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EFFECT OF THE AGE OF PLANTATION AND HARVEST TERM ON CHEMICAL COMPOSITION AND ANTIOXIDANT AVCTIVITY OF STINGING NETTLE (*Urtica dioica* L.)

WPŁYW WIEKU PLANATCJI I TERMINU ZBIORU NA SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNĄ POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.)

Abstract: In 2004–2006 field experiment aimed at the assessment of the effect of plantation age and harvest term on chemical composition and antioxidant activity of stinging nettle.

The research showed that the highest content of pigments (chlorophyll *a*+*b* and carotenoids) and calcium were characterized plants grown on 1-year-old plantation, whereas the highest amount of magnesium and potassium contained the oldest plants. The most considerable antioxidant activity featured stinging nettle harvested in May, while the following harvests brought about the decrease in this property. Antioxidant activity depends on polyphenols content.

Keywords: stinging nettle, antioxidant activity, chemical composition

Stinging nettle constitutes a valuable source of numerous biologically active substances: vitamins (A, B, C, K), macro- and microelements (P, Mg, Ca, K, Fe, Se), tannins, polyphenols, silicic acid and volatile oil [1, 2]. The mentioned properties provide different ways of its utilization – as medicinal or fibrous plant, leafy vegetable used in soups and omelets, as well as fodder for animals [3, 4].

Medicinal properties of stinging nettle have been known and applied for a long time, among others, in prophylaxis and treatment of lung diseases, rheumatism or cirrhosis of liver [5]. It is also used as mild duretic, antimycoric and bactericide agent. Main application of stinging nettle, however, is connected with obtaining chlorophyll, a raw

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material in pharmaceutical and food industry, as well as cosmetics production [6]. A number of substances stinging nettle contains characterize antiradical and antioxidant activity. The latter one prevents uncontrolled oxidation reaction, inhibit oxidation processes which take place in the cells and normalize redox potential, thus protecting human organism from such civilization diseases as artheroma, hypertension or neoplasms [7, 8]. The presence of chemical components in plants is not permanent and it depends on such factors as environmental conditions, phase of plant growth, plant form and organ, harvest term, age of plantation, as well as conditions of raw material storage [9].

The purpose of the investigation conducted in the years 2004–2006 was the assessment of the effect of plantation age and harvest term on yielding, chemical composition and antioxidant activity of stinging nettle.

Material and methods

Field experiment was carried out in Horticultural Research Station in Piastow, on sandy clay soil of pH = 7.8, containing 1.8 % humus, 138 mg P, 96 mg Mg, 220 mg K and 1538 mg Ca in 1 dm^3. Stinging nettle seedlings were produced in multicells filled with the mixture of peat substrate and loamy soil. In the last week of March 3–4 seeds were sown into each cell of 76.5 cm^3 volume. After germination seedlings were thinned, leaving one best developed plant in each pot. 7-week-old ready seedlings were planted on the field in the second decade of May, in spacing 50 × 25 cm. In the subsequent years experimental plots were fertilized with nitrogen in the amount of 200 $kg\ N \cdot ha^{-1}$, half of which was applied before planting, while in further years of cultivation fertilization took place before the beginning of plant growing period and the remaining part – after the first harvest of herbaceous plant. The experiment was established according to randomized pattern of split-plot method in three replications and the area of one plot for harvesting equaled 1 m^2. Herb harvest took place at the beginning of blossom stage, twice in the first year (in the middle of July and September) and three times in the subsequent years (in the half of May, July and September) using electric knife-mower for hedges. Samples of leaves were collected from every harvest. There was estimated level of dry matter, chlorophyll, caroteniods, polyphenols, nitrates, total N, macroelements and proteins in nettle leaves as well as antioxidant activity of raw products. Fresh leaves were blended with BOSCH blender and extracted with 100 cm^3 of methanol (80 %). The content of total phenolic compounds was assayed using Folin-Ciocalteu method [10], chlorophyll *a+b* and total carotenoids were estimated using spectrophotometer, due to the method by Ruminska et al [11].

Dry matter was estimated by drying to constant mass at 105 ^{o}C. There was also assayed nitrates content (using potentiometry method), total N by Kjeldahl method and protein content was calculated with the use of 6.25 coefficient, as well as macroelements P, K, Mg and Ca following standard method [12]. W 2005 and 2006 antioxidant activity was assessed by DPPH [13], ABTS [14] and FRAP [15] tests in leaves samples collected from all treatments.

Results and discussion

The content of dry matter in stinging nettle leaves was to a low degree dependent on plantation age and it ranged average from 27.93 to 28.15 % (Table 1).

Table 1

The effect of plant age and harvest term on chemical composition of stinging nettle

Age of plant and harvest term		Dry matter [%]	Chlorophyll *a*+*b*	Carotenoids	Polyphenols
			[mg · g^{-1} d.m.]		
1-year-old	July	30.20	9.57	1.37	13.20
	September	26.09	12.86	1.61	8.13
Mean		28.15	11.22	1.49	10.67
2-year-old	May	28.79	8.39	1.32	20.76
	July	29.30	8.63	1.07	15.92
	September	25.71	8.57	1.00	8.96
Mean		27.93	8.23	1.13	15.21
3-year-old	May	32.40	7.38	1.12	19.06
	July	26.69	10.80	1.61	15.19
	September	25.03	12.90	1.47	9.11
Mean		28.13	10.36	1.40	14.45
Mean	May	30.59	7.88	1.22	19.91
	July	28.82	9.67	1.35	14.77
	September	25.61	11.44	1.36	8.73
Mean		28.34	9.66	1.31	14.47
LSD α = 0.05 for:					
age of plant		ns	0.89	0.36	1.56
term of harvest		ns	1.12	0.12	1.15

Higher diversity of this parameter was observed in I particular cuts. The leaves of stinging nettle harvested in September contained the lowest amount of dry matter, while the highest quantity of dry matter was obtained from May harvest. Chlorophyll *a*+*b* and carotenoids content was related to both plantation age and herb harvest term. Higher content of pigments was recorded in younger plants (one-year-old). In the second and third year of cultivation their level was relatively even. Higher content of carotenoids was reported in the first and third year of cultivation, while in two-year-old plants their content was considerably lower. Concentration of chlorophyll *a*+*b* increased in leaves in subsequent cuts, similar results was observed in carotenoids content of 1- and 3-year-old plantation whereas in 2-year-old nettle highest level of carotenoids was noticed in first cut. Weglarz and Karaczun [16] did not observe any correlation between herb harvest term and the content of carotenoids and chlorophyll in stinging nettle leaves. In the research presented by these authors, however the content of these pigments did decrease according to the age of plants (older plants from 3-year-old and 4-year-old plantations).

Polyphenols content in stinging nettle leaves from 1-, 2- and 3-year-old plantation was similar and it ranged from 14.45 to 15.19 mg · g^{-1} d.m. Regardless the age of plantation, polyphenols content decreased in the subsequent cuts and plants harvested in

May contained average 20.84 mg · g^{-1} d.m. of polyhenols, while those of September harvest – 8.73 mg · g^{-1} d.m. Węglarz and Karaczun [16] examined correlation between plantation age and harvest term regarding the content of flavonoids. They reported no effect of plantation age on flavonoids content, as well as diminishing content of flavonoids in raw material coming from the subsequent cuts.

The level of total nitrogen and total protein in stinging nettle leaves gradually increased in the subsequent years, according to plant age (Table 2). On average, their highest value was recorded in 3-year-old plants and it ranged from 3.86 to 4.11 % d.m. for total N and from 24.12 to 25.68 % for total protein. Stinging nettle is a plant of slight tendency to nitrates accumulation since their average content in leaves, regardless plantation age and harvest term, did not exceed average 400 mg.

Table 2

The effect of plant age and harvest term on total nitrogen, protein and nitrates content of stinging nettle

Plant age at harvest	Nitrogen – total			Protein			Nitrates		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
	[% d.m.]						[mg · kg^{-1} f.m.]		
1-year-old		3.10	3.48		19.37	21.75		158	288
2-year-old	2.77	2.88	3.43	17.31	18.00	21.43	133	121	125
3-year-old	3.86	3.92	4.11	24.12	24.50	25.68	175	375	123
Mean	3.32	3.30	3.67	20.72	21.25	23.56	154	218	178.66
LSD α = 0.05 for:									
age of plant		0.22			1.16			12	
term of cut		0.11			1.07			22	

Our own investigation proved that stinging nettle is a valuable source of macroelements. In the conditions of cultivation on the soil rich in P, K, Ca and Mg their content in dry matter of raw material was as 0.4 % P, 1.77 % K, 3.48 % Ca and 0.34 % Mg.

The content of phosphorus in 1-, 2- and 3-year-old stinging nettle leaves was similar, while the amount of magnesium and potassium increased and Ca content decreased, as plants were getting older. There was not observed any apparently directed effect of herb harvest term on P and K content in stinging nettle leaves and Mg amount in the leaves originating from the subsequent cuts increased, while the one referring to Ca decreased. Weglarz and Karaczun [16] also recorded the decrease in Ca content in the leaves coming from older, 3- and 4-year-old plantation. Our own investigation proved Ca value in the leaves of stinging nettle, cultivated on the soil of pH = 7.8, was relatively high and amounted 4.96–5.79 % in 1-year-old plants, 3.51–3.88 % in 2-year-old and 1.34–2.33 % in 3-year-old plants (Table 3). In the experiment conducted by Weglarz and Karaczun [16] this value gradually decreased according to plantation age from 2.06 % Ca in 1-year-old plants to 1.87 % Ca in 4-year-olds, while in the work by Szewczuk and Mazur [17], who cultivated stinging nettle on acid soil of pH = 5.6, it ranged average merely 0.5.

Table 3

The effect plant age and harvest term on macroelements content in leaves of stinging nettle

Age of plant	P			K			Ca			Mg		
	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
	[% d.m.]											
1-year-old		0.44	0.41		1.39	1.58		5.79	4.96		0.28	0.31
2-year-old	0.43	0.34	0.31	1.89	1.85	1.00	3.88	3.75	3.51	0.22	0.28	0.29
3-year-old	0.37	0.33	0.44	2.02	2.33	2.45	2.33	2.16	1.34	0.39	0.36	0.59
Mean	0.40	0.37	0.39	1.96	1.86	1.68	3.11	3.90	3.27	0.31	0.31	0.40

Antioxidant activity measured by FRAP and DPPH test reached the highest values in stinging nettle harvested in the earliest period, in May and it decreased with the subsequent cuts. In 1-year-old plants this parameter achieved the highest value at the first cut performed in the half of June. Yet no effect was recorded, as far as the age of plantation was taken into account, on antioxidant activity of raw material analyzed. Jamroz et al [7], determining antioxidant properties of hop cultivars, proved strict positive correlation between polyphenols content and antioxidative activity. Similarly, in authors own investigation it was possible to confirm high antioxidant activity of stinging nettle at considerably elevated level of phenolic compounds. These results were also in agreement with the data obtained by Katsube et al [18] in research involving berry fruits. Antioxidant activity, measured according to FRAP – quick and easy to use test, ranged from 45.9 to 130 $\mu M \cdot 100\ g^{-1}$ (Table 4).

Table 4

Effect of plant age and harvesting term on antioxidant activity of stinging nettle

Plant age and harvest term		DPPH	FRAP	ABTS
		[μM Trolox $\cdot g^{-1}$ d.m.]		
1-year-old	July	0.6	84.6	17.3
	September	0.4	45.9	34.0
Mean		0.5	65.3	25.6
2-year-old	May	1.2	129.6	23.7
	July	0.8	102.1	20.8
	September	0.5	50.0	37.9
Mean		0.8	93.9	27.5
3-year-old	May	1.2	126.5	22.5
	July	0.6	93.9	20.6
	September	0.4	51.9	38.6
Mean		0.7	90.8	27.2
Mean	May	1.2	128.0	23.1
	July	0.7	93.5	19.6
	September	0.4	49.3	36.8
Mean		0.8	90.3	26.5

Assuming classification introduced by Wojdylo et al [18] for the assessment of 32 herbaceous plants, the values obtained place this species in the group of plants featuring good and high antioxidant activity. The research by Gulcin et al [5], conducted *in vitro*, also proved that water extract of stinging nettle is a significant source of antioxidants and that this raw material can be utilized by pharmaceutical industry as diet supplement. According to the authors quoted above main source of antioxidants are polyphenols [5].

Conclusions

1. Chlorophyll content in stinging nettle leaves decreased with age of plantation, while polyphenols content showed constant level in the course of the whole cultivation period.
2. Higher amounts of polyphenols were accumulated in stinging nettle leaves harvested in May and July, while chlorophyll and carotenoids content reached the highest values in raw material harvested from September cut.
3. There was not found any directed dependence between harvest term and the content of phosphorus and potassium in stinging nettle leaves, while the amount of magnesium gradually decreased in raw material from the subsequent cuts.
4. The content of total nitrogen and total protein in this herb leaves increased according to both plantation age and in the subsequent cuts.
5. The highest antioxidant activity featured stinging nettle harvested in the earliest term, in May and June, while the lowest one was recorded in raw material from September harvest.

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WPŁYW WIEKU PLANATCJI I TERMINU ZBIORU NA SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNĄ POKRZYWY ZWYCZAJNEJ (*Urtica dioica* L.)

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Abstrakt: W badaniach polowych przeprowadzonych w latach 2004–2006 oceniano wpływ wieku plantacji i terminu zbioru ziela na skład chemiczny i aktywność antyoksydacyjną pokrzywy zwyczajnej.

Największą zawartość barwników (chlorofilu *a*+*b* i karotenoidów) oraz wapnia stwierdzono w roślinach jednorocznych, natomiast najwięcej magnezu i potasu zawierały rośliny najstarsze. Największą aktywność antyoksydacyjną wykazywał surowiec pokrzywy zbieranej w maju, natomiast zmniejszała się ona w zielu z następnych pokosów. Stwierdzono, że aktywność antyoksydacyjna surowca zależy od zawartości w nim polifenoli.

Słowa kluczowe: pokrzywa zwyczajna, aktywność antyoksydacyjna, skład chemiczny