene and 1-decanol, were detected only in Lithuanian *T. farfara* oil, not in French oil. The content of 1-nonene in French oil was four times higher than in the Lithuanian oil. On the other hand, the percentage of α-phellandrene, α-pinene, p-cymene and 1-dodecene was higher in the French oil than in Lithuanian oil (Table 1). It is important to note that the climatic conditions of the two studied area's countries are clearly different; mean temperature and rainfall during the month of collection were higher in Midi-Pyrenées, France (10.5 °C and 97.1 mm) than in Kaunas, Lithuania (6.9 °C and 34.0 mm). Comparison of the percentages of main components in *T. farfara* oils analyzed in our study and those previously reported in the literature is summarized in Table 2. By comparison with Lithuanian origin, *T. farfara* oil analyzed in our study and that previously reported [4] have roughly the same chemical profile, with n-tricosane (22.7–21.7%) as the first major compound. However, 1-nonene (8.1 ± 0.3%) was the second major compound in our study, but in the literature this compound was absent and n-pentacosane was the second major compound. Also, n-undecane and n-pentacosane were found at concentrations of 7.8 ± 0.2% and 1.3 ± 0.0%, respectively; however, their percentage was different in data published by Judzentiene *et al*. [4]: n-undecane and n-pentacosane reached 3.9% and 9.4%, respectively. In addition, the organs and the collection area of *T. farfara* plants investigated in our study (flowers and stems from Kaunas area) were not the same as those studied by Judzentiene *et al*. [4] (flowers or stems from Vilnius and Kedainiai areas).

Table 1: Chemical composition (%) of flower and stem essential oils of *Tussilago farfara* from two origins.

No	Compound	R.I.^{ap}	R.I.^{p}	Content (%)	
				Lithuania	France
1	1-nonene	890	922	8.1 ± 0.3^{b}	34.1 ± 0.0^{a}
2	α-pinene	935	1024	3.7 ± 0.1^{a}	4.2 ± 0.2^{a}
3	camphene	951	1068	–	tr
4	1-decene	988	1040	–	2.1 ± 0.0
5	α-phellandrene	1004	1163	0.8 ± 0.1^{a}	1.4 ± 0.3^{a}
6	p-cymene	1029	1270	0.1 ± 0.0^{b}	0.8 ± 0.0^{a}
7	n-undecane	1100	1100	7.8 ± 0.2^{a}	4.8 ± 0.1^{b}
8	α-fenchocamphorone	1105	1106	8.6 ± 0.0	7.5 ± 0.0
9	1-dodecene	1191	1190	0.2 ± 0.0^{b}	0.5 ± 0.0^{a}
10	n-dodecane	1200	1200	7.2 ± 0.1	6.5 ± 0.1
11	n-decanal	1201	1475	2.8 ± 0.1	1.9 ± 0.1
12	(2E, 4E)-nonadienol	1219	1771	1.4 ± 0.0	1.1 ± 0.0
13	(3E)-decen-1-one	1237	1784	0.8 ± 0.0	0.5 ± 0.0
14	(4E)-decen-1-ol	1262	1797	1.7 ± 0.0	1.1 ± 0.0
15	1-decanol	1273	1275	0.1 ± 0.0	–
16	1-tridecene	1291	1365	0.1 ± 0.0^{b}	0.3 ± 0.0^{a}
17	n-tridecane	1300	1300	6.8 ± 0.0	5.9 ± 0.0
18	α-copaene	1376	1489	0.2 ± 0.0	tr
19	β-cubebene	1387	1522	0.2 ± 0.0^{b}	0.3 ± 0.0^{a}
20	1-tetradecene	1389	1414	0.9 ± 0.1	–
21	n-tetradecane	1400	1400	3.0 ± 0.1	2.8 ± 0.0
22	β-caryophyllene	1419	1589	1.9 ± 0.1^{a}	0.3 ± 0.0^{b}
23	γ-elemene	1439	1470	0.2 ± 0.0^{b}	0.4 ± 0.0^{a}
24	α-humulene	1454	1665	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}
25	germacrene D	1485	1689	0.7 ± 0.1^{a}	0.6 ± 0.0^{a}
26	β-selinene	1489	1696	0.7 ± 0.0^{a}	0.6 ± 0.1^{a}
27	n-pentadecane	1500	1500	0.5 ± 0.1	–
28	β-bisabolene	1509	1715	1.2 ± 0.1^{a}	0.7 ± 0.1^{b}
29	δ-cadinene	1522	1737	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}
30	spathulenol	1576	2128	2.1 ± 0.1^{a}	0.7 ± 0.1^{b}
31	caryophyllene oxide	1585	1918	1.3 ± 0.1^{a}	0.2 ± 0.0^{b}
32	tetradecanol	1680	2177	–	0.2 ± 0.0
33	n-octadecane	1800	1852	2.3 ± 0.0	2.0 ± 0.0
34	n-nonadecane	1900	1948	1.4 ± 0.0	1.1 ± 0.0
35	phytol	2112	2547	1.4 ± 0.1^{a}	0.4 ± 0.0^{b}
36	n-docosane	2200	2200	1.0 ± 0.0	1.0 ± 0.0
37	n-tricosane	2300	2300	21.7 ± 0.1	1.4 ± 0.0
38	n-tetracosane	2400	2400	0.7 ± 0.0	0.5 ± 0.0
39	n-pentacosane	2500	2500	1.3 ± 0.0	0.9 ± 0.0
40	n-hexacosane	2600	2600	5.1 ± 0.1	4.3 ± 0.1
Total				98.7 ± 0.1^{a}	91.5 ± 0.5^{b}

^{ap} Retention indices relative to C8–C28 alkanes on a DB5 column
^{p} Retention indices relative to C8–C28 alkanes on a Carbowax 20M column
Values with different letters (a, b) are significantly different at $P<0.05$; trace (tr) < 0.05%

On the other hand, significant differences were found between the main constituents of *T. farfara* in the oils studied in this work and those reported by Liu *et al*. [3] and Hãdãrugã *et al*. [10] of Chinese and Romanian origin, respectively (Table 2). Indeed, Chinese *T. farfara* bud oil contained a reasonable amount of E-cycloundecene (8.5%), which was not found in the studied oils of Lithuanian and French origin. Moreover, β-bisabolene was the major constituent (13.9%) in Chinese oil, but did not exceed 0.7% and 1.2% in French and Lithuanian oil, respectively. Romanian *T. farfara* leaf oil contained two main compounds, α-bisabolol (20.7%) and chamazulene (7.2%), which were not found in Lithuanian and French oils. These significant differences could be related to climatic conditions or the studied parts of plants from these countries, which do have not the same chemical composition. Also, the chemotype may be an additional factor responsible for this difference [7, 11].

Finally, this study reveals that essential oils of Lithuanian, French, Chinese and Romanian origin were very different in their chemical composition; perhaps these differences were dependent on many factors such as climatic conditions, soil effect, where and when the samples were collected, prevailing conditions during plant development and their genetic peculiarities.

Table 2: Comparison of main essential oil constituents of *Tussilago farfara* (L.) from different plant parts and different geographic origins.

Compound	Lithuania					France	China	Romania
	Ka-FS	V-F	V-S	Ke-F	Ke-S	M-FS	Ku-B	CL
1-nonene	8.1	–	–	–	–	34.1	0.1	–
n-tricosane	21.7	21.6	22.7	19.4	7.2	–	–	–
β-bisabolene	1.2	0.6	–	0.8	–	0.7	13.9	–
E-cycloundecene	–	–	–	–	–	–	8.5	–
α-bisabolol	–	–	–	–	–	–	–	20.7
chamazulene	–	–	–	–	–	–	–	7.2
References	This study	[4]				This study	[3]	[10]

Collection area: Ka: Kaunas, V: Vilnius, Ke: Kedainiai, M: Midi-Pyrénées, Ku: Kuandonghua, C: Câmpeni; plant part: FS: flower and stem, F: flower, S: stem, B: bud, L: leaf; tr: trace < 0.05%

4. Conclusion

The present study provides new information about the chemical composition of *T. farfara* flower and stem essential oil from two geographical origins (Lithuania and France). Microscopy analysis revealed that stems contained secretory pockets (glandular trichomes) which could be useful organs for essential oil supply, reinforcing the proposal of using all aerial parts of *T. farfara* plants for essential oil production. The percentage of the main oil compounds in the studied *T. farfara* collected in remote habitats was significantly different; however, comparison of the obtained data with previously studied *T. farfara* oils revealed that there are different plant chemotypes. Therefore, further investigations are necessary to ascertain our results by studying a collection of accessions from different geographic regions cultivated under same climatic conditions.

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6. References

1. Bensky D, Clavey S, Stoger E, Gamble A. Chinese herbal medicine. In: Niemeier G (Ed.), Materia Medica, 3rd ed., Eastland Press, Seattle, 2004, 442.
2. Uzun E, Sariyar G, Adsersen A, Karakoc B, Ötük G, Oktayoglu E *et al*. Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. J. Ethnopharmacol. 2004; 95:287-296.
3. Liu Y, Yang X, Wu B. GC–MS analysis of essential oil constituents from buds of *Tussilago farfara* L. J Chin Pharm Sci. 2006; 15:10-14.
4. Judzentiene A, Budien J. Volatile oils of flowers and stems of *Tussilago farfara* L. from Lithuania. J Essent Oil Bear Pl. 2011; 14:413-416.
5. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publ. Corp., Carol Stream, IL, 2011.
6. Berlyn GP, Miksche JP. Botanical Microtechnique and Cytochemistry, Iowa State University Press, Ames, Iowa, 1976, 326.
7. Commission Nationale de Pharmacopée. Pharmacopée Française, 10th ed., Ordre National des Pharmaciens, Paris, 1972.
8. SAS Institute, Inc. SAS/STAT User's Guide, version 6, 4th ed., SAS Inst. Inc., Cary, NC, 1990, 2.
9. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific opinion on the substantiation of health claims related to *Tussilago farfara* L. and function of the upper respiratory tract and immune health (ID 2497) pursuant to Article 13 of Regulation (EC) No 1924/2006 on request from the European Commission. EFSA J. 2009; 7(9):1301(12 pp.). doi: 10.2903/j.efsa.2009.1301.
10. Hãdãrugã NG, Hãdãrugã DI, Tatu C, Gruia A, Costescu C, Lupea AX. Multivariate analysis (PCA) in compositae biocompounds class. J Agroaliment Proc Technol. 2009; 15:201-210.
11. Roche J, Alignan M, Bouniols A, Cerny M, Vear F, Mouloungui Z *et al*. Sterol content in sunflower seeds (*Helianthus annuus* L.) as affected by genotypes and environmental conditions. Food Chem. 2010, 121.