Content of pyrrolizidine alkaloids in the leaves of coltsfoot (*Tussilago farfara* L.) in Poland

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Abstract

Coltsfoot (*Tussilago farfara* L.) is a common species, widely used in European and Chinese traditional medicine for the treatment of respiratory diseases. However, raw material from this plant contains hepatotoxic pyrrolizidine alkaloids (PAs). The aim of the study was to determine the variability of the level of PAs (senkirkine and senecionine) in leaves of coltsfoot originated from natural populations in Poland. In the phytochemical analysis, 20 samples of *T. farfara* were used. This plant material was obtained from the Garden of Medicinal Plants in Plewiska near Poznań and originated from different regions of Poland. Coltsfoot leaves were harvested in the middle of July of 2010 and then dried at room temperature. The alkaloid content was detected using the HPLC-DAD method. The amount of PAs in leaves of *T. farfara* changed in a wide range from 0.06 to 1.04 μg g⁻¹ of dry matter (DM). The content of senkirkine and senecionine was positively correlated (*r* = 0.68, *P* = 0.001). There was no statistically significant correlation between the amount of PAs as well as leaf weight and water content in leaves of *T. farfara*. Our results showed that a medium-sized leaf of coltsfoot (0.33 g DM) may contain from 0.02 to 0.34 μg of PAs (on average 0.14 μg). The level of PAs was not associated with the region of Poland, but phytochemical similarity of samples was usually visible at the local scale. Coltsfoot leaves are characterized by a high variability of the content of toxic PAs, much higher than in the case of the main active compounds, especially flavonoids and mucilage. This phytochemical variability is mainly genetically determined (samples came from a garden collection), and it can be increased by environmental factors. Our investigations indicate that Polish natural populations of *T. farfara* may provide raw material with a low level of toxic PAs.

Keywords: *Tussilago farfara*, medicinal plants, toxicity, pyrrolizidine alkaloids, senkirkine, senecionine

Introduction

Coltsfoot (*Tussilago farfara* L., Asteraceae) is a perennial herbaceous plant with thin, branched rhizomes and yellow flowers (capitulum) developing in early spring before the leaves. This is an anemochoric pioneer species colonizing disturbed and ruderal areas such as roadides, clay slopes, fields and fallow lands, riverside gravels and others. Coltsfoot is native and widespread from Europe to western and northern Asia and North Africa as well as it was introduced to North America. In Poland it occurs commonly throughout the country, from the lowlands to the dwarf-pine belt [1–3].

*T. farfara* belongs to the oldest herbs in traditional medicine, mainly used as a cough suppressant as well as for treating obstructive lung diseases: asthma, bronchitis, and emphysema. In Poland and other European countries, coltsfoot leaves (*Farfarae folium*) are the basic raw material, but in folk Chinese medicine flower buds (known as Kuan-Dong-Hua) are applied [4–10]. Experimental studies indicate the antioxidant, antimicrobial and anti-inflammatory properties of coltsfoot extracts [11–15]. Mucilage (water-soluble polysaccharides) and phenolics (flavonoids, tannins, and phenolic acids) are the major bioactive compounds of these extracts [16–19].

Unfortunately, a wide use of coltsfoot in phytotherapy is restricted because of the presence of toxic alkaloids: senkirkine and senecionine. These 1,2-unsaturated PAs (pyrrolizidine alkaloids) may cause damage to the liver and even liver cancer in humans [4,20]. The published data on the content of PAs in coltsfoot leaves are relatively few and very diverse [21–23]. In this context, it seems interesting to determine the variability of the amount of pyrrolizidine alkaloids and the safety of raw material obtained from natural sites.

The aim of this study was to determine the variation in the content of hepatotoxic PAs (senkirkine and senecionine) in leaves of *T. farfara* collected from natural populations in various regions of Poland.
Material and methods

Plant material

Plant material originated from the collection in the Garden of Medicinal Plants, Plewiska near Poznań, Poland, founded in 2008–2009 [24]. In the study, 20 samples of *T. farfara* were used. These samples represented natural populations of coltsfoot from different regions of Poland (Fig. 1): Western Pomerania (localities 1–2), the Lubusz region (3), Greater Poland (4–12), Upper Silesia and Dąbrowa Basin (13–15), Lesser Poland (16–18), and the Cieszyn Silesia (19–20). The investigated populations of *T. farfara* occurred in different types of habitats, mainly anthropogenic ones such as roadsides and roadside ditches as well as fallow lands.

Fig. 1 Distribution of investigated populations of coltsfoot in Poland with the level of pyrrolizidine alkaloids – PAs. Groups of samples – according to the cluster analysis (Fig. 3). Location of coltsfoot populations and type of occupying habitats: 1 – Krąpiel near Stargard Szczeciński (roadside); 2 – Sicko near Choszczno (roadside); 3 – Deszczno near Gorzów Wielkopolski (roadside); 4 – Slomowo near Oborniki Wielkopolskie (roadside); 5 – Poznań-Umultowo I (fallow land); 6 – Poznań-Umultowo II (roadside ditch); 7 – Poznań-Świerczewo I (heap of sand); 8 – Poznań-Świerczewo II (abandoned garden); 9 – Plewiska near Poznań (abandoned garden); 10 – Szyszlowo near Konin (fallow land); 11 – Kamienica near Konin (fallow land); 12 – Jóźwin near Konin (fallow land); 13 – Siemianowice Śląskie (mining dump); 14 – Katowice (ruin of the house); 15 – Siewierz near Zawiercie (roadside ditch); 16 – Falniów near Miechów (roadside ditch); 17 – Małoszów near Miechów (roadside ditch); 18 – Modlnica near Oborniki (site after earthworks); 19 – Brenna near Cieszyn (fallow land); 20 – Puńców near Cieszyn (edge of stream).

Coltsfoot leaves (usually 60–100 leaves per sample) were harvested in the middle of July of 2010 and dried at room temperature (23–25°C, 50–55% Rh). For each sample, the average fresh, air-dry and dry leaf weight was determined. Water content (%) in the raw material was measured after drying it at 105°C in a HR73 halogen moisture analyzer (Mettler Toledo).

Alkaloid analysis

The alkaloid content (senkirkine and seneconine) was determined by high performance liquid chromatography with a photodiode array detector (HPLC-DAD, Agilent 1100). Air-dried and powdered coltsfoot leaves (5.0 g) were extracted with 50% (v/v) methanol acidified with citric acid to a pH of 2–3. Next, the samples were extracted with dichloromethane and diethyl ether. After bringing the aqueous layer to a pH of 9–10, it was extracted with dichloromethane. The dichloromethane layer was evaporated to dryness; the residue was dissolved in dichloromethane and purified on a Diol SPE column (500 mg, 3 ml). Alkaloids were eluted with methanol from the SPE column, and samples were evaporated to dryness and dissolved in methanol (the modified method of Lebada et al. [22]).

Quantitative analysis was performed on a Hypersil BDS C8 column – 250 × 4.6 mm, 5 μm (Thermo Electron) at a temperature of 40°C. To separate alkaloids, a gradient elution was used; phase A: 5 mM hexane-1-sulfonic acid sodium salt in 1% (v/v) aqueous phosphoric acid (pH = 3.2), and phase B: 100% acetonitrile. The following gradient program was applied: 0–7 min – 20% B; 7–25 min – increase to 40% B; 25–28 min – to 60% B; 28–31 min – to 80% B; 31–38 min – 80% B; 38–40 min – decrease to 20% B. The mobile phase flow-rate was 0.8 ml min⁻¹. Senkirkine and seneconine were detected at 220 nm (the modified method of Mróczek et al. [23]).

The method of alkaloid extraction was validated with ICH rules. In a first step, linearity of calibration curves was checked. Recurrence of time retention and peak area for all compounds was analyzed. The analytical method was characterized by a high recurrence and precision. The precision of data for the samples made for 6 repeats. The accuracy of this method was conducted for 3 different levels. Percentage of the recovery ranged in 83.54–94.75%.

The regression analysis for the alkaloids was performed at 5 concentration levels. The average and relative standard deviation (RSD) for those results were determined. The coefficient of determination (R²) of the calibration curve for senkirkine (Y = 76885.71x – 55.91) was 0.9997, and for seneconine (Y = 45134.0x – 179.61) it was R² = 0.9985.

Chemicals and reagents

Dichloromethane, diethyl ether, and phosphoric acid were purchased from POCh. The Diol SPE column and nanopure water were from J. T. Baker. Hexane-1-sulfonic acid sodium salt, acetonitrile and methanol were from Merck. Senkirkine and seneconine were obtained from ChromaDex. The calibration standards were freshly prepared before each analysis. Standards were dissolved in methanol in the range of 0.001–0.1 mg ml⁻¹.

Statistical analysis

Statistica 7.1 software [25] was used for calculations. The Shapiro-Wilk test was employed to assess the normality of variable distribution. For the skewed distribution of variables, square root transformation of data was performed and then Pearson's coefficient of correlation was used. The Kruskal–Wallis test was applied to determine the statistical significance of intergroup differences. The phytochemical similarity of coltsfoot samples was determined based on UPGMA cluster analysis with Euclidean distance. The content of senkirkine and seneconine was standardized.

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Results

The content of toxic PAs in leaves of T. farfara plants originating from different populations in Poland changed in a wide range from 0.06 to 1.04 µg g⁻¹ dry matter (DM) of raw material. This phytochemical variability was much greater than the variation in the average leaf weight of coltsfoot samples. The water content in the leaves of this species was characterized by high stability (Tab. 1). There was no statistically significant correlation between the content of PAs as well as leaf weight and water content in coltsfoot leaves, but the amounts of senecionine and senkirkine were relatively strongly correlated (Fig. 2). The cluster analysis showed two main groups of samples with a different content of alkaloids. In group I (a very low level of PAs), the average amount of senecionine was nearly five times lower, while that of senkirkine more than two times lower than in group II (a low level of PAs). There was also one sample (group III) with a high level of PAs (Fig. 3). The geographical distribution of the studied populations of T. farfara indicates that the content of pyrrolizidine alkaloids is probably not associated with the region of Poland, but phytochemical similarity of samples is usually visible at the local scale (Fig. 1).

Discussion

According to the literature, the content of toxic PAs in coltsfoot leaves is very changeable and ranges from 0.1 to 368 µg g⁻¹ [21–23,26,27]. It seems that such a large variation of the amount of PAs can arise from the different origin of plant material (commercial samples or samples from natural sites) and its preparation (raw material drying method) as well as from different extraction methods (with water or methanol, citric acid, ammonia, etc.) and analytical methods (capillary zone electrophoresis, gas or liquid chromatography). However, phytochemical analysis of plant material from various provenances in Europe shows the high variability in the content of PAs in coltsfoot [22,27]. Our study of samples from natural populations in Poland confirms these results (Tab. 1). It is interesting that our previous research on this plant material indicated a relatively low variation in the content of the main active compounds in coltsfoot, especially flavonoids and mucilage [24].

Coltsfoot is a common and widespread medicinal plant, which is still collected from natural sites in Poland. This species is not only important in traditional phytotherapy [5,28], but it also belongs to edible plants formerly consumed as green vegetables [29–31]. Leaves of T. farfara are traditionally used for wrapping sarma rolls in western Ukraine and Romania [32,33]. The use of coltsfoot in phytotherapy requires the safety assessment of its raw material for humans, but it is difficult. Therefore, only preparations free of PAs have been legalized in Austria since 1994. In Germany, the daily intake of these compounds is limited to 1 µg. In the case of herbal tea, the content of PAs in coltsfoot is restricted to 10 µg per day [22]. According to the Australia New Zealand Food Authority [34], the provisional tolerable daily intake (PTDI) of toxic PAs is 1 µg kg⁻¹ body weight (b.w.) per day. The lowest known dose of PAs that causes long-term toxicity in humans (acute hepatic veno-occlusive disease) is 15 µg kg⁻¹ b.w. per day [35]. The reported results show that a medium-sized leaf of coltsfoot (0.33 g DM) may contain from 0.02 to 0.34 µg (on average 0.14 µg) of toxic PAs.
The amount of pyrrolizidine alkaloids detected in Polish populations of *T. farfara* is similar to the HPLC-data of Mroczek et al. [23] and it is a low level compared to other published results (e.g. Lebada et al. [22]). However, a PA-free clone of coltsfoot, registered as the variety *T. farfara* 'Wieni', is known in Austria and Germany [27].

In the context of the use of coltsfoot leaves as food, the problem of the thermal stability of PAs is interesting. The information on this subject is insufficient, but it is known that 2 h extraction at about 100°C does not cause decomposition of senecionine and senkirkine. At higher temperatures (at gas chromatography method conditions), PAs are unstable [23]. For example, senkirkine undergoes degradation at temperature above the melting point, i.e. 198°C [36].

Based on the available literature and our phytochemical data, it can be concluded that coltsfoot is characterized by a high variability of the content of toxic PAs, much higher than in the case of the main active compounds. It can be assumed that this phytochemical differentiation is mainly genetically determined (samples came from a garden collection in the similar growing conditions), and it can be increased by environmental factors. Nonetheless, it appears from our research that Polish natural conditions), and it can be increased by environmental factors.

**References**

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