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## Antioxidant Properties and Composition of Deodorized Extracts of *Tussilago farfara* L.

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**Abstract:** Residues obtained after isolating essential oil very often constitute more than 99% of the total raw material. Such residues are poorly exploited although they may represent a potential sustainable source for valuable natural products. This study investigated antioxidant properties and the composition of bioactive compounds (total phenolics, flavonoids, flavonols) present in the deodorized extracts of *Tussilago farfara* flowers and stems collected in Lithuania and South of France, which were isolated with acetone, methanol or ethanol. Online HPLC/UV/DPPH scavenging assay showed that among 8 identified by HPLC/MS compounds, dicaffeoylquinic acids and quercetin pentoside were the major radical scavengers in the *T. farfara* extracts.

**Keywords:** *Tussilago farfara* L.; Deodorized extracts; Antioxidants; Radical-scavenging capacity.

### 1. Introduction

There is an increasing demand for natural bioactive compounds which may be used in foods, nutraceuticals, pharmaceuticals, cosmetics and other products; therefore comprehensive bio-prospecting, i.e. collection, investigation and utilisation of diverse biological resources remain an important scientific and practical task. Plant kingdom is a tremendous renewable resource of biologically active compounds, whereas biodiversity of plant chemical profiles offers a vast list of phytochemicals which has to be properly assessed. Therefore, the studies of plant chemical composition and discovery of new components are of great interest for scientists and society [1]. The processing of plant origin raw materials results in the production of by-products that may be rich sources of natural products, including antioxidatively active phenolic compounds [2]. The availability of phenolic compounds from agricultural and industrial residues, their extraction and antioxidant activity were reviewed more than 10 years ago [3]. Phenolic compounds with antioxidant activity were reported in various agricultural by-products, such as rice [4], buckwheat [5] and almond hulls [6].

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The residues obtained during essential oil distillation may constitute up to 99.5% of the raw material and therefore represent a potential source for antioxidants and other valuable substances, however currently such residues are under exploited. The content of phenolic and other valuable compounds in plants depends on several factors, such as cultivation zone, climatic conditions, vegetation phase, genetic peculiarities. Therefore, the composition and the quality of plant material grown in various sites and geographical zones may be remarkably different [7]. *Tussilago farfara* (Asteraceae), commonly called coltsfoot has been traditionally used as a medicinal herb to treat lung ailments, such as asthma as well as various coughs by way of smoking. *T. farfara* leaves and flowers possess expectorant activity and are used for chronic dry cough and various pulmonary diseases [8]. Volatile constituents of *T. farfara* were studied previously, while to the best of our knowledge the residues remaining after their distillation from the plant were not evaluated until now [9-10]. The extracts of *T. farfara* were shown to exhibit various activities, such as antioxidant and antimicrobial as well as inhibitory effects on NO synthesis in LPS-activated macrophage and diacylglycerol acyltransferase activity [11-12]. Some phenolics, mucopolysaccharides and water-soluble polysaccharides were isolated from the flower buds of *T. farfara* [13], however the presence of flavonoids has not been reported until now.

It should be noted that besides beneficial bioactive compounds present in the herb, it was found to contain toxic pyrrolizidine alkaloids (PAs), mainly senkirkine, which was recognised by The International Agency for Research on Cancer (IARC) as possessing "sufficient or limited evidence" for the carcinogenicity [14]. As a result, in order to minimize the amount of toxic PAs ingested, the German health authorities limited the daily intake of toxic PAs to 1 µg [15]. Most recently Committee on Herbal Medicinal Products recommended to keep exposure to PAs as low as practically achievable, as recommended by IPCS 1988, EFSA 2007, BfR 589 2007 [16]. To achieve this requirement some free of the toxic compounds *T. farfara* clones were selected and introduced [17].

Deodorized plant residues may contain a number of bioactive compounds, which was shown by the studies of some other species [18]. So far as there is no information on the properties of *T. farfara* by-products, the main aim of this study was to evaluate the antioxidant potential of *T. farfara* deodorized residues obtained after hydrodistillation of flowers and stems collected from two different geographical origins.

## 2. Materials and Methods

### 2.1. Plant material

The flowers and stems of coltsfoot (*Tussilago farfara* L.) were collected in Midi-Pyrénées (south-west of France 43° 36' 16.2" N/ 1° 26' 38.4" E) and in Kaunas Botanical Garden of Vytautas Magnus University, Lithuania (54° 52' 14" N/ 23° 54' 40" E), during April, 2010 at flowering phase. Climatic conditions in both locations were favourable for the plant development; however the mean temperature and the amount of rainfalls were higher in Midi-Pyrénées (10.5°C and 971 mm) than in Kaunas (6.9°C and 340 mm). The plants were dried at ambient temperature in a ventilated room.

### 2.2. Preparation of extracts

The volatile compounds were removed by hydrodistillation and the remaining solid residue was dried at 30 °C and extracted with acetone, methanol or ethanol; the extracts obtained are further referred as AE, ME and EE, respectively. The yield of the extracts varied from 10.3 to 12.8% (w/w). The extractions were performed in triplicate.

### 2.3. DPPH<sup>•</sup> radical scavenging assay

Radical scavenging capacity (RSC) of *T. farfara* extracts against stable 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH<sup>•</sup>, Sigma-Aldrich Chemie, Steinheim, Germany) was determined on a UV/Vis spectrophotometer Spectronic Genesys 8 (Rochester, USA) at 515 nm by a slightly modified method of Brand-Williams and co-workers [19]. Extract solutions were prepared by dissolving dry *T.*

*farfara* extract in methanol. The solution of DPPH<sup>•</sup> in methanol ( $6.5 \times 10^{-5}$  M) was prepared daily before measurements. Two mL of this solution were mixed with 50  $\mu$ L of extract solution in a 1 cm path length microcuvette and after 30 min the decrease in absorbance was read. Blank sample with the same amount of methanol and DPPH<sup>•</sup> was prepared and measured daily. The RSC was calculated by the following formula:  $I = [(AB - AA) / AB] \times 100$ , where I is DPPH<sup>•</sup> inhibition, %; AB is the absorbance of a blank sample (t=0 min); AA is the absorbance of extract solution (t=30 min). The amount of extract required to decrease the initial DPPH<sup>•</sup> concentration in the reaction by 50% is referred as an effective concentration, IC<sub>50</sub>. The measurements were carried out in triplicate for each solvent.

#### 2.4. ABTS<sup>•+</sup> radical cation decolourisation assay

The RSC of extracts was also measured by ABTS<sup>•+</sup> radical cation assay [20]. Stock solution of ABTS (2 mM) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g NaCl, 0.27 g KH<sub>2</sub>PO<sub>4</sub>, 1.42 g Na<sub>2</sub>HPO<sub>4</sub> and 0.15 g KCl in 1 L of ultra-pure water. If the pH was lower than 7.4, it was adjusted with NaOH. Ultra-pure water was used to prepare 70 mM solution of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>. ABTS<sup>•+</sup> radical cation was produced by reacting 50 mL of ABTS stock solution with 200  $\mu$ L of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution and allowing the mixture to stand in the dark at room temperature for 15–16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, ABTS<sup>•+</sup> solution was diluted with PBS to obtain the absorbance of  $0.800 \pm 0.030$  at 734 nm. Three mL of ABTS<sup>•+</sup> solution were mixed with 30  $\mu$ L ethanol solution of *T. farfara* extract in 1 cm path length microcuvette. The absorbance was read at ambient temperature after 1, 4, 6 and 10 min. PBS solution was used as a blank sample. All determinations were performed in triplicate. The percentage decrease of the absorbance at 734 nm was calculated by the formulae:  $I = [(AB - AA) / AB] \times 100$ , where I is ABTS<sup>•+</sup> inhibition, %; AB is the absorption of blank sample (t=0 min); AA is the absorption of extract solution (t=10 min). The RSC was expressed as a percentage of inhibition of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) mM equivalent per 1000 g using prepared calibration curve. The extent of quenching of ABTS<sup>•+</sup> radical by extracts was compared with standard amounts of Trolox. The concentrations of Trolox standard used for calibration curve were 0.001, 0.01, 0.1, 0.5, 1, 1.5 mM/L.

#### 2.5. Ferric reducing antioxidant power (FRAP)

The ability of plant extracts to reduce ferric ion (FRAP assay) is another indicator frequently used for assessing antioxidant power [21]. Ferrous ion (Fe<sup>2+</sup>) produced in this assay forms a blue complex (Fe<sup>2+</sup>/TPTZ) absorbing at 593 nm. Briefly, the reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O at 10:1:1 (v/v/v). Firstly, 300  $\mu$ L of freshly prepared FRAP reagent was heated to 37 °C and an absorbance (A<sub>0</sub>) of a blank reagent was read at 593 nm in a Biotek EL808 microplate reader (Vermont, USA). Then 10  $\mu$ L of 0.1% extract solution in water and 30  $\mu$ L H<sub>2</sub>O were added (final dilution of samples in the reaction mixture was 1:34) and the absorbance (A) was recorded every 1 min during the whole monitoring period which lasted up to 30 min. The change in the absorbance ( $\Delta A_{593}$  nm) between the final reading and A<sub>0</sub> was calculated for each sample and related to the  $\Delta A_{593}$  nm of a Fe<sup>2+</sup> reference solution which was measured simultaneously.

#### 2.6. Determination of total phenolic compounds (TPC)

The content of TPC in extracts was determined with Folin–Ciocalteu reagent [22]. Calibration curve was prepared by using 1 mL reference gallic acid solutions in ethanol (0.025, 0.075, 0.100, 0.175 and 0.350 mg/mL), which were mixed with 5 mL of a standard Folin–Ciocalteu reagent and diluted with distilled water (1:10) and 4 mL of 7.5% sodium carbonate solution in distilled water. The absorption was read after 30 min at 765 nm. The concentration of TPC was expressed in mg of gallic acid equivalents (GAE) per g of plant extract.

### 2.7. Determination of flavonoids

The percentage of flavonoids was measured using rutin as a reference [23]. One mL of plant extract solution in 95% ethanol (10 g/L) and 1 mL of aluminum trichloride solution in 95% ethanol (20 g/L) were pipetted into a 25 mL volumetric flask and made up with 95% ethanol. The absorbance was read at 415 nm after 40 min at 20 °C. Blank samples were prepared from the mixture of 1 mL of plant extract and 1 drop of diluted acetic acid. The absorbance of a reference solution, which was prepared by using 1 mL of rutin solution instead of plant extract, was measured simultaneously. Rutin solution was prepared from 0.05 g of dried at 130-150 °C for 3 h rutin, which was diluted in 100 mL of 95% ethanol. All determinations were performed in triplicate. The percentage of flavonoids in plant extracts was calculated by formula:  $X=(A \times m_o \times 100 \times 10)/(A_o \times m \times 100)$ , where A is the absorbance of extract;  $A_o$  is the absorbance of rutin; m is the weight of pure extract (g);  $m_o$  is the weight of rutin (g).

### 2.8. Determination of flavonols

The content of flavonols was determined by the previously reported method [24]. Series of reference rutin solutions containing 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, and 0.5 mg/mL of rutin were prepared. Two mL of reference were mixed with 2 mL of aluminum trichloride solution (20 g/L) in 95% ethanol and 6 mL of sodium acetate solution in ethanol (50 g/L) were added. The absorbance was read at 440 nm after 2.5 h at 20 °C and the calibration curve on the dependence of the absorbency on the concentration of rutin was drawn. Plant extract samples were prepared under the same conditions by using 2 mL of extract (10 g/L) in 95% ethanol instead of rutin. All determinations were performed in triplicate. The percentage of flavonols was calculated by the formula:  $X=(C \times V \times 100)/(m \times 100)$ , where C is the concentration of rutin, determined from the calibration curve (mg/mL); V is the volume of plant extract (mL); m is the weight of pure plant extract (g).

### 2.9. On-line radical scavenging assay (HPLC/UV/DPPH)

The extracts were analyzed on a HPLC/UV system supplemented with DPPH<sup>•</sup> radical scavenging detector. Two chromatograms were recorded simultaneously, one of which representing UV absorbance of effluent at 265 nm prior to the reaction, while a mirror chromatogram was obtained at 517 nm after reaction of the effluent with DPPH<sup>•</sup> solution in the reaction coil. The mobile phase was supplied to the column by a model 9012 HPLC gradient pump (Varian, USA) at a flow rate 0.75 mL/min. The samples (20 µL) were injected into the HPLC system by means of Cheminert C1 injector (Valco Instruments, USA). Reverse-phase LiChroSpher RP-18e 5 µm 12.5×0.4 cm column and 0.5×0.4 cm precolumn (Merck, Germany) were used for separation. The DPPH<sup>•</sup> reagent was prepared by dissolving 0.01 M DPPH<sup>•</sup> in 0.1 M sodium citrate buffer (pH=7.6), methanol and acetonitrile (50:25:25 v/v). It was continuously supplied into a reaction coil (3 m of 0.25 mm id fused-silica capillary) by a model 2200 HPLC pump (Bischoff, Germany) at a flow rate of 0.75 mL/min. The signals were acquired at 265 and 517 nm wavelengths by means of Linear 206 PHD and Linear UVIS 200 UV-VIS detectors, respectively. Solution A (bidistilled water with 0.05% TFA) and B (methanol with 0.05% TFA) were used as a mobile phase components for gradient elution. The extracts were separated using the following gradient: 10% of B at 0 min, 25% of B at 5 min, 40% of B at 25 min, 95% of B at 40 min, 95 % of B at 43 min and 10% of B at 44 min. Clarity chromatography software (DataApex, Czech Republic) was used for data acquisition.

### 2.10. LC-MS analysis

LC system consisted of a Thermo-Fisher Spectra System (TFSP, San Jose, CA) P1000XR pump, a TFSP 6000LP Photodiode Array Detector and a TFSP AS 3000 autosampler. Separation of compounds was performed on a Varian Pursuit XRs 5 C18 column (250 mm×4.6 mm ID, 5 µm) using a linear gradient of 0–30 min., 3–97% of acetonitrile (A) to bidistilled water with 0.1% TFA (v/v) (B), 30–35 min., 20–80% of A to B, 35-45 min., 3-97% of A to B. Flow rate was 1 mL/min and 10 µL of the sample was injected.

Mass spectra were acquired using a Thermo-Fisher LCQ mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an atmospheric pressure chemical ionization source (APCI) using both positive and negative ion mode. The APCI source operating parameters were as follows: the capillary and APCI vaporizer temperatures were set to 250 °C and 450 °C, respectively, and the spray was stabilized with nitrogen sheath and auxiliary gas (80 and 25 arbitrary units, respectively). Discharge current was 5  $\mu$ A and capillary voltage was +15 V and -15 V in the positive and negative ion mode, respectively. The mass spectra were acquired in the data dependent mode with wideband activation (i.e. the most intense ion obtained for each scan in the full mass spectrum is further submitted to 38% collision energy for MS/MS).

### 2.11. Statistical analysis

For antioxidant activity measurements, mean values and standard deviations were calculated from at least three replicates using MS Excel 2003. Statistical analysis was performed by using one-way analysis of the variance (ANOVA), followed by the Duncans' post hoc test to compare the means showing significant variation ( $p < 0.05$ ). All the data were subjected to variance analysis using the GLM procedure of SAS [25]. Analyses were performed using MSStat (ANALYT MTC, Muehlheim, Germany, MS Statistical Software Version 3.02u); looking for differences in the plant origins.

## 3. Results and Discussion

### 3.1. Antioxidant properties of extracts in electron/hydrogen transfer based assays

DPPH $\cdot$  and ABTS $^{2+}$  radical scavenging and FRAP assays were used for assessing antioxidant activity of *T. farfara* extracts (Table 1). These methods are simple and widely used for the fast screening of plant antioxidant properties and they provide quite reliable preliminary information on the presence of antioxidatively active constituents in the extracts. In general, the reaction is based on the ability of radicals to accept either an electron or hydrogen atom. The extracts were isolated consecutively by using increasing polarity solvents, acetone, methanol or ethanol. Ethanol and methanol are quite similar protonic solvents; however both of them were tested because ethanol is more acceptable for the isolation of food and pharmaceutical grade ingredients. The results demonstrated that extract RSC was dependent on the solvent polarity, the type of free radical used in the reaction and the origin of plant material. The IC<sub>50</sub> values of *T. farfara* extracts in DPPH $\cdot$  assay were from 0.15 (EE) to 0.27 (AE) mg/mL for the extracts isolated from the plants grown in Lithuania and 0.22 (EE) to 0.39 (AE) mg/mL for the plants grown in France, showing that EE possessed stronger RSC than AE and ME in this assay. In this case the extracts isolated with polar solvents were stronger DPPH $\cdot$  radical scavengers, which is in agreement with previously published results [26-28]. However, EE extracts were less effective in scavenging ABTS $^{2+}$  than AE and ME. Although the principle of DPPH $\cdot$  and ABTS $^{2+}$  assays is similar, the latter is applicable both for lipophilic and hydrophilic antioxidants; in addition DPPH $\cdot$  can be dissolved only in organic solvents (e.g., methanol), while ABTS $^{2+}$  is soluble in both aqueous and organic media. Therefore, ABTS $^{2+}$  assay can be performed in hydrophilic and lipophilic systems [20].

FRAP assay is a versatile method and can be readily applied to aqueous, alcohol and acetone extracts of different plants. In this assay, the antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron and the results are expressed in mg ferrous iron equivalents per mL of sample. In FRAP assay, similarly to ABTS $^{2+}$ , acetone extracts were significantly stronger antioxidants than ME and EE. The ferric reducing antioxidant power of different extracts was in the range of 0.44–0.52 mg Fe (II)/mL for Lithuanian origin plants and 0.39–0.62 mg Fe (II)/mL for French origin plants. In FRAP assay a ferric salt (Fe III) is used as an antioxidant and its redox potential (0.70 V) is comparable to that of ABTS $^{2+}$  (0.68 V), therefore the results of TEAC and FRAP assays showed similar trends [29].

**Table 1.** Antioxidant characteristics, the content of total phenolic compounds, flavonoids and flavonols in the extracts isolated from *T. farfara*

Characteristics	Lithuania			France		
	Acetone (AE)	Methanol (ME)	Ethanol (EE)	Acetone (AE)	Methanol (ME)	Ethanol (EE)
DPPH <sup>•</sup> scavenging (IC <sub>50</sub> ), mg/mL	0.27±0.00 <sup>a</sup>	0.20±0.01 <sup>a</sup>	0.15±0.00 <sup>a</sup>	0.39±0.01 <sup>b</sup>	0.35±0.02 <sup>b</sup>	0.22±0.01 <sup>b</sup>
ABTS <sup>•+</sup> scavenging, %	31.7±0.60 <sup>b</sup>	28.3±0.20 <sup>b</sup>	19.6±0.30 <sup>b</sup>	17.2±0.25 <sup>a</sup>	16.0±0.10 <sup>a</sup>	13.1±0.15 <sup>a</sup>
TEAC, %, equivalent 1mmol Trolox	0.92±0.03 <sup>b</sup>	0.81±0.01 <sup>b</sup>	0.55±0.02 <sup>b</sup>	0.46±0.01 <sup>a</sup>	0.45±0.00 <sup>a</sup>	0.34±0.01 <sup>a</sup>
FRAP, mg/mL	0.52±0.00 <sup>a</sup>	0.45±0.00 <sup>a</sup>	0.44±0.00 <sup>b</sup>	0.62±0.02 <sup>b</sup>	0.47±0.01 <sup>a</sup>	0.39±0.01 <sup>a</sup>
Total phenolic compounds, mg GAE/g	94.8±0.35 <sup>a</sup>	94.6±0.22 <sup>b</sup>	66.4±0.15 <sup>b</sup>	123.2±0.12 <sup>b</sup>	66.2±0.30 <sup>a</sup>	48.8±0.22 <sup>a</sup>
Total flavonoids, mg RE /g	9.20±0.17 <sup>a</sup>	5.95±0.03 <sup>b</sup>	3.25±0.09 <sup>b</sup>	9.18±0.03 <sup>a</sup>	5.12±0.09 <sup>a</sup>	2.20±0.07 <sup>a</sup>
Total flavonols, mg RE /g	0.70±0.01 <sup>b</sup>	0.53±0.02 <sup>b</sup>	0.39±0.01 <sup>b</sup>	0.49±0.01 <sup>a</sup>	0.27±0.02 <sup>a</sup>	0.19±0.01 <sup>a</sup>

Superscript letters indicate if the values for the extracts isolated with the same solvent from Lithuanian and French origin plants are statistically different

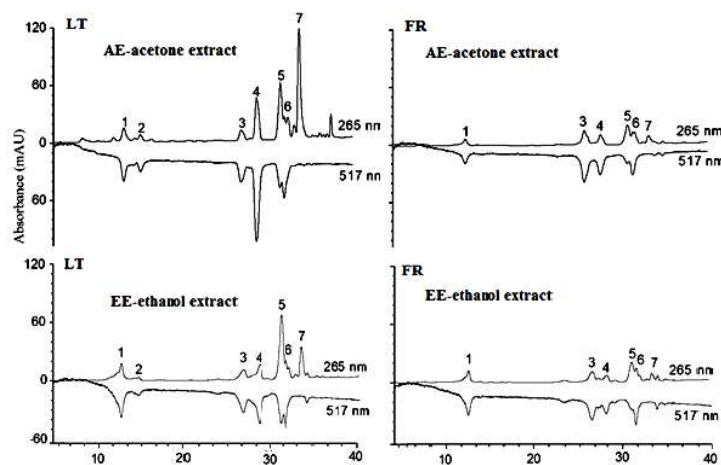
Comparing antioxidant activity indicators it may be observed that the extracts isolated from Lithuanian origin *T. farfara* in most cases possessed higher RSC than French origin plants, although in FRAP assay these differences were negligible. The differences in climatic conditions (the amount of rainfall during plant development was nearly three times less abundant in Lithuania than in France, the average temperature was also lower in Lithuania) might have the impact on these differences, however, other factors, such as plant chemotype, soil, sunlight may also have the impact on plant properties and composition. Determination of the effects of these factors on *T. farfara* properties would require special experimental design which was beyond the scope of this study.

### 3.2. Content of total phenolic compounds (TPC), flavonoids and flavonols

Phenolic compounds are very important plant antioxidants due to the presence of hydroxyl groups in their structure. It was established that phenolic compounds are the major constituents with antioxidant activity in many plants which are able to adsorb and neutralize the free radicals [30]. Among them flavonoids is the most abundant group of natural constituents found in various plants, which exhibit antioxidant activity through radical scavenging or chelating mechanisms [31]. The content of polyphenols, flavonoids and flavonols in the extracts isolated from two origin plants by three different solvents is presented in Table 1. The highest concentration of all three compound groups was in AE followed by ME and EE except for ME isolated from *T. farfara* from Lithuania; it contained similar amount of TPC to AE. It is interesting noting that AE of French origin plants contained higher amount of TPC than AE of Lithuanian origin *T. farfara*, while the content of TPC in ME and EE was higher in Lithuanian origin plants. However, the sums of TPC in AE+ME and AE+EE were similar (189.4 mg/g) or very close (161.2 and 171.0 mg/g) for both origin plants. So far as acetone and alcohol were used as consecutive extraction solvents it may be suggested that individual phenolic compounds are present in the plants of two origins at different proportions. The content of flavonoids, expressed in rutin equivalents was from 2.20 to 9.48 mg/g. In general, these results are in agreement with previously published data [26-27]. It is obvious that acetone does not provide exhaustive extraction of phenolic compounds, flavonoids and flavonols from the solid plant residue obtained after hydrodistillation; polar solvent should be applied as a second solvent.

The RSC and reducing capacity of plant extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom [32]. Usually the content of TPC measured in the extracts isolated from whole plant material is in a good correlation with RSC of such extracts. However, for *T. farfara* extracts isolated from the

hydrodistillation residue such correlations were more complex. Strong correlation ( $R^2$ ) was observed between TPC, total flavonoids and FRAP; 0.8341 and 0.7964, respectively, suggesting that phenolic compounds might be important contributors to the antioxidant properties of these extracts. However, the correlation between TPC, total flavonoids and RSC was very weak, less than 0.5: in DPPH<sup>•</sup> assay 0.269 and 0.3803, respectively; in ABTS<sup>•+</sup> assay 0.1905 and 0.3036, respectively. The correlation coefficient between total flavonols and FRAP (0.3368) or DPPH<sup>•</sup> (0.005) was found to be very weak, but strong correlation was observed between total flavonols and RSC in ABTS<sup>•+</sup> assay (0.7949).



**Figure 1.** HPLC–UV–DPPH chromatograms of acetone (AE) and ethanol (EE) extracts of *T. farfara* from Lithuanian (LT) and French origin (FR).

### 3.3. Assessment of radical scavengers by the on-line HPLC-DPPH<sup>•</sup> method

Flavonoid glycosides quercetin-3-O- $\beta$ -L-arabinopyranoside and quercetin-3-O- $\beta$ -D-glucopyranoside [33] as well as quinic, chlorogenic and dicaffeoylquinic acids were reported previously in *T. farfara* flower bud extract from China [11]. The on-line HPLC-DPPH<sup>•</sup> method was used for the detection of radical scavenging components. The ESI-MS of the AE, ME and ET of *T. farfara* extracts from two origins (Figure 1) were qualitatively different. Compounds 3 and 4 showed [M-H]<sup>-</sup> signal at  $m/z$  515 with fragmentation at  $m/z$  353 and 179, from which a molecular formula  $C_{25}H_{24}O_{12}$  was assigned. The ion at  $m/z$  353 indicated a chlorogenic acid fragment derived from the loss of a caffeoyl group. The ion at  $m/z$  179 indicated fragments of caffeic acid moieties. These MS data indicated that the compounds 3 and 4 were dicaffeoylquinic acid isomers. HPLC/MS data was not sufficient to determine exact isomers of dicaffeoylquinic acid; it is known that both of them are common components of *T. farfara* and exhibit antioxidant activity [11]. Usually, the location of the caffeoyl groups in dicaffeoylquinic are at C-3'; C-4' or C-3'; C-5'.

The ESI-MS of the *T. farfara* extracts from Lithuania gave ions corresponding to: 1-( $m/z$  191)-quinic acid, 2-( $m/z$  354)-chlorogenic acid, 3-4 ( $m/z$  515)-dicaffeoylquinic acid, 5-( $m/z$  609)-quercetin-3-rutinoside (rutin), 6-( $m/z$  434)-quercetin-pentoside and 7-( $m/z$  447)-kaempferol-glucoside. In the extracts of French origin chlorogenic acid was not detected. Quinic, chlorogenic and dicaffeoylquinic acids and rutinoside were previously isolated from the flower buds of *T. farfara* [11], whereas, to the best of our knowledge, quercetin-pentoside and kaempferol-glucoside were not previously reported in this plant. In general, the RSC of *T. farfara* extracts from the plants grown in Lithuania was slightly higher than those of French origin (Table 1). The profiles of chromatograms of *T. farfara* extracts from different growing locations obtained by HPLC–DPPH<sup>•</sup> method were also different (Figure 1): the number of negative peaks in the extracts of Lithuanian *T. farfara* origin was higher. Dicaffeoylquinic acids and quercetin-pentoside were major active compounds with total RSA 39.6, 10 and 17.1% (AE from Lithuania) and 16.9, 13.8 and 13.1% (AE from France), respectively (Table 2). Phytochemical composition of plant secondary metabolites depends on several factors, such as cultivation area, climatic conditions, vegetation phase, genetic peculiarities and others; therefore evaluation of plant properties from different geographical regions was in the focus of numerous studies [30-31].



**Table 2.** Composition and radical scavenging capacity of *Tussilago farfara* constituents evaluated by the on-line HPLC-UV-DPPH' method

Plant origin	Active compound	Acetone extract			Methanol extract		Ethanol extract	
		RT, min	Peak area, mV×s	% of total RSC	Peak area, mV×s	% of total RSC	Peak area, mV×s	% of total RSC
FR	Quinic acid	13.1	365.3	8.4	7.2	0.4	718.8	18.3
	CGA	-	-	-	-	-	-	-
	diCQA	26.8	150.9	16.9	863.3	46.3	871.4	22.2
	diCQA	28.6	1226.2	13.8	645.3	44.9	513.6	13.1
	Rutin	31.3	539.8	9.8	-	-	261.1	6.6
	Q-pent	31.9	1159.3	13.1	58.0	6.3	800.5	21.6
	K-3-O-glc	34.2	862.9	5.8	12.4	0.7	154.5	3.9
LT	Quinic acid	12.9	458.3	8.3	387.9	9.4	1054.8	15.9
	CGA	15.0	185.3	3.6	208.0	6.3	126.8	2.6
	diCQA	26.7	915.6	10.0	699.3	12.6	834.1	11.7
	diCQA	28.5	3385.3	39.6	917.7	23.4	1657.7	24.9
	Rutin	31.1	756.6	12.5	411.7	11.5	942.9	18.9
	Q-pent	31.9	1464.6	17.1	987.1	15.7	1065.4	16.0
	K-3-O-glc	34.2	87.3	1.0	141.7	2.8	141.5	4.2

CGA: chlorogenic acid, diCQA: dicaffeoylquinic acid, Q-pent: quercetin-pentoside, K-3-O-glc: kaempferol-glucoside.

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