Phytochemical variability of coltsfoot (*Tussilago farfara* L.) in Poland

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S u m m a r y

Coltsfoot leaves are a traditional raw material, rich in polysaccharides and phenolics. The variability of the contents of these main compounds was determined, using plant material originated from 22 natural populations of *Tussilago farfara* L. growing in various regions of Poland. In the years 2008–2009, plants from each investigated population were collected in the Garden of Medicinal Plants in Plewiska near Poznań (Poland). Coltsfoot leaves were harvested in the middle of June and July of 2010, and then dried at room temperature. In these raw material we quantified swelling index (describing mucilage content) and spectrophotometrically: the amounts of total polyphenols, polyphenols unadsorbed on hide powder (non-tannin phenolics) and tannins (expressed as pyrogallol equivalent) as well as the sum of hydroxycinnamic acid derivatives (expressed as rosmarinic acid) and flavonoids (expressed as quercetin). The demonstrated results show the relatively high and balanced contents of the basic active compounds, especially flavonoids (0.7–1.3%) and polysaccharides (swelling index: 8.0–14.5). In addition, it was found that flavonoids and mucilage in coltsfoot leaves fluctuate in only a small range (V=11–13%), regardless of overshadow and the harvest time of raw material.

Key words: *Tussilago farfara*, *medicinal plants*, *mucilage, polyphenols, tannins, flavonoids*

INTRODUCTION

Coltsfoot – *Tussilago farfara* L. (*Asteraceae)* is native to Europe, Asia and North Africa as well as brought into North America and Australia [1-3]. In European countries, leaves (*Farfarae folium*) are the main raw material of this species. In traditional Chinese medicine the flower buds are applied [4-7]. Coltsfoot is primarily used for the treatment of cough and bronchial infection [8]. Its leaf extracts and phenolic components show antimicrobial activity [9, 10]. There are some observation that *Tusillago farfara* displays antiinflammatory actions due to its inhibition of arachidonic acid metabolism and nitric oxide synthesis in lipopolisaccharide-stimulated macrophages [11]. The ethyl acetate fraction of coltsfoot significantly attenuates the neuronal damage induced by arachidonic acid and shows antioxidant effects [12, 13]. Moreover, a diterpene isolated from *Tussilago farfara*, named tussilagone, is a potent respiratory and cardiovascular stimulant [14].

The major components of raw material are polysaccharides and oligosaccharides [2, 15, 16]. Other constituents are tannins, flavonoids, phenolic acids, sterols, choline, bitter compounds, sesquiterpenes, chromones and essential oils [2, 7, 17-20]. In some studies, pyrrolizidine alkaloids in coltsfoot (senecionine and senkirkine) were found, known for hepatotoxic, carcinogenic and mutagenic activities [21-24].

The aim of present study was to determine the variability of the contents of the main active compounds (mucilage and phenolics) in leaves of *Tussilago farfara* originated from natural populations in Poland.

MATERIAL AND METHODS

Plant material

Plant material was obtained from 22 natural populations of *Tussilago farfara* originated from different regions of Poland: Western Pomerania (Pomorze Zachodnie), the Lubusz region (Ziemia Lubuska), Greater Poland (Wielkopolska), Silesian Upland (Wyżyna Śląska), the Kraków-Częstochowa Upland (Wyżyna Krakowsko-Częstochowska) and the Cieszyn Silesia (Śląsk Cieszyński). In the years 2008–2009, 16 coltsfoot specimens (clumps) from each investigated population were planted on the plots with an area of 1.7 m^2 (1.3 x 1.3 m), located in the Garden of Medicinal Plants, Plewiska near Poznań (Institute of Natural Fibres and Medicinal Plants). Plant material (usually 60–100 leaves per plot) was harvested in the middle of June and July of 2010. Average fresh and air-dry weights of leaf for each plot (population) were measured. Samples were dried at room temperature (23–25°C) and relative humidity of 50–55%. In order to determine the influence of overshadow, we compared morphological and phytochemical parameters of coltsfoot leaves on plots under tree canopy (density of tree layer: 95%) with plants growing in full sun, 3 m from the border of tree crown.

Phytochemical analysis

The investigations concern the contents of mucilage and total polyphenols as well as phenolic acids, tannins and flavonoids in dry matter (DM) of coltsfoot leaves. Swelling index was measured for 1.0 g of raw material, according to Polish Pharmacopoeia, 8th edition [25]. The amounts of total polyphenols and polyphenols unadsorbed on hide powder (non-tannin polyphenols) were determined spectrophotometrically with the Folin-Ciocalteu reagent, for 0.7 g of air-dry coltsfoot leaves [25]. The tannin content (expressed as pyrogallol) was calculated as a difference between total polyphenols and non-tannin polyphenols. Total amount of hydroxycinnamic acid derivatives (0.2 g of sample) was indicated with Arnov's reagent [25]. The content of these phenolic acids was expressed as a rosmarinic acid equivalent. The level of flavonoids (expressed as quercetin) was quantified for 0.25 g of powdered leaves, using the Christ-Müller's method [26]. The absorbance was measured on a Cintra 20 UV-VIS spectrometer (GBC) at λ=760.0 nm (total phenols and non-tannin phenols), λ=505.0 nm (phenolic acids) as well as λ=425.0 nm (flavonoids).

Statistical analysis

The Wilcoxon's and Student's tests were used to determine the statistical significance of the differences between investigated groups of samples. To check the normality of variable distribution the Shapiro-Wilk test was applied. For these analysis Statistica 7.1 software was used [27].

RESULTS

Obtained results show that coltsfoot leaves from garden collection were characterized by the relatively high and balanced contents of the main active compounds, especially flavonoids and mucilage (tab. 1). The highest variability coefficient was noted in non-tannins to tannins ratio as well as content of non-tannin polyphenols, and it was similar to morphological differentiation of coltsfoot leaves (fresh and air-dry weight). Two investigated factors: the harvest time of raw material and the overshadow of plants had an important influence on the phytochemical and (or) morphological variability of leaves of *Tussilago farfara*. The average amounts of phenolic acids, total polyphenols and non-tannin phenolics were lower in July than in June by 17–23% (fig. 1). In coltsfoot leaves harvested in July we determined slightly smaller content of non-tannin polyphenols in relation to tannins (Wilcoxon's test: $p=0.0438$) as well as water amount too ($p=0.0496$). In turn, the overshadow of plants caused 28–35% smaller mean contents of phenolic acids, total polyphenols and non-tannin phenolics than in leaves of coltsfoot growing on light (tab. 2). However, under overshadow leaves of *Tussilago farfara* were characterized by bigger fresh and air-dry weight as well as higher water content. The contents of mucilage,

flavonoids and tannins were similar, regardless of overshadow and the harvest time of raw material (statistical significance of differences was not detected).

Table 1.

Phytochemical and morphological variability of coltsfoot leaves harvested in the middle of June and July of 2010

Variables	Mean ±SD	Min.	Max.	V [%]
Swelling index	10.5±1.4	8.0	14.5	13
Phenolic acids [%]	5.60±1.33	3.61	8.05	24
Total polyphenols [%]	5.59±1.18	3.13	7.46	21
Non-tannin polyphenols [%]	3.41±0.97	1.37	5.29	28
Tannins [%]	2.18±0.50	1.12	3.28	23
Non-tannin polyphenols/tannins	1.63±0.54	0.43	3.36	33
Flavonoids [%]	1.07±0.12	0.73	1.29	11
Fresh weight of leaf [g]	2.28±0.69	1.39	4.60	30
Air-dry weight of leaf [g]	0.34±0.09	0.21	0.68	28
Water content in leaves [%]	91.0±0.9	89.4	93.5	1

Phenolic acid content – sum of the hydroxycinnamic acid derivatives expressed as rosmarinic acid equivalent; total polyphenols, non-tannin polyphenols and tannins – expressed as pyrogallol equivalent; non-tannin polyphenols/tannins – the ratio of non-tannins to tannins; flavonoids – expressed as quercetin equivalent; SD – standard deviation; V – variability coefficient. The content of all compounds – in dry matter (DM) of raw material.

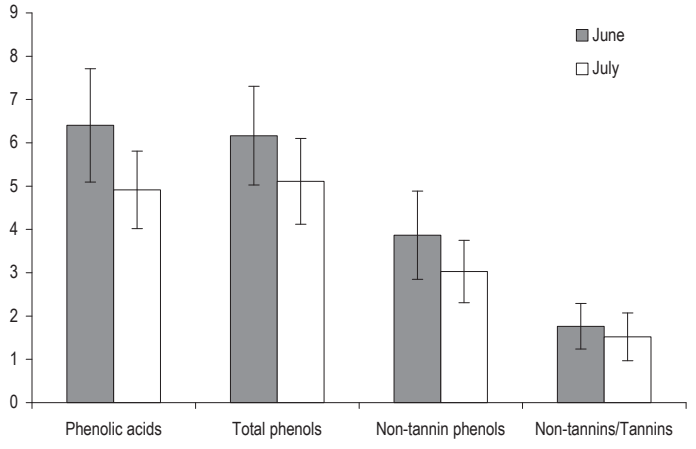

Figure 1.

Effect of the harvest time of the coltsfoot leaves (June–July of 2010) on the content of phenolics [% DM] and the proportion of non-tannin polyphenols to tannins (mean ±SD)

Wilcoxon's test: phenolic acids – p=0.00045; total phenols – p=0.00039; non-tannin phenols – p=0.00089; non-tannins/tannins – p=0.043805; n=40.

Table 2.

Effect of overshadow on the phytochemical and morphological variability of coltsfoot leaves (mean ±SD)

Variables	Light	Shadow	p-value
Swelling index	10.6±1.7	11.3±0.5	N.S.
Phenolic acids [%]	**6.67**±1.04	4.81±0.63	**
Total polyphenols [%]	**6.67**±1.12	4.71±0.73	**
Non-tannin polyphenols [%]	**4.07**±0.97	2.64±0.86	*
Tannins [%]	2.60±0.45	2.07±0.58	N.S.
Non-tannin polyphenols/tannins	1.60±0.47	1.41±0.69	N.S.
Flavonoids [%]	1.03±0.16	1.00±0.11	N.S.
Fresh weight of leaf [g]	1.90±0.29	**3.31**±0.72	***
Air-dry weight of leaf [g]	0.28±0.03	**0.46**±0.11	**
Water content in leaves [%]	90.8±0.7	**92.0**±1.1	*

Student's test: *** – p≤0.001; ** – p≤0.01 * – p≤0.05; N.S. – not significant; n=16.
The content of all compounds – in dry matter (DM) of raw material.

DISCUSSION

Traditional use of coltsfoot in phytotherapy is related to the content of mucilage, essential oils and polyphenolics, especially tannins and flavonoids in raw material. According to the literature, the swelling index for coltsfoot leaves amounts about 10–15. The content of mucilage (polysaccharides) is 6–10%, tannins – about 4.5–5% and flavonoids – about 0.8% [2, 6, 15, 28-30]. In general, these data confirm results of our investigations (tab. 1), although, it was found that the content of tannins can be lower (1.1–3.3%) and of flavonoids – a little higher (0.7–1.3%). It is interesting that the amount of flavonoids in leaves of *Tussilago farfara* is similar to the typical flavonoid raw materials, such as *Betulae folium* and *Tiliae inflorescentia* [31]. In addition, our investigations show that flavonoids in coltsfoot leaves fluctuate in only a small range (tab. 1), regardless of overshadow (tab. 2) and the harvest time of raw material (fig. 1). Observed stability of flavonoid content has indicated their chemotaxonomic value known for *Asteraceae* [32, 33] and other groups of plants [34, 35].

Coltsfoot is the common and widespread species (also in Poland) and it can be harvested for raw material from natural sites in great amounts [28, 36]. For this reason, finding of natural variability of major active compounds of *Tussilago farfara* is very important, especially in practice. Unfortunately, in the available literature there is a small information on the content of the basic components in coltsfoot leaves. A lot of studies concern flowers (capitulum) or flower buds of *Tussilago farfara* [11, 17-20, 37-40]. In coltsfoot leaves, phytochemical investigations are

mainly connected with pyrrolizidine alkaloids [5, 8, 21, 41, 42], and sometimes – with individual compounds of water soluble polysaccharides [16, 43, 44]. From our research, it appears that Polish natural populations of coltsfoot give a high quality raw material (*Farfarae folium*), rich in mucilage and phenolics. It seems that the amounts of polysaccharides, flavonoids and tannins are conditioned to a considerable degree by the genetic factors, regardless of environmental influence, for example overshadow (tab. 2).

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ZMIENNOŚĆ FITOCHEMICZNA PODBIAŁU (*TUSSILAGO FARFARA* L.) W POLSCE

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S t r e s z c z e n i e

Liście podbiału stanowią tradycyjny surowiec zielarski, bogaty w polisacharydy i związki fenolowe. W niniejszych badaniach określono poziom zróżnicowania zawartości wspomnianych związków czynnych. Jako materiał roślinny wykorzystano 22 naturalne populacje *Tussilago farfara* L., pochodzące z różnych regionów Polski. W latach 2008–2009 pobrano rośliny z każdej badanej populacji i założono kolekcję pochodzeniową w Ogrodzie Roślin Leczniczych w Plewiskach koło Poznania. Liście podbiału do analiz fitochemicznych zbierano w połowie czerwca i lipca 2010 r., a następnie suszono w temperaturze pokojowej. W pozyskanym surowcu zielarskim oznaczono wskaźnik pęcznienia (określający zawartość śluzów) oraz spektrofotometrycznie: poziom polifenoli ogółem, polifenoli nie wiążących się z proszkiem skórzanym i garbników (w przeliczeniu na pirogalol), a także sumę pochodnych kwasu hydroksycynamonowego (w przeliczeniu na kwas rozmarynowy) i flawonoidów (w przeliczeniu na kwercetynę). Prezentowane wyniki badań wskazują na relatywnie wysoką i wyrównaną zawartość głównych związków czynnych, szczególnie flawonoidów (0,7–1,3%) i polisacharydów (wskaźnik pęcznienia: 8,0–14,5). Stwierdzono, iż poziom wspomnianych grup związków zmienia się w małym zakresie (V=11–13%) i nie zależy od ocienienia roślin i terminu zbioru surowca.

Słowa kluczowe: *Tussilago farfara*, *rośliny lecznicze*, *śluzy, polifenole, garbniki, flawonoidy*