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ACCUMULATION OF PHENOLIC COMPOUNDS IN UNDERGROUND ORGANS OF DROPWORT (*Filipendula vulgaris* Moench)

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Abstract. Herb and underground organs of dropwort have been used as medicinal raw materials. Decrease of natural resources of this species results in the necessity to introduce it into cultivation. In this study accumulation of biomass and phenolic compounds in underground organs of cultivated dropwort during two years of plant vegetation was evaluated. Underground organs were harvested at the end of the first year of plant vegetation and in the second year: at the beginning of vegetation, at the stage of blooming, and at the end of vegetation. Phenolics were determined by HPLC. At the end of the second year the weight of air-dry rhizomes with tuberous roots reached 188.3 g per plant and it was almost five times higher than in the first year. Underground organs of dropwort appeared to be a rich source of flavan-3-ols and gallic acid. There was no clear relation between the stage of plant development and accumulation of phenolics in these organs.

Key words: plant development, term of harvest, biologically active compounds, flavan-3--ols, phenolic acids, flavonol glycosides

INTRODUCTION

Dropwort (*Filipendula vulgaris* Moench, syn. *Filipendula hexapetala* Gilib., family *Rosaceae*) is a perennial growing wild in Europe, Asia, and north-western Africa, mainly on dry nonacidic grasslands and sunny slopes [Weidema et al. 2000]. In Poland, before the Second World War this species had been used in official and folk medicine.

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After the war, because of the problems with obtaining sufficient amount of raw material, it was replaced by meadowsweet (*Filipendula ulmaria* /L./ Maxim.). The plant produces the rosette of fern-like, odd-pinnate leaves, and flowering shoots up to 80 cm high with pinkish-white flowers arranged in paniculate inflorescences. In central Europe the blooming period lasts from May to August. The underground organs consist of rhizomes with tuberous roots. Both herb and underground organs of dropwort are known in folk medicine as anti-inflammatory, antirheumatic, diuretic, and diaphoretic agents. The decoction of underground organs has often been used as an astringent, in diarrhoea and haemorrhoids [Radulović et al. 2007, Tiță et al. 2009, Šarić-Kundalić et al. 2010]. In the recent years some new aspects of biological activity of dropwort have become the object of scientific interest, e.g. antioxidant activity [Maksimović et al. 2007, Imbrea et al. 2010, Smirnova et al. 2010], anxiolytic action, and protection of the brain against hypoxic injury [Vengerovsky et al. 2011].

So far, the raw materials of dropwort have been harvested from wild growing plants, which results in the diverse quality of raw materials originating from different natural sites [Lempiäinen 1982]. Dropwort is grown as an ornamental plant, because of the attractive leaves and inflorescences (especially in case of the full-blossomed cultivar 'Plena'). However, the studies on cultivation of this species for medicinal purposes and on the effect of agrotechnical factors on the quality of raw materials harvested are scarce [Lempiäinen 1978, Bączek et al. 2010].

The aim of this study was to determine the effect of time of harvest on the yield and quality of underground organs of dropwort, with special regard to the content of phenolic compounds that play an important role in the biological activity of this raw material.

MATERIAL AND METHODS

The field experiment was carried out in two two-year cycles (2007–2008 and 2008–2009) at the Experimental Station of the University of Agriculture in Krakow, on brown alluvial soil of $pH_{(KCl)}$ 6.11. The phytochemical analyses were done at the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW.

The object of the study were one- and two-year-old plants of dropwort (*Filipendula vulgaris* Moench). The seeds collected from the natural site in Podlasie area (Drohiczyn, N 52º 23.825' E 022º 40.338') were sown in the greenhouse in March. The seedlings were planted out in the field in May. Plant density was 70 × 25 cm. The experiment was established in the randomized block design in 3 replications. The single plot area was 5.6 m^2. The forecrop was late cabbage grown at the full manure rate. Phosphorus and potassium fertilization was applied (regarding the results of soil analysis in autumn) to obtain phosphorus content 70 mg · dm^{-3}, and potassium content 200 mg · dm^{-3} of soil. Triple superphosphate and potassium chloride were applied in two doses: in autumn and in spring, before planting out seedlings. In the first year of plant vegetation nitrogen fertilizer (magnesium nitrochalk) in the rate of 60 kg N · ha was also used in two doses: before planting and at the stage of intensive vegetative growth. In the second year, at the

beginning of plant vegetation 60–80 kg N · ha was applied, according to the results of soil analysis.

The factor of the experiment was the term of harvest. Underground organs of plants were harvested at the end of the first year of vegetation (October) and in the second year: at the beginning of vegetation (April), at the stage of blooming (July), and at the end of vegetation (October). They were dried at 40ºC. The weight of underground organs per plant was determined and the raw materials obtained were subjected to chemical evaluation.

For the determination of phenolic compounds 1.0 g of air-dry, grounded raw material was extracted exhaustively with 100 ml of methanol in Büchi Extraction System B-811. After evaporation of solvent, the residue was dissolved in 10 ml of methanol, filtered (through a Supelco Iso-Disc™ Syringe Tip Filter Unit, PTFE membrane, diameter 25 mm, pore size 0.45 μm), and subjected to HPLC. The analysis was carried out using the Shimadzu chromatograph equipped with autosampler SIL-20, photodiode array detector SPD-M10A VP DAD, and Class VP 7.3 chromatography software. A Phenomenex Luna® C18(2), 250 × 4.6 mm, 5 μm column was used. The gradient of solvents A (10% acetonitrile in deionised water adjusted to pH 3 with phosphoric acid) and B (55% acetonitrile in deionised water adjusted to pH 3 with phosphoric acid) was applied as follows: 0 min – 15% B, 30 min – 75% B, 30.01 min – 95% B, 35 min – 95% B, 35.01 min – 15% B. The flow rate was 1.0 ml · min^{-1}, injection volume 10 μl, and column temperature 31ºC. Time of analysis was 40 min. UV spectra were recorded in the range of 190–450 nm. Peaks were identified by comparison of retention time and spectral data with adequate parameters of standards (purchased from ChromaDex). For the quantitative analysis of each compound the five-point calibration curve was generated by chromatographing different volumes (0.5–10 μl) of a methanolic standard solution (prepared according to the instructions of Chromadex Tech Tip 0003: Reference Standard Recovery and Dilution) in triplicate. The detection wave was: 206 nm (/+/-catechin, /-/-epicatechin, /-/-epigallocatechin, /-/-epigallocatechin gallate), 254 nm (ellagic acid, hyperoside, rutoside), 264 nm (astragalin), 280 nm (gallic acid), 300 nm (salicylic acid), and 370 nm (quercetin and spiraeoside). Quantification was based on the peak area. The content of the determined compounds was calculated in mg · 100 g dry matter.

The results of field studies and chemical analyses were subjected to the one-way analysis of variance and Tukey's test at $\alpha = 0.05$.

The presented results are mean values from two cycles of the experiment.

RESULTS AND DISSCUSSION

As it was mentioned before, underground organs of dropwort have been collected for medicinal purposes from the wild growing plants, as yet. There is little information on cultivation trials of this species. On web pages of some horticultural companies it is possible to find elementary data on cultivation of ornamental cultivars of dropwort. In Finland some field experiments concerning the development of underground organs of dropwort and their chemical composition have been carried out [Lempiäinen 1978,

Lempiäinen and Henriksnäs 1979, Lempiäinen 1982]. However, they were focused on nutritional value of dropwort tubers. It was found that application of lime, N and a multinutrient fertilizer increased the number of tubers per plant, but reduced tuber size. The digestible protein and starch content of the tubers were also significantly affected by the fertilization applied [Lempiäinen 1978].

There are no reports on accumulation of secondary metabolites, responsible for medicinal value of dropwort, in cultivated plants. Such studies have been undertaken a few years ago at the Department of Vegetable and Medicinal Plants, Warsaw University of Life Sciences – SGGW. Only some results have been published as yet – those concerning the effect of flowering shoots removal in the second year of plant vegetation on the yield of leaves and underground organs and accumulation of phenolic compounds in these organs [Bączek et al. 2010].

In the present study the accumulation of biomass and phenolic compounds in underground organs of cultivated dropwort during two years of plant vegetation was evaluated. After the first year of vegetation the weight of air-dry underground organs was relatively low (38.40 g · plant^{-1}). Similar yield of dropwort roots was obtained by Lempiäinen [1978] in the experiment carried out in Finland. In the second year a considerable increase in biomass of underground organs was observed, especially after plant blooming. At the end of vegetation in the second year the weight of air-dry rhizomes with tuberous roots (188.30 g · plant^{-1}) was almost five times higher than in the first year (tab. 1).

Table 1. Air-dry weight of underground organs (g · plant^{-1})
Tabela 1. Powietrznie sucha masa organów podziemnych (g · roślina^{-1})

Age of plants Wiek roślin	Stage of development Faza rozwojowa	Weight of underground organs (g · plant^{-1}) Sucha masa organów podziemnych (g · roślina^{-1})
1-year-old 1-roczne	end of vegetation koniec wegetacji	38.40 c
2-year-old 2-letnie	beginning of vegetation początek wegetacji	47.90 c
	blooming kwitnienie	74.80 b
	end of vegetation koniec wegetacji	188.30 a

Values marked with the same letter do not differ significantly at $\alpha = 0.05$
Wartości oznaczone tymi samymi literami nie różnią się istotnie przy $\alpha = 0,05$

In the raw material twelve phenolic compounds were identified (tab. 2–4), including three phenolic acids (ellagic, gallic and salicylic acid), four flavan-3-ols (/+/-catechin, /-/-epicatechin, /-/-epigallocatechin, and epigallocatechin gallate), and five other flavonoids: quercetin (belonging to flavonols), its three glycosides – hyperoside (quercetin 3-O-galactoside), rutoside (quercetin 3-O-rutinoside), and spiraeoside (quercetin 4'-O-glucoside), as well as astragalin (kaempferol 3-O-glucoside).

Table 2. Content of identified flavan-3-ols in underground organs (mg · 100 g^{-1})
Tabela 2. Zawartość zidentyfikowanych flawan-3-oli w organach podziemnych (mg · 100 g^{-1})

Age of plants Wiek roślin	Stage of development Faza rozwojowa	(+)-C	(-)-EC	(-)-EGC	EGCG
1-year-old 1-roczne	end of vegetation koniec wegetacji	477.30 a	198.78 ab	552.48 c	83.42 b
2-year-old 2-letnie	beginning of vegetation początek wegetacji	489.88 a	192.36 b	623.20 b	99.50 a
	blooming kwitnienie	452.22 b	207.12 a	640.85 a	108.04 a
	end of vegetation koniec wegetacji	403.78 c	164.54 c	506.49 d	75.58 c

C – catechin, katechina; EC – epicatechin, epikatechina; EGC – epigallocatechin, epigalokatechina; ECG – epigallocatechin gallate, galusan epigalokatechiny
Values in columns marked with the same letter do not differ significantly at α = 0.05 – Wartości oznaczone tymi samymi literami nie różnią się istotnie przy α = 0,05

Irrespective of the stage of plant development the dominating phenolics were: (-)-epigallocatechin and (+)-catechin. However, their content in underground organs changed during plant development. The content of (-)-epigallocatechin increased till the blooming period in the second year of plant vegetation, and then significantly decreased. The content of (+)-catechin was highest at the beginning of the second year of vegetation, and then systematically decreased. The content of two other identified flavan-3-ols (/-/-epicatechin and epigallocatechin gallate) was also significantly lowest at the end of the second year of plant vegetation (tab. 2). (+)-Catechin, (-)-epicatechin and their 4-benzyl thioethers have been previously identified as components of oligomeric proanthocyanidins present in dropwort roots. (+)-Catechin was the main constitutive unit of these proanthocyanidins [Oszmianski et al. 2007].

Table 3. Content of identified phenolic acids in underground organs (mg · 100 g^{-1})
Tabela 3. Zawartość zidentyfikowanych kwasów fenolowych w organach podziemnych (mg · 100 g^{-1})

Age of plants Wiek roślin	Stage of development Faza rozwojowa	Ellagic acid Kwas elagowy	Gallic acid Kwas galusowy	Salicylic acid Kwas salicylowy
1-year-old 1-roczne	end of vegetation koniec wegetacji	11.26 a	215.82 c	0.86 b
2-year-old 2-letnie	beginning of vegetation początek wegetacji	9.48 b	236.21 b	0.85 b
	blooming kwitnienie	6.05 d	299.20 a	0.92 a
	end of vegetation koniec wegetacji	7.91 c	177.60 d	0.92 a

Values in columns marked with the same letter do not differ significantly at α = 0.05
Wartości oznaczone tymi samymi literami nie różnią się istotnie przy α = 0,05

Among the identified phenolic acids gallic acid was the dominating one (tab. 3). The highest content of this compound was characteristic for the underground organs collected at the stage of plant blooming. Then, till the end of vegetation, significant decrease in its content was observed. On the contrary, the content of ellagic acid in underground parts was the highest at the end of the first year of plant vegetation, and the lowest at the stage of blooming in the second year. Gallic acid has been previously reported as a constituent of herb and underground organs of dropwort, together with seventeen other phenolic acids (e.g. p-coumaric, syringic, vanillic, ferulic, and 3,4-dimetoxycinnamic) present either in a free form or as components of more complex structures [Sokołowska-Woźniak 1998, cit. after Smolarz and Sokołowska-Woźniak 2001]. Oszmianski et al. [2007] found ellagic acid, and not gallic acid, as a product of degradation of hydrolysable tannins occurring in dropwort root. Salicylic acid has not been listed as a constituent of dropwort roots, although it belongs to the main constituents of the essential oil from the aerial parts of this species [Pavlovic et al. 2007]. In the present study this compound was found in underground organs of dropwort in very low quantities showing only slight changes during the investigated period of plant development (tab. 3).

Table 4. Content of identified flavonols and glycosides of flavonols in underground organs (mg · 100 g^{-1})

Tabela 4. Zawartość zidentyfikowanych flawonoli i glikozydów flawonoli w organach podziemnych (mg · 100 g^{-1})

Age of plants Wiek roślin	Stage of development Faza rozwojowa	Quercetin Kwercetyna	Astragalin Astragalina	Hyperoside Hiperozyd	Rutoside Rutozyd	Spiraeoside Spireozyd
1-year-old 1-roczne	end of vegetation koniec wegetacji	1.24 a	19.58 a	10.91 c	26.45 c	9.95 a
	beginning of vegetation początek wegetacji	1.15 ab	19.09 a	11.93 b	59.23 a	9.10 a
2-year-old 2-letnie	blooming kwitnienie	0.58 c	19.70 a	13.57 a	45.85 b	8.06 b
	end of vegetation koniec wegetacji	1.07 b	17.10 b	10.24 c	28.96 c	9.27 a

Values in columns marked with the same letter do not differ significantly at α = 0.05
Wartości oznaczone tymi samymi literami nie różnią się istotnie przy α = 0,05

Among several biological activities of dropwort, antioxidant properties have gained a considerable scientific interest [Smirnova et al. 2010]. Antioxidant capacity of dropwort herb extracts was found to be correlated with flavonoid content [Imbrea et al. 2010]. Antioxidant activity of particular flavonoids, e.g. spiraeoside, was also reported [Maksimović et al. 2007]. There is only one report concerning the presence of flavonoids in underground organs of dropwort. According to this report, rhizomes of dropwort contain quercetin, hyperoside, rutoside, spiraeoside, avicularin, isoquercitrin, and quercitrin [Smolarz et al. 1999]. The presence of the first four above mentioned compounds

in the underground organs of dropwort has been confirmed in the present study. Astragalin has been found in this raw material for the first time. The content of all identified flavonol glycosides and free quercetin was low. The accumulation of particular compounds proceeded differently. Rutoside content was the highest at the beginning of the second year of plant vegetation, and the lowest at the end. Hyperoside content was the highest at the stage of plant blooming, whereas quercetin content was lowest at this time. Astragalin and spiraeoside content were relatively stable during the investigated period of plant development, with slight decrease at the end of the second year of plant vegetation and at the stage of blooming, respectively. The presence of quercetin, rutin, and spiraeoside has been previously reported in the above-ground parts of dropwort, together with other flavonoids (kaempferol, apigenin, luteolin, dihydroquercetin, isoquercitrin, and avicularin) [Vengerovsky et al. 2011].

In summary, the analysis of changes in the content of identified phenolic compounds in underground organs during two-year cultivation of dropwort indicated that there was no clear relation between the stage of plant development and accumulation of these compounds. Nevertheless, in the case of those occurring in the greatest amounts (flavan--3-ols and gallic acid), the content determined at the end of plant vegetation in the second year of cultivation was lower than at the three earlier stages of plant development, i.e. blooming, beginning of vegetation, and end of vegetation after the first cultivation year. The decrease in the content of those compounds between plant blooming and end of vegetation might result from the intense increase in mass of underground organs observed in the same period of plant growth.

CONCLUSIONS

1. It is possible to obtain high yield of underground organs of dropwort in the second year of plant cultivation.
2. The most intense increase in mass of underground organs was observed between the stage of plant blooming and end of vegetation in the second year.
3. Underground organs of dropwort are a rich source of phenolic compounds, especially flavan-3-ols and gallic acid.
4. There was no clear relation between the stage of plant development and accumulation of identified phenolic compounds in underground organs of dropwort.

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GROMADZENIE SIĘ ZWIĄZKÓW FENOLOWYCH W ORGANACH PODZIEMNYCH WIĄZÓWKI BULWKOWEJ (*Filipendula vulgaris* Moench)

Streszczenie. Ziele i organy podziemne wiązówki bulwkowej stosowane są jako surowce lecznicze. Zmniejszenie zasobów naturalnych tego gatunku skłania do wprowadzenia go do uprawy. W pracy badano – w warunkach uprawy – przyrost masy organów podziemnych i gromadzenie się w nich związków fenolowych w ciągu dwóch lat wegetacji roślin. Organy podziemne zbierano pod koniec pierwszego roku oraz w drugim roku: na początku wegetacji, w fazie kwitnienia i pod koniec wegetacji. Związki fenolowe oznaczano metodą HPLC. Pod koniec drugiego roku wegetacji masa organów podziemnych wynosiła 188,3 g na roślinę i była prawie pięciokrotnie wyższa niż w pierwszym roku. Organy podziemne wiązówki bulwkowej okazały się bogatym źródłem flawan-3-oli i kwasu galu-

sowego. Nie stwierdzono wyraźnej zależności pomiędzy fazą rozwoju roślin a zawartością związków fenolowych w organach podziemnych.

Słowa kluczowe: rozwój roślin, termin zbioru, związki biologicznie czynne, flawan-3--ole, fenolokwasy, glikozydy flawonoli

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