The content of flavonoids and polyphenolic acids in inflorescences of Sandy Everlasting [*Helichrysum arenarium* (L.) Moench] from natural stands and plantations

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S u m m a r y

During the years 2007–2008 the content of flavonoids and polyphenolic acids in inflorescences of *Helichrysum arenarium* (L.) Moench was analyzed in specimens from natural stands and plantations. Plants cultivated in experimental plots originated from *in vitro* plantlets. The amount of flavonoids in inflorescences from experimental plots was higher and attained its maximum at 1.19% compared with 0.84% observed for plants from natural stands. In herbal material collected in 2008 the content of flavonoids was lower. The tendency was not observed for polyphenolic acids. Within the study period their amount ranged over the interval 0.74–2.82%. It was also found that dry mass of inflorescences from natural stands was higher than that for cultivated plants by 30.9%. In contrast, the density of generative shoots was 5.2-fold higher in natural stands. It was shown that Sandy Everlasting plantations could yield up to 3.4-fold more herbal material which is of a better quality (contains more biologically active compounds) than that from natural stands.

Key words: *Helichrysi inflorescentia, active compounds, TLC, density of generative shoots, yield of herbal material.*

INTRODUCTION

Helichrysum arenarium (L.) Moench is a perennial belonging to the sunflower family (*Asteraceae*). The species is under partial protection in Poland. Its inflorescences are a source of valuable herbal material known since medieval times and used in medicinal and cosmetic preparations. Dried plants can be used for bouquets and Easter decorations.

Compounds present in the inflorescences of Sandy Everlasting exhibit cholagogue and biligenic activity and protect the liver from toxins; they are also effective antioxidants [1-4]. According to the Polish Pharmacopoeia [5], the material referred to as *Helichrysi inflorescentia* should contain at least 0.5% of flavonoids (calculated as quercetin).

Since the 1970s there have been some unsuccessful attempts at growing Sandy Everlasting in the fields [6]. Population studies conducted lately [7-10] have only explained the reason why those attempts failed. The next step in the study on cultivation of Sandy Everlasting was determining methods of acquiring plantlets from tissue cultures and then their adaptation to greenhouse and field conditions [11-13].

The primary aim of this study was to compare the content of flavonoids and flavonoid glycosides in inflorescences from plants growing in natural stands with those of *in vitro* origin which were cultivated in plantations. The second goal was to determine the dry mass of inflorescences per shoot as well as the number of generative shoots and the inflorescence yield per unit (1 m^2).

MATERIAL AND METHODS

During growing seasons 2006–2008 the number of generative shoots per area unit (1 m^2) in phytocoenoses of *H. arenarium* and in experimental plots were determined. The shoots were then collected for further analysis.

Two natural *H. arenarium* populations were examined:

- population 1 located in the outskirts of Bydgoszcz (Fordon district);
- population 2 situated in the village of Łosiny (Bory Tucholskie forest complex).

Two observation plots were established in each of the two populations.

Experimental fields were set up in 2005 at UTLS Experimental Station in Mochełek near Bydgoszcz. Specimens of Sandy Everlasting originating from tissue cultures [12] were adapted to five different mineral and organic soil media [13]:

- highmoor peat supplemented with perlite;
- a medium for growing chrysanthemums;
- clayey soil;
- soil from a natural stand of *H. arenarium*;

- sterilized soil from a natural stand of *H. arenarium*.

Characteristics of soils and description of weather conditions within the study years can be found elsewhere [10, 11].

Room temperature dried inflorescences of Sandy Everlasting (samples of 100 g each) were subject to phytochemical analysis. The analysis included:

- identification of herbal material using thin layer chromatography TLC according to procedures described for Sandy Everlasting in Polish Pharmacopoeia VI [5];
- determining the content of flavonoids (calculated as quercetin) by Christ-Müller's method [5];
- determining the amount of polyphenolic acids (calculated as chlorogenic acid) by modified spectrophotometric method (European Pharmacopoeia VI [14]) with the use of Arnov's reagent. Analytical method was validated according to ICH regulations. Precision (RSD=3.0%; n=6), linearity (R^2=0.99998) and accuracy were evaluated. Recovery levels were within the acceptable range (98-102%).

Determining the content of polyphenolic acids (calculated as chlorogenic acid)

Reference solution A: 0.60 ml of chlorogenic acid (0.2 mg/ml in 50% ethanol, v/v) was placed in a 10 ml volumetric flask, and diluted with ethanol (50%, v/v) to 1.2 ml. Then 2.0 ml of hydrochloric acid (0.5 mol/l), 2.0 ml of sodium hydroxide (8.5%, m/v) were added; the solution was mixed and diluted with water to 10 ml.

Reference solution B: 0.60 ml of chlorogenic acid (0.2 mg/ml in 50% ethanol, v/v) was placed in a 10 ml volumetric flask, ethanol (50%, v/v) was added to 1.0 ml. Then 2.0 ml of hydrochloric acid (0.5 mol/l), 2.0 ml of Arnov's reagent and 2.0 ml of sodium hydroxide (8.5%, m/v) were added. The solution was mixed and diluted with water to 10 ml. Absorbance at λ=525 nm was measured against reference solution A.

Analyzed solution: 0.1 g of powdered material was placed in a 250 ml round-bottom flask. 40.0 ml of ethanol (50%, v/v) was added. The mixture was warmed and gently boiled for 30 min under a reflux condenser. Then the mixture was cooled to room temperature and filtered through a soft paper filter to a 50 ml volumetric flask. The paper was rinsed with 10.0 ethanol (50%, v/v). The solution was mixed and diluted to 50 ml with the same solvent.

To three 10 ml volumetric flasks 1.0 ml aliquots of the analyzed solution were added. Two flasks were then supplemented with 2.0 ml of hydrochloric acid (0.5 mol/l), 2.0 ml of Arnov's reagent, 2.0 ml of sodium hydroxide (8.5%, m/v), and filled up with water to 10 ml (analyzed solutions). To the third flask 2.0 ml of hydrochloric acid (0.5 mol/l), 2.0 ml of sodium hydroxide (8.5%, m/v) were added. The solution was mixed and diluted with water to 10 ml (reference solution C). Absorbance of the analyzed solutions was measured against reference solution C, at λ=525 nm.

The content of polyphenolic acids (calculated as chlorogenic acid) was calculated as follows:

$$X\,[\%] = \frac{A_{RB} \times c_{RW} \times R \times 100\%}{A_{RW} \times n},$$

where
A_{RB} – absorbance of the studied solution;
c_{RW} – concentration of chlorogenic acid [mg/ml];
R – dilution;
A_{RW} – absorbance of the reference solution;
n – the amount of material [mg].

Dry mass of flower heads per shoot and the yield of herbal material were also determined.

RESULTS AND DISCUSSION

The identity of *H. arenarium* inflorescence samples was confirmed using thin layer chromatography (TLC) method. Location, fluorescence and size of main bands present on chromatograms of the solutions studied corresponded with the bands on the reference chromatogram described in Polish Pharmacopoeia [5]. The following bands were recognized in the material studied: a blue-gray fluorescent band of chlorogenic acid (R_f 0.48–0.55), a yellow-orange fluorescent band of astralgin and apigenin-7-glucoside (R_f 0.68–0.72), a red-brown fluorescent band of salipurposide (R_f 0.79–0.84) and a blue-gray fluorescent band of caffeic acid (R_f 0.090–0.094). In addition, on the chromatograms the presence of other bands of weaker intensity was observed.

Two main classes of compounds present in *Helichrysi inflorescentia*, i.e. flavonoids and polyphenolic acids, were selected as quantitative markers. The content of flavonoids was determined as the total amount calculated as quercetin [5]. The content of polyphenolic acids was calculated as chlorogenic acid according to the validated method of the second author (tab. 1).

The content of flavonoids in inflorescence samples increased in 2008 compared with the previous year (tab. 1). The amount in flower heads from population 2 was 0.49% in 2007 and increased to 0.83% in the next year. The increase for population 1 was less pronounced (from 0.70% in 2007 to 0.83% in 2008). This means that the content of flavonoids in 2008 was practically the same in both populations. In turn, the level of flavonoids recorded in inflorescence samples from experimental plots was lower in 2008. The mean difference between the two study years was 0.3%. The highest difference in the content of flavonoids (0.43%) was found for plants adapted to medium for chrysanthemums, and those adapted to soil from natural stands of *H. arenarium*. The lowest difference (0.14%) was recorded for herbal material collected from the sterilized natural soil.

Table 1.

Content of flavonoids and polyphenolic acids in samples of *Helichrysi inflorescentia* collected in the years 2007–2008

origin of inflorescences	flavonoids (recalculated for quercetin) [%]		polyphenolic acids (recalculated for chlorogenic acid) [%]	
year	2007	2008	2007	2008
natural stands:				
- population 1	0.70	0.84	1.61	2.70
- population 2	0.49	0.83	0.74	2.82
cultivation of *in vitro* plants in experimental fields on different medium types:				
- peat + perlite	1.06	0.83	1.68	1.82
- a medium for chrysanthemums	1.07	0.64	1.99	1.48
- clayey soil	1.19	0.84	2.54	1.96
- soil from a natural stand of *H. arenarium*	1.03	0.60	1.42	1.56
- sterilized soil from a natural stand of *H. arenarium*	0.95	0.81	2.22	1.86

The content of flavonoids in inflorescences from experimental plots was higher than that in flower heads from natural populations and attained the values of 1.19% and 0.84%, respectively.

The level of flavonoids in herbal material harvested in July 2004 from 22 natural populations of *H. arenarium* growing in the middle part of Bug Valley determined by Bryksa-Godzisz et al. [15] varied over the interval 0.15–0.70%. A higher content of flavonoids in *Helichrysi inflorescentia* (varying from 0.37 to 1.10%), similar to that described in the present work, is reported by Czinner et al. [1].

Inflorescences collected from natural stands had, in turn, a higher content of polyphenolic acids (tab. 1). In general, the content of polyphenolic acids in plants from natural stands was higher in 2008; the highest level (2.82%) was found in herbal material taken from population 2. In contrast, a higher content of polyphenolic acids in 2008 was found in plants from experimental fields only for two media: peat supplemented with perlite and the soil from a natural stand (the difference between 2007 and 2008 was 0.14%). The most pronounced drop in the level of polyphenolic acids was observed for sandy clayey; the difference between the first and the second year of study was –0.58%.

A lower level of polyphenolic acids in herbal material studied, varying from 0.45 to 1.51%, is reported by Bryksa-Godzisz et al. [15].

Dry mass of inflorescences, the number of shoots per area unit (1 m^2), and the yield of herbal material depended on the origin of *H. arenarium* specimens (tab. 2). Dry inflorescence mass of plants from natural stands was higher by 30.9%, on average, than that of plants from experimental plots. In natural stands, the highest dry inflorescence mass per shoot was found in 2006 (the population 2: 10.78 g), whereas the highest value of the parameter recorded for the experimental plots was 7.16 g (peat + perlite, 2007). These results were associated with pluviothermal conditions and the age of plantations [11].

The density of shoots was higher (5.2-fold, on average) in experimental plots than in natural stands (tab. 2). The highest number of generative shoots per area unit was observed on medium for chrysanthemums (maximum of 519.87 shoots per 1 m^2; 370.51, on average). In 2008, the fourth year of cultivation, the density dropped by 90.04% compared to the value recorded in 2006. The decrease in 2007 was only 15.84%. It could be brought about by the age of plants and weather conditions in winter 2007/2008 and early spring 2008 (when plots were temporarily flooded).

Table 2.

Selected features of Sandy Everlasting from natural stands and experimental plots

origin of *H. arenarium*	year (of cultivation)			mean
	2006 (2nd)	2007 (3rd)	2008 (4th)	
dry mass of inflorescences per shoot [g]				
natural stands:				
- population 1	6.10	5.33	3.82	5.08
- population 2	10.78	8.44	4.42	7.88
cultivation of *in vitro* plants in experimental fields on different medium types:				
- peat + perlite	5.66	7.16	3.14	5.32
- a medium for chrysanthemums	5.03	6.85	2.18	4.69
- clayey soil	5.04	7.14	2.00	4.73
- soil from a natural stand of *H. arenarium*	3.96	5.04	1.74	3.58
- sterilized soil from a natural stand of *H. arenarium*	3.07	6.69	2.44	4.07
number of generative shoots per 1 m^2				
natural stands:				
- population 1	87.50	41.50	12.50	47.17
- population 2	158.50	31.50	8.50	66.17
cultivation of *in vitro* plants in experimental fields on different medium types:				
- peat + perlite	491.82	443.28	29.30	321.47
- a medium for chrysanthemums	519.87	513.67	78.00	370.51
- clayey soil	432.00	404.67	44.70	293.79
- soil from a natural stand of *H. arenarium*	471.43	297.56	35.51	268.17
- sterilized soil from a natural stand of *H. arenarium*	360.50	256.08	39.21	218.60
yield of herbal material per 1 m^2 [kg m^{-2}]				
natural stands:				
- population 1	0.53	0.22	0.05	0.27
- population 2	1.71	0.27	0.04	0.67
cultivation of *in vitro* plants in experimental fields on different medium types:				
- peat + perlite	2.78	3.17	0.09	2.01
- a medium for chrysanthemums	2.61	3.52	0.17	2.10
- clayey soil	2.18	2.89	0.09	1.72
- soil from a natural stand of *H. arenarium*	1.87	1.50	0.06	1.14
- sterilized soil from a natural stand of *H. arenarium*	1.11	1.71	0.10	0.97

The yield of herbal material from experimental plots was 3.4-fold higher on average than that from natural stands (tab. 2). The highest yield of air dry inflorescences, obtained in 2007 from the medium for chrysanthemums, was 3.52 kg m^{-2}. The mean yield in the subsequent years of cultivation was 2.11, 2.56 and 0.10 kg m^{-2}. The mass of air dry flower heads collected from natural stands was 1.12, 0.25 and 0.05 kg m^{-2}, respectively.

In their 3-year field experiment with Sandy Everlasting growing from seeds at Stryków Experimental Station, Pacholak and Załęcki [6] obtained the yield of 0.05, 0.19 and 0.22 kg m^{-2}. The mean yield of inflorescences was 0.15 kg m^{-2} which constitutes only 9.43% of the average yield of herbal material originating from the *in vitro* cultures obtained it the experiments conducted at the UTLS Experimental Station in Mochełek on sandy clayey medium.

A plantation of *H. arenarium* specimens of *in vitro* origin may give a significantly higher yield than that obtained from natural stands or plantations started from achenes. Moreover, *Helichrysi inflorescentia* obtained from plantations started from tissue cultures is of much better quality, i.e. contains higher amounts of active compounds.

CONCLUSIONS

1. Chlorogenic acid, astralgin, apigenin-7-glucoside, salipurposide, and caffeic acid were found in inflorescences of *H. arenarium* in plants from natural stands and experimental fields. The content of flavonoids in herbal material from cultivated plants was higher than that in inflorescences collected from natural stands, up to 1.19% and 0.84%, respectively.
2. The content of polyphenolic acids in inflorescences of *H. arenarium* varied from 0.74 to 2.82% and was higher in material collected from natural stands.
3. In comparison to natural stands, up to 3.4-fold more herbal material, containing higher amounts of flavonoids, can be harvested from Sandy Everlasting plantations originating from *in vitro* cultures.

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ZAWARTOŚĆ FLAWONOIDÓW I POLIFENOLOKWASÓW W KWIATOSTANACH KOCANEK PIASKOWYCH [*HELICHRYSUM ARENARIUM* (L.) MOENCH] POCHODZĄCYCH ZE STANOWISK NATURALNYCH ORAZ UPRAWY POLOWEJ

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Streszczenie

Badano rośliny *Helichrysum arenarium* (L.) Moench pochodzące ze stanowisk naturalnych oraz otrzymane metodą *in vitro* i uprawiane na poletkach doświadczalnych. Zawartość flawonoidów w kwiatostanach pochodzących z uprawy była wyższa i wynosiła maksymalnie 1,19%, gdy w zebranych ze stanowisk naturalnych dochodziła tylko do 0,84%. W 2008 roku, w materiale zielarskim pochodzącym z uprawy, zaobserwowano spadek zawartości flawonoidów, natomiast zawartość polifenolokwasów nie wykazywała zmian zgodnych z tą tendencją. Ich zawartość wahała się od 0,74 do 2,82%. Stwierdzono także, że pędy generatywne zebrane ze stanowisk naturalnych miały kwiatostany o suchej masie wyższej o 30,9% od tychże pochodzących z uprawy. Natomiast zagęszczenie pędów kwiatostanowych na poletkach uprawowych było 5,2 raza wyższe w stosunku do stanowisk naturalnych. Uprawa pozwala również na osiąganie 3,4 raza wyższych plonów surowca zielarskiego. Oznacza to, że w porównaniu ze stanowiskami naturalnymi, z plantacji kocanek piaskowych pochodzących z kultur tkankowych można otrzymać więcej surowca zielarskiego charakteryzującego się wyższą zawartością substancji czynnych.

Słowa kluczowe: *Helichrysi inflorescentia, substancje czynne, TLC, zagęszczenie pędów generatywnych, plon surowca zielarskiego*